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# Comparison of antioxidant activity of extracts of hop leaves harvested in different years

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## Summary

**Introduction:** Hop (*Humulus lupulus* L.) is a common plant in Europe, with many beneficial health effects. In addition to the use in brewing, hops are a valuable source of active substances used in conventional and folk medicine, such as humulones and lupulones, as well as antioxidants, including phenolic compounds.

**Objective:** The aim of the study was to evaluate and compare the antioxidant activity of alcoholic extracts of fresh hop leaves collected in 2017 and 2018.

**Material and methods:** The raw material consisting of fresh hop leaves was extracted using ultrasound-assisted extraction. Methyl, ethyl and isopropyl alcohol at three concentrations were used as extractants. The antioxidant activity of extracts was determined using DPPH and FRAP methods. Total phenolic content was evaluated using the Folin-Ciocalteu technique.

**Results:** All the extracts showed antioxidant potential as well as the phenolic content. Regardless of the harvesting year and methods of evaluation, the highest antioxidant activity and the total polyphenol content were observed for extracts prepared in undiluted methanol, obtained during one hour lasting extraction.

**Conclusion:** The results of the studies have suggested that hop leaves can be a potential source of health-promoting antioxidants.

**Key words:** *Humulus lupulus* L., DPPH, Folin-Ciocalteu method, FRAP, antioxidants, ultrasound-assisted extraction

**Słowa kluczowe:** *Humulus lupulus* L., DPPH, metoda Folin-Ciocalteu, FRAP, antyoksydanty, ekstrakcja wspomagana ultradźwiękami

## INTRODUCTION

Common hop (*Humulus lupulus* L.) is a dioecious, perennial climbing plant of the *Cannabaceae* family. It is found mainly in deciduous forests and thickets of Europe and western Asia, but also in other temperate climate zone areas. It develops underground rhizomes with flagellums, and its above-ground shoots can reach up to 10 m in height as wrapping around the support [1-3]. The female flowers without the perianth form strobiles – characteristic inflorescences resembling the appearance of cones, whereas the male with the flowers are grouped in bunches. Female hops are cultivated for industrial purposes, as a valuable brewing or medicinal raw material [1, 2, 4].

In addition to primary metabolites present in different parts of the hop, including sugars (glucose, fructose, raffinose and maltose), lipids (sitosterol derivatives), amino acids (tryptophan), peptides and proteins, this plant is a source of many valuable secondary metabolites with biological activity. They include terpenoids (mono- and sesquiterpenes), a large group of phenolic compounds (chalcones, flavanones, flavonols, flavan-3-ols, phenolic acids, tannins, stilbenes and lignans), as well as alkaloids, bitter acids (humulones and lupulones) and others (tab. 1) [1, 2, 5, 6].

These compounds are responsible for among other antibacterial, antioxidant, chemopreventive,

anticollagenic, estrogenic, anti-inflammatory action. They also could influence on enzyme activity and factors involved in the processes of tumorigenesis or cell apoptosis activity [2, 7]. Flower cones, known as hops, besides brewing, are used in conventional and folk medicine, homeopathy or cosmetology, as diastolic, analgesic, antiulcer, antiallergic, regenerating, bactericidal, fungicidal and diuretic agents. In addition to beer production, they are also used to make liqueurs, oils, infusions, capsules, dragees or ointments [7, 8]. Hops are also a source of lupulin – a substance with a sedative and hypnotic effect, from the surface of female cones, which fills the hops glandular hair – so-called hops glands [2, 7, 9]. Similar properties show farnesene, humulone and lupulone – used to treat mood disorders, including anxiety or insomnia [3, 4]. Hop preparations inhibit the activity of the cerebral cortex and excessive agitation. Due to their estrogenic activity, which is connected to 8-prenylnaringenin content, hops extracts are also used to relieve the menopause symptoms [3, 10]. *H. lupulus* L. compounds, in particular xanthohumol and lupulone, show antibacterial and anti-inflammatory activity, which may affect the acne causes and bacterial infections symptoms, by reducing the development of the genus *Staphylococcus*, *Streptococcus* or *Propionibacterium* [11-13]. The mentioned xanthohumol may also inhibit the multiplication of virus causing viral bovine diarrhea, cytomegalovirus, herpes simplex or HIV, as

Table 1.

