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EXPERIMENTAL PAPER

Application of green-extraction technique to evaluate of antioxidative capacity of wild population of fireweed (*Epilobium angustifolium*)

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Summary

Introduction: Fireweed (*Epilobium angustifolium* (L.) Holub) is a common weed growing on meadows, roadside and agricultural wasteland, creating vast, rapidly spreading fields.

Objective: The aim of the study was to evaluate the antioxidant activity of extracts from fresh fireweed, harvested at three ripening stages.

Methods: Analysis of antioxidative activity was carried out by DPPH, ABTS and FRAP methods. Total polyphenol and total flavonoid content were also determined. Plant material was extracted using ultrasound-assisted green extraction technique with methanol, ethanol and isopropanol at different concentrations and water.

Results: The highest antioxidant activity evaluated by DPPH, ABTS and FRAP was found for the extracts prepared in 70% ethanol. The highest content of total polyphenols were observed in extracts in 70% ethanol, whereas the highest content of flavonoids extracts in undiluted methanol.

Conclusion: *Epilobium angustifolium* harvested at fruit ripening stage seems to be a valuable source of antioxidants.

Keywords: ***Epilobium angustifolium, antioxidant activity, total polyphenol content, total flavonoid content, green-extraction technique***

Słowa kluczowe: ***Epilobium angustifolium, aktywność antyoksydacyjna, ogólna zawartość polifenoli, ogólna zawartość flawonoidów, zielona metoda ekstrakcji***

INTRODUCTION

Fireweed (*Epilobium angustifolium* (L.) Holub) belongs to the family of *Onagraceae* and occurs mainly in North America, Asia and Europe. This plant belongs to the genus *Epilobium*, consisting of more than 200 species, with 26 different varieties of this plant in Europe [1-3]. Its major natural habitat are coniferous forests, although it can often be found on porches, gravel lands, wastelands, roadsides, meadows and pastures, as well as in highly insolated areas [4]. This plant is an undesirable weed, especially in fields and meadows, due to its high competitiveness to other plant species to access nutrients and water. It has been recommended in traditional medicine for a long time as a popular raw material with analgesic, antibacterial and anti-inflammatory activity. Moreover, it has been also used as an adjuvant in the prevention and alleviation of symptoms of prostatic hyperplasia. Due to the sweet taste, this plant is popular in Russia as a infusion from fermented leaves and used as an adjuvant for gastric ulcerations and inflammations [5-6]. Many authors have also observed antioxidant properties of *E. angustifolium*. An increasing consumption of plants with antioxidant capacity seems to be important. The formation of an excessive amount of reactive oxygen species (ROS) can cause an oxidation of proteins, lipids and DNA and can lead to cell damage. As a consequence, susceptibility to many so-called civilization diseases such as, for example, cardiovascular and neoplastic diseases, as well as diabetes, osteoporosis and neurodegenerative diseases has been observed [7-9].

One of modern extraction techniques used to obtain plant material, inter alia active ingredients with antioxidant properties, is the application of ultrasound-assisted extraction, which could be classified as so-called green extraction method. Recently, this technique has been increasingly used due to its high efficiency. Extraction of plant material occurs in a short time, along with reduced consumption of solvents, coupled with lower environmental pollution [10]. It is an affordable, simple and efficient method, as compared to traditional extraction techniques. Ultrasounds exert a mechanical effect to allow an increase of solvent penetration into the sample matrix and to increase the contact area between solid and liquid phase. As a result, the substances contained in the plant material quickly diffuse to the extraction solvent [11]. The aim of the study was to evaluate the antioxidant activity and the total polyphenol and flavonoid content of extracts of fireweed, harvested from the natural state, in three plant ripening stages. The ultrasound-assisted extraction was performed in 15, 30 and 60 minutes with use of four solvents (water, methanol, ethanol and isopropanol in different proportions) to obtain the extracts.

MATERIAL AND METHODS

Reagents. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 2,2-azino-bis (ethylbenzothiazolin-6-sulfonoic acid (ABTS), 2,4,6-tripyridyl-S-triazine (TPTZ) and rutin were purchased from Sigma Aldrich (USA); Folin-Ciocalteu reagent from Merck (Germany); 99.5% acetic acid, sodium acetate anhydrous, potassium persulfate, potassium acetate, aluminum chloride, 36% hydrochloric acid, as well as ethanol, methanol and isopropanol (all of analytical grade) were obtained from Chempur, Piekary Śląskie (Poland).

Plant material. The plant material consisted of fresh fireweed harvested in 2017 from a natural site, which was an agricultural wasteland located near a big city (N 53°23'18", E 14°28'56"). Plants were harvested in three developmental stages: the first – intensive growing (second week of May), the second – massive blooming (second week of July) and the third – fruit ripening stage (fourth week of August). The harvested material was subjected to extraction immediately. The plant material was identified by Anna Nowak, who graduated from Agriculture University, Szczecin, Poland. Her PhD thesis concerned plant physiology and her research was closely relater to this field of interest.

