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EVALUATION OF ANTIOXIDATION PROPERTIES OF NATURAL POLYPHENOL WATER EXTRACTS FROM SELECTED PLANTS IN A MODEL SYSTEM

Summary

In the recent times plants are becoming more popular. Their beneficial pro-health properties result from the content of numerous characteristic compounds. Reports are confirming their beneficial antioxidant, antibacterial and anti-inflammatory properties. The aim of this study was to compare the antioxidant potential and the characteristics of water extracts obtained from plants: yarrow herb (AM) *(Achillea millefolium* L.*), knotweed herb* (PA) *(Polygonum aviculare* L.*), horsetail herb* (EA) *(Equisetum arvense* L.*), and motherwort herb* (LC) *(Leonurus cardiaca* L.*). Extracts osmolality was from* $0{,}142^{a}\pm0.02$ *mOsm/kg* H_2O *for the AM extract to* $0{,}176^{c}\pm0{,}01$ *mOsm/kg* H_2O *for the EA extract. The test with the use of the Folin-Ciocalteu reagent showed that the EA extract had the highest content of reducing compounds (*$54{,}24^{d}\pm2{,}19$ *mg GAE/g dw). It was shown that the highest activity against the DPPH radical was obtained for the extract from Equisetum arvense* L. *(*$5.97^{c} \pm 0.11$ *mg TE/1 g dw), and this extract also showed the highest antioxidant capacity measured with the radical ABTS method (*$7.23^{b}\pm0.06$ *mg TE/g dw). These analyses were also confirmed by the tests carried out using the FRAP test, EA extract also showed 40% higher activity compared to the lowest AM extract. It has been shown that the investigated herbal raw materials can be an attractive raw material with antioxidant properties that can be used in application in food technology.*

***Key words**: antioxidant, FRAP, Folin-Ciocalteu, ABTS, DPPH, yarrow herb, knotweed herb, horsetail herb, motherwort herb*

OCENA AKTYWNOŚCI PRZECIWUTLENIAJĄCEJ WODNYCH EKSTRAKTÓW Z WYBRANYCH SUROWCÓW ROŚLINNYCH W UKŁADACH MODELOWYCH

Streszczenie

Surowce roślinne cieszą się coraz większą popularnością. Ich korzystne właściwości prozdrowotne wynikają z zawartości licznych charakterystycznych związków. Dostępne są doniesienia, potwierdzające ich korzystne właściwości antyoksydacyjne, przeciwbakteryjne i przeciwzapalne. Celem niniejszej pracy było porównanie potencjału przeciwutleniającego oraz charakterystyka ekstraktów wodnych uzyskanych z następujących roślin: ziele krwawnika (AM) (Achillea millefolium L.), ziele rdestu ptasiego (PA) (Polygonum aviculare L.), ziele skrzypu (EA) (Equisetum arvense L.) oraz ziele serdecznika (LC) (Leonurus cardiaca L.). Osmolalność ekstraktów wynosiła od $0{,}142^{a}\pm0{,}02$ *mOsm/kg* H_2O *dla ekstraktu AM do* $0{,}176^{c}\pm0{,}01$ *mOsm/kg* H_2O *dla ekstraktu EA. Test z odczynnikiem Folin-Ciocalteu wykazał, że ekstrakt EA charakteryzował się najwyższą zawartością związków redukujących (*$54{,}24^{d}\pm2{,}19$ *mg GAE/g sm). Najwyższą aktywność wobec rodnika DPPH uzyskano dla ekstraktu z Equisetum arvense L. (*$5{,}97^{c}\pm0{,}11$ *mg TE/1g sm), ekstrakt ten wykazał także najwyższą zdolność antyoksydacyjną mierzoną metodą z kationorodnikiem ABTS (*$7{,}23^{b}\pm0{,}06$ *mg TE/g sm). Analizy te zostały również potwierdzone w teście z użyciem metody FRAP, ekstrakt EA wykazywał o 40% wyższą aktywność w porównaniu z najniższym uzyskanym wynikiem (AM). Wykazano, że badane surowce zielarskie mogą być atrakcyjnym źródłem związków polifenolowych o właściwościach przeciwutleniających, które mogą znaleźć znacznie szersze niż dotychczas zastosowanie w technologii żywności.*