Selected examples of secondary metabolites present in *H. lupulus* L. [1, 2, 5, 6]

TERPENOIDS	
Monoterpenes	d-limonene, $\beta$ -myrcene, $\alpha$ -, $\beta$ -pinene, $\beta$ -phellandrene
Sesquiterpenes	$\alpha$ -humulene, $\beta$ -caryophyllene, $\alpha$ -, $\beta$ -farnesene $\alpha$ -, $\beta$ -, $\delta$ -selinene, $\alpha$ -zingiberene
Other terpenoids	$\alpha$ -, $\beta$ -, $\delta$ -amyrin, lupeol
PHENOLIC COMPOUNDS	
Chalcones	xanthohumol, xanthohumols B, C, D, E, G, H, I, M, desmethylxanthohumol, xanthogalenol, flavokavine
Flavanones	isoxanthohumol, naringenin, 8-prenylnaringenin
Flavonols and their glycosides	quercetin, kaempferol, myricetin, rutin
Flavan-3-ols	catechin, epicatechin, epigallocatechin, gallic acid
Tannins	catechin and epicatechin derivatives, prodelphinidin B <sub>3</sub> , procyanidin B <sub>1</sub> , B <sub>2</sub> , B <sub>3</sub> , B <sub>4</sub> , C <sub>1</sub> , C <sub>2</sub>
Phenolic acids	<i>p</i> -coumaric acid, ferulic acid, cinnamic acid, hydroxycinnamic acid, gallic acid
Lignans	isolariciresinol, pinoresinol
Stilbens	resveratrol
ALKALOIDS	
Tetrahydro- $\beta$ -carboline	
BITTER ACIDS	
$\alpha$ -Acids – humulones	ad-, co-, pre-, post-, deoxy- and isohumulone, humulone A, B, C
$\beta$ -Acids – lupulones	ad-, co-, pre-, postlupulone, lupulone C, E, G i H

well as *Trichophyton* species dermatophytes. The active substances contained in hops have a chemopreventive effect, so may be valuable ingredients of an anti-cancer diet rich in antioxidants [13, 14]. Hops stipule extracts can be successfully used to prevent caries and periodontal diseases [15].

Moreover, various parts of this plant favorably affect urinary and digestive system, reduce cholesterol level, risk of ulcers development and high blood pressure, seal blood vessels and have a positive effect in relieving rheumatic pains [2, 7, 11, 12, 16].

The aim of the study was to evaluate and compare antioxidant activity of alcoholic extracts of fresh *H. lupulus* L. leaves collected in 2017 and 2018. The effect of solvent and extraction time on antioxidant potential of extracts was evaluated.

## MATERIAL AND METHODS

Reagents used in this research were purchased from Sigma-Aldrich, USA: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-S-triazine (TPTZ); from Merck, Darmstadt, Germany: iron(II) sulphate heptahydrate, the Folin-Ciocalteu reagent and gallic acid; from Chempur, Poland: iron(III) chloride hexahydrate, sodium carbonate anhydrous, 36% hydrochloric acid, sodium acetate anhydrous, methanol, 99.5% acetic acid and isopropyl alcohol – all of analytical grade and from Lineal Chemicals, Poland – 96% (v/v) ethyl alcohol.

The raw material consisted of wild *H. lupulus* L. leaves and was collected from natural sites in Szczecin. To evaluate antioxidant activity and polyphenols content at the beginning of hop vegetation, upper and young leaves were used to obtain the extracts. Fresh hop leaves harvested in May in two consecutive years were applied as raw material. 0.5 g of fresh material was extracted with 10 cm<sup>3</sup> of solvent using ultrasound-assisted extraction of 40 kHz in 15, 30 and 60 minutes at a room temperature. Polar solvents as methanol (MeOH), ethanol (EtOH) and isopropanol (IsoProOH) as well as their aqueous solutions in three concentrations for each: 40, 70 and 96-99.5%(v/v) were applied for extraction.

The antioxidant activity as well as total polyphenols content of extracts were evaluated with methods based on spectrophotometric measurements – DPPH, FRAP and Folin-Ciocalteu (F-C) method, using previously described techniques [17-18]. Three independent samples were prepared for each extract. The obtained results are presented as

arithmetic mean ± standard deviation (SD).

To evaluate antioxidant activity, the DPPH method was applied, where 0.3 mM ethanolic solution of DPPH was diluted to the absorbance of 1.00±0.02. Then, to 2850 µl of diluted reagent, 150 µl of extract was added and incubated at a room temperature for 10 min. Absorbance was taken at 517 nm using 10 mm cuvettes. Based on the absorbance and calibration curve for Trolox as a standard, Trolox equivalent activity concentration (TEAC) was calculated and expressed as mg Trolox/g raw material. Moreover, the percentage of extracts radical scavenging activity – RSA was also calculated based on formula:

$$\text{RSA}[\%] = (1 - A_s/A_o) \cdot 100\%$$

where  $A_s$  is the absorbance of tested sample and  $A_o$  – the absorbance of blank sample.