Ultrasound-assisted extraction. The vegetable raw material was extracted using the following solvents:

aqueous ethanol (40% *v/v*), 70% *v/v*) and undiluted), aqueous methanol (40% *v/v*), 70% *v/v* and undiluted), aqueous isopropanol (40% *v/v*), 70% *v/v* and undiluted) as well as water. Extraction was performed using an ultrasound bath for 15, 30 or 60 minutes. The obtained extracts were evaluated for their antioxidant activity and for total polyphenol and flavonoid content using spectrophotometric methods.

DPPH radical scavenging activity. The scavenging activity of DPPH stable free radicals was measured as described previously [12, 13]. Shortly, a sample of 0.15 cm^3 of the plant extract was mixed with 2.85 cm^3 of 0.3 mM DPPH radical solution dissolved in 96% *v/v* ethanol. Measurement of antioxidant activity was performed after 10 min. of incubation in dark at a room temperature. Absorbance at 517 nm was measured. The results are presented as radical scavenging activity (RSA) [%].

ABTS radical scavenging activity. The procedure applied to evaluate ABTS radical scavenging activity was described previously [14]. Shortly, 7 mM solution of ABTS (2,2-azine-bis (3-ethylbenzthiazoline-6-sulfonic acid) in a 2.45 mM aqueous solution of potassium persulfate was used to prepare the stock solution. After dissolving the components, solution was incubated for 24 hours in dark at a room temperature, then diluted with 50% *v/v* methanol to obtain an absorbance of 1.000±0.005. The abovementioned solution was added to the test extract in a ratio of 1:100 by vol. The absorbance of the samples was measured at 734 nm. As previously, the results were expressed as trolox equivalent antioxidant capacity - TEAC.

FRAP assay. The ability to reduce ions Fe^{3+} to Fe^{2+} (Ferric ion Reducing Antioxidant Power – FRAP) was determined as previously described [15]. In this method, the working solution consisted of 0.3 M acetate buffer (pH 3.6) mixed with 0.01 M 2,4,6-tripyridyl-S-triazine (TPTZ) in 0.04 M HCl and 0.02 M ferric chloride (10:1:1 by vol.). One volume of plant extract was mixed with 29 parts of this solution. The absorbance was measured at a wavelength of 593 nm and the results were expressed as TEAC.

Total polyphenols content (TPC). Total polyphenol content was determined with Folin-Ciocalteau method as described previously [12]. Shortly, to 0.15 cm^3 of the extract 0.15 cm^3 of tenfold diluted Folin-Ciocalteu reagent, 1.35 cm^3 of 0.01 M sodium carbonate solution and 1.35 cm^3 water was added and mixed. After 15 min of incubation in dark at a room temperature, the absorbance was measured at 765 nm. Gallic acid (GA) was applied as a standard and results were expressed as gallic acid equivalents (GAE) in mg gallic acid/g of raw material.

Total flavonoid content. The total flavonoid content in the test samples was determined using the colorimetric method described by Berreira *et al.* [16]. Shortly, to 2.5 cm^3 of plant extract, 1.25 cm^3 distilled water and 0.075 cm^3 of 5% sodium nitrite solution were added. After 5 min, 0.15 cm^3 of 10% aluminum chloride and 0.5 cm^3 of 1 M sodium hydroxide aqueous solution were added followed by 1.35 cm^3 water. As a reference substance, rutin (RU) was used. The results were expressed in mg RU/g of raw material. Spectrophotometric measurements were taken at 510 nm.

Statistical analysis. The statistical analysis of the results was carried out with the Statistica 12 program package (StatSoft) using a one-way analysis of ANOVA variance, with the significance level $p<0.05$. Inter-group differences were determined by Tukey's test (n=3). The Pearson correlation coefficient between the results obtained with individual methods of antioxidative capacity assessment and total polyphenols and flavonoids content were also calculated. To evaluate the differences between individual ripening stages, the Wilcoxon test was used.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

When assessing the antioxidant activity of the plant raw material, the solvent used, the extraction time, as well as the method of evaluation of antioxidant potential seems to be important [13, 14]. In the present study, the extraction of the raw material was carried out by ultrasound assisted extraction, classified as the so-called green extraction method. In recent years, more and more attention has been paid to the use of ecological methods to obtain plant extracts. Such techniques are aimed primarily to less solvent consumption, and thus less interference in the natural environment. The ultrasound extraction technique has been previously used by many researchers. Li *et al.* and Goula et al. isolated carotenoids from carrot root (*Dacutus carota*) and pomegranate fruit (*Punica granatum*) [17, 18]. Chemat et al. found that isolation of active substances using an ultrasonic bath was a key technology to achieve sustainable "green" chemistry due to, among others, a short time of extraction and low energy consumption [19].