***Słowa kluczowe:** przeciwutleniacze, FRAP, Folin-Ciocalteu, ABTS, DPPH, ziele krwawnika, ziele rdestu, ziele skrzypu, ziele serdecznika*

1. Introduction

Currently, there is a growing interest in the possibilities of using plant materials in medicine, as well as in the design of functional food. The biological activity of raw materials depends on the content of individual phytochemicals that are found in various anatomical parts of plants: fruits, shoots, buds, leaves, roots, and bark [8, 12].

New raw materials that can gain importance not only as an innovation in functional food design are yarrow herb (*Achillea millefolium* L.), knotweed herb (*Polygonum aviculare* L.), horsetail herb (*Equisetum arvense* L.), and motherwort herb (*Leonurus cardiaca* L.). It is common in many parts of the world and does not require special growing conditions. They can be used to design innovative dietary supplements or functional foods.

Food should not only provide essential nutrients but should also be considered in terms of the pro-health benefits that can be obtained from it. Herbs are a good source of polyphenolic compounds with high antioxidant activity, and the polyphenols they contain protect cell structures against damage caused by free radicals, which contribute to faster aging of the body.

The current results of scientific research confirm the beneficial properties of extracts from all tested raw materials [1, 3, 6, 15]. The research confirmed that these extracts contain polyphenolic compounds and show antioxidant activity. Thus, preparations of yarrow herb (*Achillea millefolium* L.), bird knotweed herb (*Polygonum aviculare* L.), horsetail herb (*Equisetum arvense* L.) and motherwort herb (*Leonurus cardiaca* L.) can be a valuable raw material, used in the food and pharmaceutical industries as a source of bioactive compounds with multidirectional antioxidant activity.

The aim of this study was to characterize phytochemicals present in the herb yarrow (*Achillea millefolium* L.), knotweed herb (*Polygonum aviculare* L.), horsetail herb (*Equisetum arvense* L.), and motherwort herb (*Leonurus cardiaca* L.), their biological properties, and also the possibility of their use in industry and food.