Ferric ion reducing power of extracts was determined using FRAP method. The working solution consisted of 1 volume of 10 mM TPTZ solution in 40 mM HCl, 1 volume of 20 mM FeCl<sub>3</sub> and 10 volumes of acetate 0.3 M buffer (pH 3.6) was prepared. Aliquots of 2320 µl of such solution with 80 µl of extract were mixed and after 15 min incubation at room temperature their absorbance in 1 cm cuvettes at 593 nm was taken. The results were expressed as FeSO<sub>4</sub> equivalents [mg FeSO<sub>4</sub>/g raw material].

Folin-Ciocalteu (F-C) method was applied to total polyphenol content evaluation of hop leaves extracts. The 10% F-C reagent solution was prepared and incubated 1 h in dark at room temperature. Then, to 2700 µl of 5mM Na<sub>2</sub>CO<sub>3</sub> 150 µl extract and 150 µl of previously prepared F-C solution was added and incubated for 15 minutes at room temperature. Absorbance was taken at 750 nm. Total polyphenol content was expressed as gallic acid (GA) equivalents [mg GA/g raw material].

Statistical significance of differences between antioxidant activity of extracts of hop leaves harvested in 2017 and 2018, as well as between the results obtained with use of different methods of evaluation (DPPH, FRAP, F-C) were assessed using the Wilcoxon test (parameter z), assuming the significance level  $\alpha=0.05$ . The Pearson correlations coefficients (r) between antioxidant potential of extracts from raw material harvested in two consecutive years were also estimated. Statistical analyses of the results were done using Statistica 13.1 (Statsoft, Poland) as well as Prostat 5.5 (Poly Software International, USA).

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

## RESULTS

Antioxidant activity of *H. lupulus* extracts of leaves collected in different years is presented in table 2. All evaluated extracts show antioxidant potential. Regardless of the collecting year and methods of evaluation, the highest properties were found for extracts prepared in concentrated methanol during one-hour ultrasound-assisted extraction. The lowest potential was found for extracts in 70% (v/v) isopropyl alcohol, where the extraction time was 15 minutes. The exception was a group of extracts made from leaves collected in 2017 and evaluated by F-C method, where the lowest polyphenols level was found for the sample prepared in 70% (v/v)

methanol in 30 min. extraction.

Antioxidant potential of hop leaves collected in 2017 evaluated with DPPH method ranged between  $0.26\pm 0.02$  and  $3.67\pm 0.13$  mg Trolox/g raw material (tab. 2), which corresponded to RSA  $11.67\pm 0.31\%$  to  $78.33\pm 2.46\%$  (fig. 1). The statistically significant differences (estimated by the Wilcoxon test) were found between the activity of extracts made from leaves collected in individual years evaluated using DPPH method ( $z=-2.427$ ,  $p=0.015$ ). Moreover, significant Pearson's correlation coefficient ( $r=0.622$ ;  $p<0.001$ ) was found between these groups of results.

The antiradical potential of leaves extracts collected in 2017 evaluated with FRAP method ranged

**Table 2.**

Total phenol content and antioxidant activity of extracts of hop leaves, collected in different years, evaluated with Folin-Ciocalteu, DPPH and FRAP methods (mean  $\pm$ SD)