In our study, commonly used techniques, such as DPPH, ABTS, FRAP were applied to evaluate

antioxidant activity. DPPH (2,2-diphenyl-1-picrylhydrazyl) forms a stable radical to be reduced in the presence of antioxidants and leads to decolourization of violet to form a pale yellow solution. The method is used to determine antioxidant potential of either individual compounds as well as plant extracts [20-22]. Another technique frequently used to assess the antioxidant activity is based on the application of ABTS (2,2-azine-bis (ethylbenzthiazoline-6-sulphonic acid) radical. This method allows to determine the antioxidant activity of both hydrophobic and hydrophilic antioxidants [22]. In the present study, antioxidant activities measured by DPPH method showed a similar tendency to results obtained by ABTS, however, the activity values expressed as TEAC using ABTS method were significantly higher as compared to DPPH method. Similar relationships between methods were also observed in our previous study on antioxidative activity of green tea [14]. In contrary, Wojdyło *et al.* observed results dissimilar to our for *E. hirsutum*, using trolox as a standard in both methods, because the values obtained by DPPH method were significantly higher, as compared to the results obtained with ABTS technique [21].

In present study, the antioxidant activity measured with use of DPPH method ranged from 13.42%±0.86 for 15 min aqueous extracts in fruit ripening stage to 96.77%±4.77 for samples prepared in 70% ethanol in 15 min (also fruit ripening stage). With this method, high values were also found for extracts prepared in 96% (30 min) and 70% ethanol (60 min) as well as in 70% methanol (15 min): 94.12%±0.87; 94.03%±0.09 and 93.80%±0.73, respectively, for material harvested in the fruit-setting state. When ABTS method was applied, the TEAC values ranged from 0.61±0.01 for extracts in undiluted isopropanol (60 min; fruit ripening stage) to 59.64±0.57 for extracts in 70 % ethanol (extraction time 60 min) harvested during intensive growing stage) (tab. 1).

E. augustifolium is known as a plant with high antioxidant activity [1, 5, 22-25]. Tóth *et al.* used the DPPH method to assess the antioxidant activity of aqueous extracts and those of 80% acetone in various species of willow (*E. parviflorum, E. roseum, E. tetragonum, E. montanum* and *E. angustifolium*). They observed antioxidant activity expressed as EC_{50} of 7.96±0.24 μM, while for ascorbic acid – EC_{50} it was 14.29±0.43 μM [25]. Wojdyło et al. found antioxidant activity of *E. hirsutum* herb extracts in 80% methyl alcohol. They analyzed 32 species of medicinal plants, belonging to 21 botanical families, to be found as wild plants in Poland. They observed antioxidant activity of fireweed to be one of the highest, as compared to other plants. The antioxidant activity of *E. hirsutum*, measured by the DPPH method, was 2021 μM trolox/100 g dry matter. In contrary, activity of garden angelica (*Archangelica officinalis*) was only 7.34±1.14 μM trolox/100 g dry matter. Similarly, the highest value of 69.5 μM trolox/100 g dry matter was also found for *E. hirsutum* with ABTS method. Based on the results of the study, the authors came into conclusion that the majority of analyzed plant species, naturally occurring in Poland, have lower activity, as compared to fireweed. In most cases, it was lower than 500 μM trolox/100 g dry matter [21]. Also Stef *et al.* compared antioxidant potential of *E. hirsutum* with several other plants. They prepared extracts of eleven medicinal plants in 50 % ethanol. The DPPH radical scavenging activity was 21.87% for *E. montanum* and it was slightly lower than that of purple coneflower (*Echinacea purpurea*): 23.43% and herb wormwood (*Artemisia absinthium*): 22.93% [26].

The antioxidant effect of plant materials can also be assessed by FRAP method, the principle of which is based on the measurement of TPTZ (iron-2,4,6-tripyridyl-S-thiazine complex) reduction by antioxidant contained for instance in plant [27]. In our study it was shown that extracts from fireweed have the ability to reduce Fe^{3+} ions, the highest TEAC values of 97.50±0.72 and 102.52±1.33 were found for extracts from the plant harvested in the intensive growing stage, prepared in 70 % ethanol in 30 and 60 min, respectively (tab. 2). Wojdyło *et al.* also demonstrated that the antioxidative activity of *E. hirsutum* extracts in 80% methanol determined by the FRAP method, was 275 μM trolox/100 g [21]. Moreover, Stef *et al.* used FRAP technique and found the ability of *E. montanum* extracts in 50 % ethanol to reduce iron ions of 4.28 Fe^{2+} mM/l [26].