2. Material and extraction

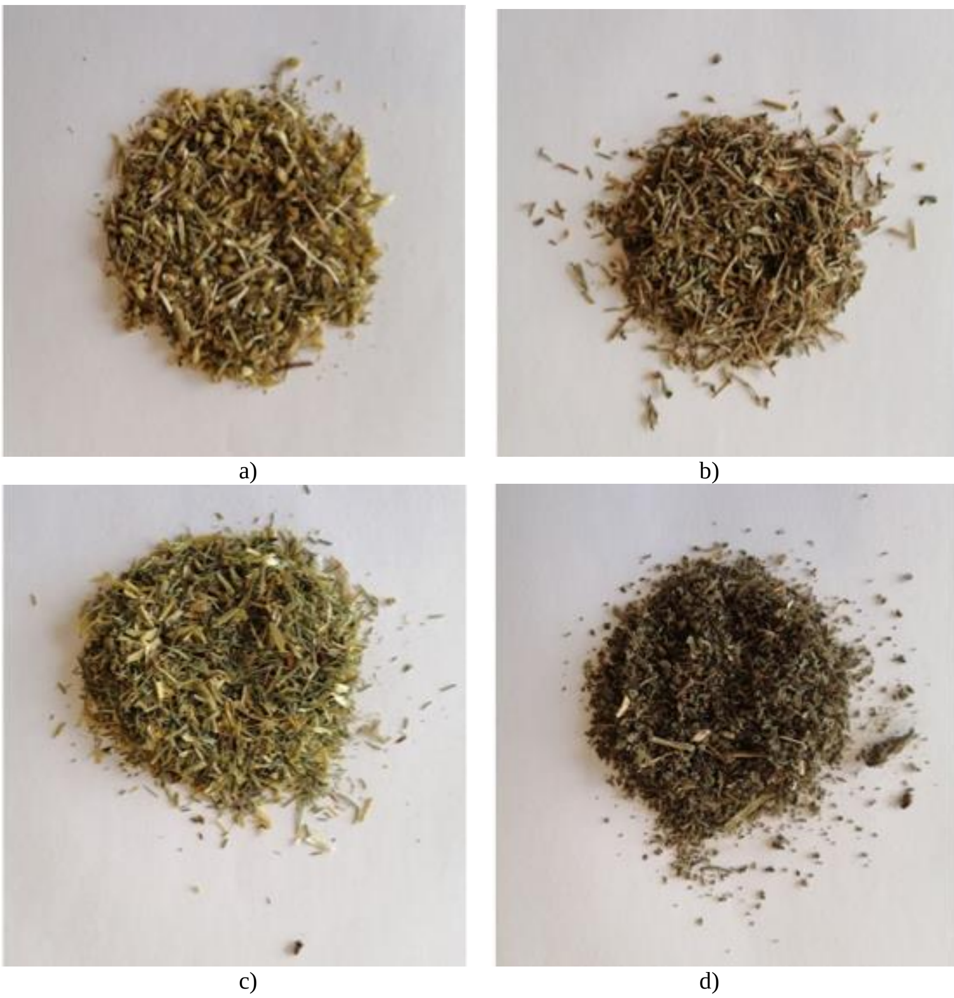
The tested material was the yarrow herb (*Achillea millefolium* L.) (AM), knotweed herb (*Polygonum aviculare* L.) (PA), horsetail herb (*Equisetum arvense* L.) (EA), and motherwort herb (*Leonurus cardiaca* L.) (LC), obtained from an orchard farm in Ozierany Małe in Podlasie, Poland (53º 13 '14,865 "N 23º 51' 9,327" E). The average amount of rainfall during the growing season was 315 nm per square meter with an average temperature of 14.7°C, and the soil in the orchard was moderately rich in macronutrients. The tested raw materials were dried at a temperature of 60°C for 240 min. Then, the dried plant material was ground and subjected to water extraction (Fig. 1).

The water extracts from tested plant materials were obtained using 1000 mL of water, at 90°C, mixed with 50 g of raw plant material and extracted for 15 min. The extracts were filtered and centrifuged (800 x *g*, 15 min), each time. The fractions were decanted and filtered (Whatman 1:11 μm) and the prepared extracts were stored at 4°C in dark tubes until examination.

3. Methods

3.1. Color and osmolality of extracts measurement

Color measurement of tested plant extracts was run in L* a* b* CEN unit system using spectrometer CM-5 (Konica Minolta, Japan) according to the methodology described by the manufacturer. As a source of light, D 65 was applied, and color temperature equaled 6504 K. The observation angle of the standard colorimetric observer was 10°. Measurements for each sample was repeated fivefold. The instrument calibration was performed with the use of a black pattern.



a) b) c) d)

Source: own study / Źródło: opracowanie własne

Fig. 1. Dried plant material: a) yarrow herb, b) knotweed herb, c) horsetail herb, d) motherwort herb
Rys. 1. Susz roślinny: a) krwawnik pospolity, b) rdest ptasi, c) skrzyp polny, d) serdecznik pospolity

3.2. Antioxidative potential analysis by spectrophotometric method

The total phenolic content (TPC) of the obtained extracts was determined using the method described by Kulczyński et al. [9]. Aliquots of 100 µL diluted in 900 µL of 40% ethanol (Sigma-Aldrich, Germany) were mixed with 1 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, Germany), followed by the addition of 1 mL of 35% sodium carbonate (POCH, Poland). Samples were vortexed for 5 s and after incubation in darkness at room temperature for 90 min, the absorbance of the reaction mixture was measured at 765 nm against blank. The TPC was expressed as mg of gallic acid (Sigma-Aldrich, Germany) equivalents (GAE) per 1 g of dry mass using the calibration curves of gallic acid.