Year of collection		2017	2018	2017	2018	2017	2018
Solvent	Extraction time [min]	DPPH [mg Trolox/g raw material]		FRAP [mg FeSO <sub>4</sub> /g raw material]		Folin-Ciocalteu [mg GA/g raw material]	
98% (v/v) MeOH	15	2.97 $\pm$ 0.03	2.65 $\pm$ 0.09	4.20 $\pm$ 0.04	4.25 $\pm$ 0.03	3.41 $\pm$ 0.14	3.20 $\pm$ 0.05
	30	3.18 $\pm$ 0.17	0.95 $\pm$ 0.01	4.28 $\pm$ 0.15	6.08 $\pm$ 0.02	3.17 $\pm$ 0.26	1.10 $\pm$ 0.04
	60	3.67 $\pm$ 0.13	3.72 $\pm$ 0.00	7.44 $\pm$ 0.04	8.55 $\pm$ 0.02	6.60 $\pm$ 0.26	6.22 $\pm$ 0.15
70% (v/v) MeOH	15	3.50 $\pm$ 0.12	2.68 $\pm$ 0.08	3.80 $\pm$ 0.43	3.22 $\pm$ 0.01	3.91 $\pm$ 0.20	1.72 $\pm$ 0.11
	30	0.98 $\pm$ 0.03	1.57 $\pm$ 0.11	0.95 $\pm$ 0.14	2.48 $\pm$ 0.01	0.51 $\pm$ 0.11	1.24 $\pm$ 0.09
	60	2.88 $\pm$ 0.17	3.62 $\pm$ 0.04	3.12 $\pm$ 0.01	4.46 $\pm$ 0.01	2.69 $\pm$ 0.25	2.80 $\pm$ 0.20
40% (v/v) MeOH	15	2.93 $\pm$ 0.17	1.46 $\pm$ 0.09	4.51 $\pm$ 0.05	2.11 $\pm$ 0.02	4.15 $\pm$ 0.23	0.95 $\pm$ 0.09
	30	2.98 $\pm$ 0.02	1.59 $\pm$ 0.07	5.03 $\pm$ 0.26	2.61 $\pm$ 0.02	4.94 $\pm$ 0.14	1.19 $\pm$ 0.01
	60	2.89 $\pm$ 0.11	1.75 $\pm$ 0.02	4.24 $\pm$ 0.03	2.65 $\pm$ 0.05	3.20 $\pm$ 0.09	1.20 $\pm$ 0.01
96% (v/v) EtOH	15	2.83 $\pm$ 0.10	1.35 $\pm$ 0.02	3.51 $\pm$ 0.01	3.78 $\pm$ 0.03	3.46 $\pm$ 0.11	2.83 $\pm$ 0.26
	30	2.46 $\pm$ 0.04	3.21 $\pm$ 0.09	3.22 $\pm$ 0.03	6.17 $\pm$ 0.03	3.04 $\pm$ 0.05	4.46 $\pm$ 0.44
	60	2.46 $\pm$ 0.19	2.53 $\pm$ 0.03	2.66 $\pm$ 0.03	6.04 $\pm$ 0.02	2.64 $\pm$ 0.28	3.94 $\pm$ 0.25
70% (v/v) EtOH	15	2.14 $\pm$ 0.18	1.39 $\pm$ 0.07	2.12 $\pm$ 0.16	1.91 $\pm$ 0.01	2.01 $\pm$ 0.24	1.03 $\pm$ 0.06
	30	3.07 $\pm$ 0.14	3.69 $\pm$ 0.02	3.62 $\pm$ 0.07	6.29 $\pm$ 0.03	3.27 $\pm$ 0.15	5.23 $\pm$ 0.33
	60	3.61 $\pm$ 0.03	3.31 $\pm$ 0.10	6.18 $\pm$ 0.11	4.09 $\pm$ 0.02	5.58 $\pm$ 0.26	2.90 $\pm$ 0.04
40% (v/v) EtOH	15	0.94 $\pm$ 0.03	0.37 $\pm$ 0.09	0.75 $\pm$ 0.10	1.24 $\pm$ 0.02	0.67 $\pm$ 0.11	0.09 $\pm$ 0.03
	30	3.11 $\pm$ 0.05	2.20 $\pm$ 0.17	2.55 $\pm$ 0.28	3.43 $\pm$ 0.01	4.12 $\pm$ 0.11	2.08 $\pm$ 0.08
	60	3.35 $\pm$ 0.08	2.66 $\pm$ 0.06	7.19 $\pm$ 0.47	3.75 $\pm$ 0.03	6.44 $\pm$ 0.21	2.34 $\pm$ 0.18
99.5% (v/v) IsoProOH	15	1.34 $\pm$ 0.02	1.22 $\pm$ 0.07	1.20 $\pm$ 0.07	3.79 $\pm$ 0.04	1.08 $\pm$ 0.16	2.00 $\pm$ 0.17
	30	1.12 $\pm$ 0.01	0.99 $\pm$ 0.05	0.70 $\pm$ 0.01	3.50 $\pm$ 0.03	0.76 $\pm$ 0.01	1.95 $\pm$ 0.18
	60	2.14 $\pm$ 0.11	0.96 $\pm$ 0.09	2.56 $\pm$ 0.08	3.86 $\pm$ 0.01	2.48 $\pm$ 0.13	2.26 $\pm$ 0.13
70% (v/v) IsoProOH	15	0.26 $\pm$ 0.02	0.13 $\pm$ 0.00	0.30 $\pm$ 0.03	1.02 $\pm$ 0.02	0.56 $\pm$ 0.11	0.02 $\pm$ 0.01
	30	2.25 $\pm$ 0.10	2.74 $\pm$ 0.14	2.44 $\pm$ 0.13	4.49 $\pm$ 0.47	2.58 $\pm$ 0.25	3.44 $\pm$ 0.33
	60	3.56 $\pm$ 0.04	1.00 $\pm$ 0.08	5.22 $\pm$ 0.02	3.58 $\pm$ 0.02	5.47 $\pm$ 0.07	2.58 $\pm$ 0.23
40% (v/v) IsoProOH	15	1.88 $\pm$ 0.11	0.47 $\pm$ 0.01	2.69 $\pm$ 0.17	1.36 $\pm$ 0.03	2.03 $\pm$ 0.26	0.54 $\pm$ 0.11
	30	0.58 $\pm$ 0.05	1.40 $\pm$ 0.01	0.86 $\pm$ 0.04	2.66 $\pm$ 0.02	0.75 $\pm$ 0.13	1.11 $\pm$ 0.05
	60	3.42 $\pm$ 0.15	3.55 $\pm$ 0.11	7.28 $\pm$ 0.05	5.88 $\pm$ 0.07	6.56 $\pm$ 0.21	3.64 $\pm$ 0.29