The active compounds of plants, such as polyphenols or flavonoids, could influence their biological activity. Such compounds are one of the most popular groups of nutrients, classified as phytochemicals, to have a protective effect against many diseases [28]. Fireweed belongs to plants rich in polyphenols and flavonoids [1, 3, 21, 25, 27, 29]. This observation has been also confirmed in our studies. The total polyphenol content ranged from 0.38±0.09 GAE for water extracts, extracted in 30 minutes (intensive growing stage) to 22.99±0.12 GAE for extracts prepared in 70% ethanol, extraction time 30 min (fruit ripening stage) (tab. 4). The highest total flavonoid content of 7.03±0.20 mg RU/g of raw material was observed for fruit ripening

Table 1.

Mean (± standard deviation) antioxidant activity of fresh *E. angustifolium* herb extracts (in %RSA) evaluated with DPPH method.

DPPH [%RSA]				
Solvent	Alcohol concentration [*v/v*]	Extraction time		
		15'	30'	60'
Intensive growing				
Methanol	99.8%	87.94±0.95ab	85.69±1.66b	88.04±0.39b
	70%	84.64±2.09b	78.32±2.50c	80.05±1.55c
	40%	72.24±1.74b	76.93±3.48b	82.06±0.57b
Ethanol	96%	87.29±1.33a	86.48±0.83b	88.52±0.48c
	70%	75.94±4.28a	83.15±0.47b	86.92±0.41b
	40%	78.42±1.86b	77.23±1.33c	83.69±1.57b
Isopropanol	99.5%	79.78±1.75b	73.36±2.50b	74.99±1.77b
	70%	81.92±0.58c	84.00±3.02b	77.95±3.96b
	40%	81.72±0.16b	85.25±0.39b	79.17±1.94b
Water		27.79±3.47b	27.42±1.15b	39.72±0.56c
Massive blooming				
Methanol	99.8%	86.32±1.82b	84.89±3.06b	89.05±2.54b
	70%	78.21±2.61c	85.43±1.24b	85.81±1.62b
	40%	73.27±0.81b	79.80±2.14b	86.65±3.94ab
Ethanol	96%	86.49±3.57a	87.10±1.30b	90.36±0.75b
	70%	78.90±2.71a	87.32±2.96b	89.59±3.07ab
	40%	77.42±1.88b	82.23±2.02b	84.28±2.34b
Isopropanol	99.5%	54.73±2.20c	33.01±3.06b	86.33±1.80a
	70%	89.74±0.45b	89.91±0.58a	88.38±2.22a
	40%	82.22±0.99b	87.38±0.53b	87.99±3.00a
Water		35.54±1.79a	39.07±2.64a	81.36±0.63b
fruit ripening				
Methanol	99.8%	92.34±3.57a	91.48±1.66a	92,62±1,10a
	70%	93.80±0.73a	91.26±0.58a	93,92±0,44a
	40%	92.88±0.38a	90.88±1.23a	90,47±0,33a
Ethanol	96%	86.53±1.18a	94.12±0.87a	93,38±0,24a
	70%	96.77±4.77a	93.70±0.15a	94,03±0,09a
	40%	91.71±0.48a	92.40±0.44a	93,42±0,05a
Isopropanol	99.5%	88.43±4.15a	77.75±4.43a	87,91±0,20a
	70%	93.35±2.32a	93.16±1.57a	92,62±1,56a
	40%	92.88±1.90a	91.33±2.52a	92,69±2,55a
Water		13.42±0.86c	17.76±2.40c	90.91±0.97a

The values marked with different letters differ significantly between the development stages ($p<0.05$, n=3)

Table 2.

Mean (± standard deviation) antioxidant activity of fresh *E. angustifolium* herb extracts (in TEAC, i.e. mg trolox/g raw material) evaluated with ABTS method.