The DPPH procedure was based on the reduction of DPPH solution absorbance (2,2-diphenyl-1-picrylhydrazyl) at wavelength 517 nm in the presence of free radicals [8]. Measurements were performed using SP-830 Plus apparatus (Metertech, Taiwan). The percentage of DPPH radical scavenging was evaluated on the basis of the standard curve for y = 321.54x + 21.54 (R^2 = 0.986) and presented as mg TE/g dw of extract.

The ABTS cation radical scavenging activity was measured according to the TEAC (Trolox Equivalent Antioxidant Capacity) test according to the methodology described by Kobus-Cisowska et al. [8]. Spectrophotometric measurement of the ability to scavenge $ABTS^{\bullet+}$ formed from ABTS (2,20-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) by oxidation with potassium persulphate was carried out at a wavelength of 414 nm using SP-830 Plus apparatus (Metertech, Taiwan). The percentage rate of $ABTS^{\bullet+}$ scavenging was calculated from the standard curve for y = 121.63x +26.33 (R^2 = 0.96) and expressed as mg TE/g dw of extract.

3.3. Ferric reducing antioxidant power (FRAP)

The antioxidant properties of the water extracts were determined using a ferric reducing/antioxidant power assay (FRAP method) according to the procedure described by O'Sullivan et al. [11]. FRAP reagent [2 mL; 0.01 M TPTZ (2,4,6-tripyridyl-s-triazine) in 0.04 M HCl, 0.02 M FeCl3. 6H2O and 0.3 M acetate buffer] was added to 1 µL of each sample diluted in 999 µL distilled H2O. A calibration curve was constructed using FeSO4×7H2O. Samples were incubated for 30 min and the absorbance was measured at 593 nm (Metertech SP880, Taiwan). Data were expressed as µM FeSO4/g dw of extract.

3.4. Statistical analysis

Statistical analysis of all results was performed using Microsoft Excel 2013 software (USA) and Statistica 13 software (StatSoft, Poland). The electrochemical results were treated as an additional factor to the model based on standard analytical techniques. The *p* values for Levene's test of independent variables were calculated.

4. Results
4.1. Characteristics of tested plant extracts

The osmolality of the extracts indicates the freezing point of the extract and its differences relative to the freezing of water, which is a measure of the osmotic pressure of the tested extract. Extracts osmolality was from $0{,}142^{a}\pm0.02$ mOsm/kg H_2O for the AM extract and 0,176±0,01 mOsm/kg H_2O for the EA extract (Table 1).

Tested plant extracts were physically and chemically characterized (Table 1). It was shown that the color of the tested extracts differed in terms of assessed parameters. Parameter L* determining the brightness was from $24.2^{a}\pm0.33$ (AM) to $27.2^{a}\pm0.18$ (PA). Parameter a*, responsible for the color change in the range from green to red, was from $8.98^{a}\pm0.21$ (PA) to $9.41^{a}\pm0.76$ (LC), whereas parameter b* responsible for the color change in the range from blue to yellow had lower values for AM sample ($1.62^{a}\pm0.19$) and the highest for EA sample ($1.92^{a}\pm0.43$).

The analyzed extracts were evaluated for their antioxidant potential by spectroscopic methods (Table 2). It was found that extracts prepared from the tested plant materials had different properties. The highest content of these compounds was found in the extract of *Equisetum arvense* L. (EA) (Table 2). The test with the use of the Folin-Ciocalteu reagent showed that the EA extract had the highest content of reducing compounds ($54.24^{d} \pm 2.19$ mg GAE/g dw).

The antioxidant activity of the tested extracts was also assessed by the FRAP method. The antioxidant capacity of the extracts decreased according to the following sequence: (EA) $287.65^{a} \pm 4.87$ > (LC) $265.92^{a} \pm 1.98$ > (PA) $254.65^{b} \pm 12.11$ > (AM) $205.43^{a} \pm 7.12$ (µM $FeSO_4$/g dw).