ABTS [TEAC, mg trolox/g raw material]				
Solvent	Alcohol concentration [*v/v*]	Extraction time		
		15'	30'	60'
Intensive growing				
Methanol	99.8%	27.26±0.30a	25.71±1.93a	10.30±1.14c
	70%	34.35±0.77b	50.00±1.46a	32.57±2.26b
	40%	25.40±0.58b	41.94±0.93b	20.07±1.08b
Ethanol	96%	2.77±0.47b	24.98±2.27a	34.75±0.68a
	70%	24.33±0.54a	59.06±0.90a	59.64±0.57a
	40%	31.02±1.29b	36.63±1.79b	57.86±0.61a
Isopropanol	99.5%	14.58±0.71b	16.23±0.27a	15.36±0.64a
	70%	9.48±1.83c	28.77±1.18b	41.34±1.27a
	40%	10.61±1.54c	23.73±0.41b	16.56±0.26c
Water		10.47±0.58a	9.90±0.23a	10.26±0.59a
Massive blooming				
Methanol	99.8%	17.97±0.59b	15.00±1.57b	20.16±0.19b
	70%	20.50±0.37c	16.74±1.21b	21.06±0.29c
	40%	20.92±0.25c	21.22±0.10c	21.25±0.01b
Ethanol	96%	7.64±0.49a	7.19±0.05c	15.22±0.58b
	70%	11.72±0.41b	21.05±0.10c	21.14±0.18c
	40%	20.11±0.23c	17.30±0.20c	20.80±0.11b
Isopropanol	99.5%	5.31±0.39c	1.97±0.41c	7.05±0.85b
	70%	21.15±0.21b	21.25±0.10c	20.95±0.42b
	40%	17.57±0.60b	20.86±0.25c	21.09±0.04b
Water		2.08±0.03b	2.58±0.09b	4.39±0.42b
Fruit ripening				
Methanol	99.8%	17.43±1.31b	22.79±1.72a	39.15±0.99a
	70%	44.57±0.57a	49.28±1.49a	54.66±1.16a
	40%	56.91±0.33a	53.69±1.24a	53.83±1.62a
Ethanol	96%	8.47±0.89a	16.97±1.20b	15.95±1.00b
	70%	6.68±0.80c	41.49±1.11b	45.13±1.48b
	40%	46.22±1.99a	40.52±1.39a	58.74±1.16a
Isopropanol	99.5%	39.31±1.99a	9.26±0.23b	0.61±0.01c
	70%	30.02±1.08a	42.51±1.36a	38.94±1.76a
	40%	32.19±1.19a	35.32±0.82a	37.30±1.08a
Water		0.67±0.07c	2.33±0.25b	10.88±0.19a

The values marked with different letters differ significantly between the development stages ($p<0.05$, n=3)

Table 3.

Mean (± standard deviation) antioxidant activity of fresh *E. angustifolium* herb extracts (in TEAC, i.e. mg trolox/g raw material) evaluated with FRAP method.

FRAP [TEAC, mg trolox/g raw material]				
Solvent	Alcohol concentration [*v/v*]	Extraction time		
		15'	30'	60'
Intensive growing				
Methanol	99.8%	17.67±0.96a	67.67±1.04a	48.82±0.39a
	70%	38.90±0.37a	55.73±0.78a	88.53±0.65a
	40%	3.06±0.04c	35.77±0.97a	50.12±0.59a
Ethanol	96%	18.49±0.51a	16.68±0.61b	31.71±0.91a
	70%	71.76±1.05a	97.50±0.72a	102.52±1.33a
	40%	44.47±0.58a	93.92±0.24a	85.07±0.85a
Isopropanol	99.5%	6.60±0.64b	7.48±0.39a	11.97±0.44a
	70%	32.01±1.16a	40.95±1.53a	53.64±1.54a
	40%	36.63±1.81a	33.91±0.89a	95.13±0.33a
Water		6.43±0.31a	12.85±1.18a	22.62±0.61a
Massive blooming				
Methanol	99.8%	19.08±0.54a	12.20±0.41c	17.95±0.20c
	70%	21.30±0.56c	15.67±0.26c	21.25±0.41c
	40%	21.13±0.27b	22.76±0.57c	20.48±0.26c
Ethanol	96%	10.78±0.43b	8.64±0.40c	16.13±0.32c
	70%	12.55±0.91b	24.25±0.35c	20.19±0.30b
	40%	19.45±0.51c	19.03±0.27c	20.90±0.50c
Isopropanol	99.5%	2.13±0.30c	0.08±0.01b	6.94±0.29b
	70%	22.39±0.44c	20.39±0.34c	19.18±0.25c
	40%	15.40±0.60c	16.84±0.21c	19.75±0.42c
Water		0.50±0.06b	0.06±0.01b	n.a.
Fruit ripening				
Methanol	99.8%	17.30±0.69a	19.45±0.64b	26.82±0.44b
	70%	24.21±0.59b	28.12±0.11b	27.91±0.36b
	40%	27.90±0.54a	26.19±0.31b	42.91±0.50b
Ethanol	96%	8.18±0.54c	26.14±0.66a	20.98±0.83b
	70%	6.47±0.29c	35.79±0.61b	20.98±0.83b
	40%	22.93±1.08b	33.70±0.47b	42.78±0.80b
Isopropanol	99.5%	25.01±0.60a	7.05±0.11a	11.43±0.03a
	70%	28.14±1.31b	26.59±0.65b	27.52±0.04b
	40%	30.12±1.07b	24.25±1.00b	26.35±0.12b
Water		n.a.	n.a.	0.95±0.09b

n.a. – no activity. The values marked with different letters differ significantly between the development stages ($p<0.05$, n=3)

Table 4

Mean (± standard deviation) total polyphenols content in fresh *E. angustifolium* herb extracts (in GAE, i.e. mg GA/g of raw material.

FOLIN-CIOCALTEU [GAE, mg gallic acid/g raw material]				
Solvent	Alcohol concentration [*v/v*]	Extraction time		
		15'	30'	60'
Intensive growing				
Mmethanol	99.8%	5.50±0.23b	8.28±0.23a	7.63±0.30b
	70%	5.73±0.33b	7.26±0.04b	7.75±0.22a
	40%	1.35±0.19b	4.98±0.16b	5.61±0.26b
Ethanol	96%	6.10±0.23a	5.75±0.33a	7.17±0.12b
	70%	8.53±0.15a	9.08±017b	7.97±0.28b
	40%	6.14±0.13b	8.11±0.21b	7.81±0.26b
Isopropanol	99.5%	2.75±0.27b	3.57±0.22a	4.07±0.10b
	70%	5.85±0.30b	8.25±0.18b	10.18±0.31a
	40%	4.53±0.27b	6.97±0.24b	10.32±0.35a
Water		0.51±0.06a	0.19±0.09a	1.06±0.16b
Massive blooming				
Methanol	99.8%	2.36±0.07c	2.19±0.09b	3.38±0.33c
	70%	1.79±0.04c	1.73±0.17c	4.10±0.14b
	40%	1.51±0.28b	2.45±0.16c	3.94±0.13c
Ethanol	96%	0.72±0.05c	1.11±0.13b	2.84±0.30c
	70%	0.86±0.10c	3.05±0.36c	2.87±0.30c
	40%	1.79±0.05c	2.65±0.08c	2.94±0.06c
Isopropanol	99.5%	0.22±0.07c	0.25±0.02b	1.24±0.27c
	70%	2.13±0.38c	2.47±0.19c	3.23±0.19c
	40%	1.74±0.05c	1.83±0.03c	3.44±0.19c
Water		n.a.	0.72±0.09a	0.46±0.05c
Fruit ripening				
Methanol	99.8%	7.72±0.04a	8.68±0.30a	8.35±0.04a
	70%	9.28±0.23a	9.96±0.21a	8.34±0.32a
	40%	7.18±0.38a	9.53±0.12a	10.77±0.24a
Ethanol	96%	3.30±0.32b	5.74±0.27a	8.94±0.21a
	70%	3.32±0.14b	11.50±0.12a	10.61±0.27a
	40%	8.49±0.29a	10.59±0.14a	10.13±0.19a
Isopropanol	99.5%	9.28±0.25a	3.85±0.35a	5.47±0.05a
	70%	10.72±0.35a	10.51±0.14a	8.02±0.10b
	40%	8.76±0.32a	9.73±0.22a	8.01±0.31b
Water		n.a.	n.a.	1.76±0.05a

n.a. – no activity. The values marked with different letters differ significantly between development stages ($p<0.05$, n=3)

Table 5

Mean (± standard deviation) total flavonoids content in fresh *E. angustifolium* herb extracts (in mg RU/g of raw material.

Flavonoids [mg rutin (RU)/g raw material]				
Solvent	Alcohol concentration [*v/v*]	Extraction time		
		15'	30'	60'
Intensive growing				
Methanol	99.8%	4.89±0.36ab	5.70±0.43b	5.26±0.16b
	70%	2.98±0.12c	4.43±0.10a	4.76±0.06a
	40%	1.14±0.08c	3.58±0.12b	3.23±0.05c
Ethanol	96%	4.06±0.09a	4.90±0.32a	3.20±0.10b
	70%	5.31±0.39a	5.89±0.42a	3.39±0.10b
	40%	3.28±0.10a	4.48±0.13a	2.90±0.11b
Isopropanol	99.5%	2.20±0.07b	2.11±0.18a	2.60±0.14b
	70%	3.49±0.09b	4.18±0.13b	6.26±0.07a
	40%	2.88±0.04b	2.80±0.15b	4.99±0.24a
Water		0.72±0.02a	1.38±0.10a	0.92±0.05a
Massive blooming				
Methanol	99.8%	4.42±0.31b	4.48±0.04c	5.97±0.35ab
	70%	3.65±0.15b	2.33±0.16c	4.94±0.31a
	40%	3.22±0.12a	3.34±0.16b	3.62±0.02b
Ethanol	96%	2.36±0.10b	2.30±0.15c	3.44±0.21b
	70%	2.24±0.19b	4.14±0.17b	3.84±0.27b
	40%	2.91±0.07b	3.00±0.09b	3.43±0.23b
Iisopropanol	99.5%	0.94±0.05c	0.85±0.12b	1.80±0.08c
	70%	4.41±0.05a	4.73±0.39b	5.06±0.07c
	40%	2.80±±0.05b	2.82±0.20b	4.96±0.07a
Water		0.81±0.03a	0.70±0.12b	1.00±0.04a
Fruit ripening				
Methanol	99.8%	5.38±0.20a	7.03±0.20a	6,21±0,51a
	70%	4.06±0.12a	3.76±0.41b	4,94±0,56a
	40%	2.78±0.14b	4.22±0.10a	4,68±0,19a
Ethanol	96%	2.32±0.08b	4.20±0.20b	5,18±0,41a
	70%	1.87±0.17b	5.25±0.21a	4,48±0,18a
	40%	2.71±0.08b	4.72±0.12a	4,27±0,46a
Isopropanol	99.5%	3.00±0.12a	2.43±0.11a	3,67±0,20a
	70%	3.85±0.30b	5.40±0.18a	5,46±0,07b
	40%	3.35±0.16a	3.71±0.27a	5,10±0,08a
Water		0,95±0,15a	0.85±0.09b	0.98±0.02a