It was shown that for the extract from *Equisetum arvense* L. (EA) the DPPH radical scavenging capacity was the highest ($5.97^{c} \pm 0.11$ mg TE/g dw), while for the *Leonurus cardiaca* L. (LC) extract it was the lowest, amounted to $3.32^{a} \pm 0.13$ mg TE/g dw.

Table 1. Characteristic of tested extracts given in CIE L*a*b* units and osmolality
Tab. 1. Charakterystyka badanych ekstraktów podana w jednostkach CIE L a* c* oraz ich osmolalność*

Sample	*Achillea millefolium* L.) **AM**	*Polygonum aviculare* L.) **PA**	*Equisetum arvense* L.) **EA**	*Leonurus cardiaca* L.) **LC**
Osmolality (mOsm / kg H_2O)	$0{,}142^{a}\pm0.02$	$0{,}152^{b}\pm0{,}03$	$0{,}176^{c}\pm0{,}01$	$0{,}161^{c}\pm0{,}01$
Freezing temperature (°C)	$-0{,}229^{a}\pm0.01$	- 0,232±0,01	- 0,265±0,01	- 0,243±0,01
L*	$24.2^{a}\pm0.33$	$27.2^{a}\pm0.18$	$25.2^{a}\pm0.43$	$24.29^{a}\pm0.55$
a*	$9.12^{a}\pm0.04$	$8.98^{a}\pm0.21$	$9.32^{a}\pm0.33$	$9.41^{a}\pm0.76$
b*	$1.62^{a}\pm0.19$	$1.68^{a}\pm0.29$	$1.92^{a}\pm0.43$	$1.63^{a}\pm0.71$

Results are mean values of three determinations ± standard deviation. Values sharing the same letter in a line are not significantly different (P ≤ 0.05).

Source: own study / Źródło: opracowanie własne

Table 2. Total polyphenols content and antioxidant activity of plant extracts determined by the DPPH, ABTS and FRAP test
Tab. 2. Całkowita zawartość polifenoli i aktywność przeciwutleniająca ekstraktów roślinnych badana metodami DPPH, ABTS oraz FRAP

Sample / activity	*Achillea millefolium* L.) **AM**	*Polygonum aviculare* L.) **PA**	*Equisetum arvense* L.) **EA**	*Leonurus cardiaca* L.) **LC**
Folin-Ciocalteu (mg GAE/g dw)	26,65 ^{a} ± 2,93	32,32 ^{b} ± 2,76	54,24 ^{d} ± 2,19	44,21 ^{c} ± 1,93
DPPH (mg TE/1 g dw)	4,43 ^{ab} ± 0,02	5,32 ^{b} ± 0,01	5,97 ^{c} ± 0,11	3,32 ^{a} ± 0,13
ABTS (mg TE/g dw)	6,53 ^{c} ± 0,04	4,43 ^{a} ± 0,08	7,23 ^{b} ± 0,06	5,12 ^{b} ± 0,02
FRAP (uM $FeSO_4$/1g dw)	205,43 ^{a} ± 7,12	254,65 ^{b} ± 12,11	287,65 ^{a} ± 4,87	265,92 ^{a} ± 1,98

Results are mean values of three determinations ± standard deviation. Values sharing the same letter in a line are not significantly different ($P \leq 0.05$).

Source: own study / Źródło: opracowanie własne

ABTS radical method, which also showed higher activity of the solution from *Equisetum arvense* L. extract, where the value of aqueous extracts (EA) was $7.23^{b} \pm 0.06$ mg TE/g dw, and was higher than for LC extract, where it was $5.12^{b} \pm 0.02$ mg TE/g dw.

These analyses were also confirmed by the tests carried out using the FRAP test, EA extract also showed 40% higher activity compared to AM extract.

5. Discussion

Less known plants or underused plant species are of great research interest due to the presence of compounds that exhibit health-promoting properties. These compounds are mainly represented by polyphenols. Research on polyphenols has made it possible to find a relationship between their structure and the ability to scavenge free oxygen radicals. The most active are anthocyanidins and flavonoids with numerous OH groups in the so-called B ring [5].