The values marked with different letters differ significantly between development stages ($p<0.05$, n=3)

stage after extraction with undiluted methanol during 30 min (tab. 5). Similar results were obtained by Deng *et al.* In their study the total polyphenol content in ethanol extracts from *E. angustifolium*, was 16.8 g GA/100 g extract [22]. Also Wojdyło *et al.* determined the total polyphenol of 4.03 mg GA/100g dry matter in extracts in 80% methanol [21].

In our previous study on different plants it was observed that the content of active substances such as polyphenols or flavonoids was correlated with antioxidant activity [12-14]. Similar results have been found in current study. Figure 1 presents selected significant Pearson correlations between antioxidant activity and the total polyphenols and flavonoids content. The

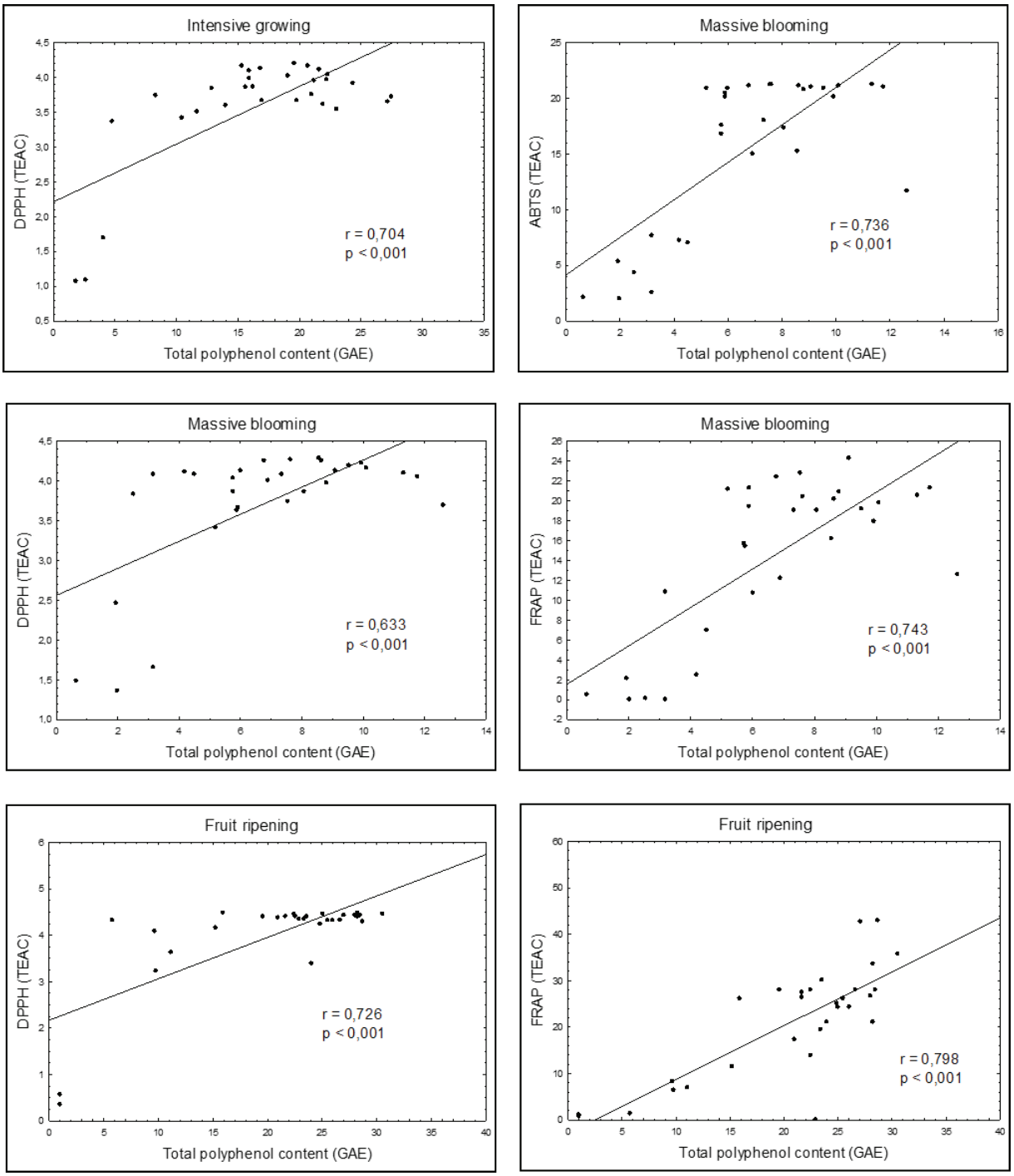

Figure 1
Correlations between antioxidant activities of fireweed extracts evaluated with different methods and total polyphenol content at different developmental stage.
TEAC – trolox equivalent antioxidant capacity (mg trolox/g raw material), GAE – gallic acid equivalent (mg gallic acid/g raw material)

highest statistically significant relationships between these parameters have been demonstrated for the massive blooming stage (fig. 1). A similar relationship was also found by Kaškoniene *et al.* They observed a significant linear relationship between antioxidant activity evaluated with DPPH and the total polyphenol content in *E. angustifolium*, the correlation coefficient was r = 0.98 [24]. Wojdyło *et al.* observed a relationship between the total polyphenols content and antioxidant activity in plants of *Labiaceae* and *Compositeae*. In the first family included, among others, sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), common balm (*Melissa officinalis*), the correlation coefficient for these plants varied from 0.83 to 0.93. However, in the case of *Compositae* family, included, among others, the elecampane (*Inula helenium*), dandelion (*Taraxacum officinalis*) or tansy (*Tanacetum vulgare*), the correlation coefficient was r=0.67–0.96 [21]. The above results suggest that the antioxidant effect of commonly used herbs depends to a high extent on their chemical composition. Other factors such as climatic and soil conditions as well as the plant's developmental stage can also influence plant biological activity [30]. The ability to scavenge free radicals may fluctuate depended on the vegetation phases. Maruška *et al.* demonstrated the highest antioxidant activity of *E. angustifolium* during the massive blooming phase [5]. In our study, in most cases, significant differences between particular vegetation phases were confirmed by Wilcoxon test. The differences between all phases of vegetation were demonstrated for DPPH, FRAP and Folin-Ciocalteu methods (p=0.001). The highest values were observed after the comparison of DPPH, ABTS and the total polyphenols and flavonoids content occurred in the fruit ripening stage, whereas for FRAP technique in the intensive growing stage. This observation was confirmed by Kujawski *et al.* who found the highest concentration of *E. angustifolium* tannins in fruit ripening stage [2]. Maruška *et al.* observed the highest flavonoids content as well as antioxidant activity in massive blooming stage of *E. angustifolium* [5]. The observed differences between the studies might be partly due to other plant growth positions, associated with different climatic conditions. Diverse habitat conditions could have a significant impact on the accumulation of active substances in plants [24]. In our study, plants were harvested in Poland, whereas Maruška *et al.* evaluated plants from Lithuania. Jüngerson *et al.* analyzed the total polyphenols content in Estonia's individual parts of *E. angustifolia*, harvested from May to October, and found the highest amount of these substances in plants harvested in July [30].

Moreover, the solvent used for extraction may also influence the isolation of active substances [14, 32, 33]. Its polarity plays a key role in the determination of antioxidant activity and could significantly affect the transfer mechanisms of a single electron or hydrogen atom [34]. In our study, four solvents were used: ethanol, methanol, isopropanol and water. The highest antioxidant capacities were found for extracts in 70% and 96% ethanol, whereas the lowest in water extracts (tab. 1, 2). In our previous study on the effect of various solvents and extraction time on antioxidant activity of green tea leaves extracts, it was found that in the case of the DPPH method undiluted methanol seemed to be the most preferred solvent for extracts obtained using ultrasound for 15 minutes, whereas for ABTS – 70% methanol, in 60 minutes. We suggested that the best solvent for extraction of, for instance, polyphenols was water after 60 min extraction [14]. In present study, extracts of plant harvested in ripening fruit stage prepared in water during 60 min showed relatively higher values as compared to other aqueous extracts (tab. 2).

CONCLUSION

E. angustifolium is characterized by high antioxidant potential and high total polyphenols and flavonoids content. An important factor determining the accumulation of biologically active ingredients and, hence, the ability to scavenge free radicals, is the time of plant harvesting. The studied plant harvested in the second half of August and extracted with more concentrated ethanol are characterized by high antioxidant activity. Accordingly, *E. angustifolium*, a potential weed, could be consider as a beneficial source of antioxidants.

Conflict of interest: Authors declare no conflict of interest.

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