There are studies available in the literature which, as in the study, indicate the antioxidant potential of the studied raw materials (*Achillea millefolium* L., *Polygonum aviculare* L., *Equisetum arvense* L., *Leonurus cardiaca* L.) [1, 3, 6, 15].

The results obtained in the study on the examined herbs confirmed that they are a rich source of biologically active substances, which was confirmed by the method with the Folin-Ciocalteu reagent. This method is the routine test for polyphenol antioxidants. This determination is characterized by great simplicity and usefulness about to with concerning standard biological materials. The disadvantage of this method is low specificity, the F-C reagent reacts with many compounds, incl. sugars, ascorbic acid, amino acids, and proteins, thus increasing the result of the analysis of phenolic compounds [2]. However, in the presented work, the overall content of these compounds allowed us to compare the tested raw materials and indicate that they differ statistically. The raw material that needs special attention is *Equisetum arvense* L.

Compounds with antioxidant activity act according to various mechanisms [14]. In recent years, the ability of naturally occurring compounds in plants to neutralize free radicals generated by the action of certain chemicals has been emphasized. The action of free radicals can lead to various damages to the human body. Therefore, the supply of compounds with antioxidant properties is an important role in the proper functioning of the body.

It is believed that the antioxidant properties of polyphenols are mainly responsible for the health-promoting effect of plant extracts, so they are used in the prevention and treatment of many diseases caused by oxidative stress [13]. Based on the effects of the interaction of these substances with the biological membrane, which is the first point of contact of various physicochemical factors with organisms, it will be possible to determine the likely molecular mechanism responsible for these effects. This mechanism has not yet been fully elucidated [13].

The study showed that the tested herbs contain compounds with antiradical and antioxidant properties. In studies conducted by Jafari and Khanavi [7], it was shown that methanol extracts from *Leonurus cardiaca* L. have antiradical activity, determined by DPPH tests to 57.75% µg mL^{-1} and 1.735 ± 0.07 µmol g^{-1}, using the ABTS radical. However, in our tests for the same water extract, the values were $3.32^{a} \pm 0.13$ mg TE/g dw in the DPPH test and $5.12^{b} \pm 0.02$ mg TE/g dw in the ABTS test.

In another study, the total content of phenolic compounds in ethanol extracts from *Polygonum aviculare* L. was determined and it was shown that it contained 677.4 ± 62.7 ($N = 8$) µg of GAE/g [6] and in our study, the total content of these compounds in water extracts was $32.32^{b} \pm 2.76$ mg GAE/g dw.

The antiradical activity of *Equisetum arvense* L. was also determined in the DPPH radical test by a group of scientists from Serbia [10], the established activity was 2.37 [EC50 (µg/mL)]. In our study, the activity in the DPPH radical test for the same plant was $5.97^{c} \pm 0.11$ mg TE/g dw.

The plant *Achillea millefolium* L. has also been the subject of many studies. Its aqueous extracts were assessed by scientists from Iran [4]. They were assessed, inter alia, in terms of the content of compounds capable of scavenging DPPH radicals, and it was shown that *Achillea millefolium* L. extract had antiradical activity at the level of 50.8%. In our research, an aqueous extract from the same plant showed such activity at the level of $4.43^{ab} \pm 0.02$ mg TE/g dw.

6. Summary

The total antioxidant effect is difficult to predict because the total antioxidant capacity is determined not only by the properties of individual compounds but above all by their interaction. Therefore, the study showed that each of the tested plants is rich in compounds from the group of polyphenols, which may also determine their various health-promoting properties. The current results of scientific research also confirm the beneficial properties of the studied herbs. The research confirmed that the extracts of yarrow herb, knotweed herb, horsetail herb, and motherwort herb contain polyphenolic compounds showing antioxidant activity. Thus, preparations using these raw materials can be a valuable raw material, used in the food or pharmaceutical industry as a source of bioactive compounds with multidirectional antioxidant activity.

7. References

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