$\alpha<0.05$

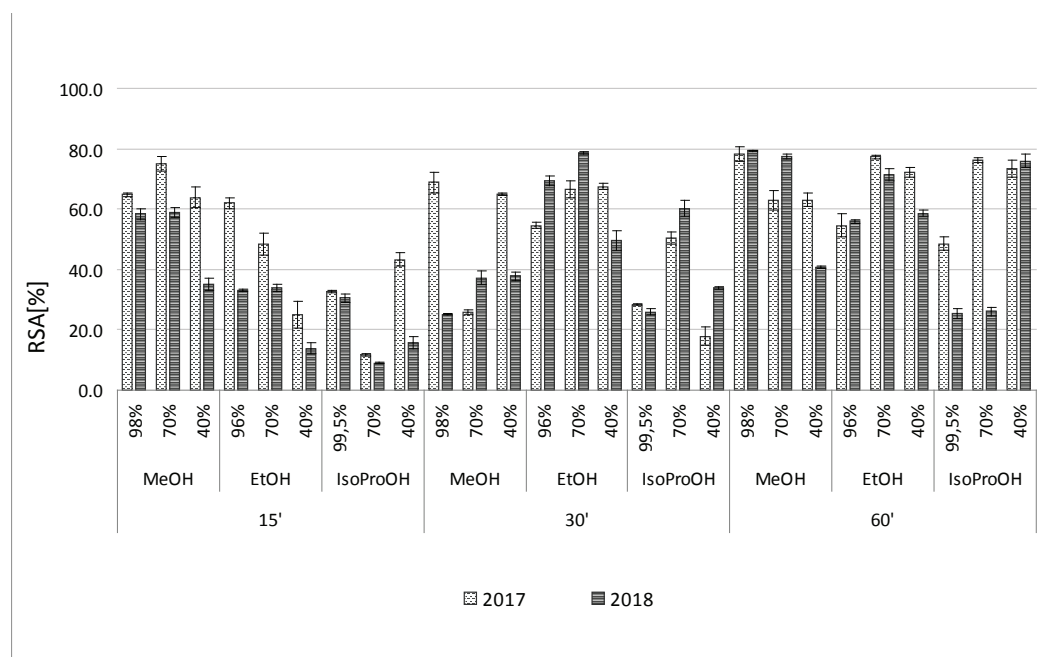


Figure 1.

Mean radical scavenging activity [%] of hop leaves extracts evaluated with DPPH method. Vertical lines represent standard deviation (SD)

from  $0.30 \pm 0.03$  to  $7.44 \pm 0.04$  mg  $\text{FeSO}_4/\text{g}$  raw material, for 2018 year from  $1.02 \pm 0.02$  to  $8.55 \pm 0.02$  mg  $\text{FeSO}_4/\text{g}$  raw material (tab. 2). Differences between the activity of leaves collected in particular years were statistically insignificant, however they correlated with each other significantly ( $r=0.515$ ,  $p=0.006$ ).

Total polyphenol content of leaves collected in 2017, evaluated with F-C method, ranged between  $0.51 \pm 0.11$  and  $6.60 \pm 0.26$  mg GA/g raw material, whereas such properties of raw material collected one year later was between  $0.02 \pm 0.01$  and  $6.22 \pm 0.15$  mg GA/g of raw material (tab. 2). Statistical significant differences were found between potential of extracts of hop leaves collected in different years ( $z=-2.258$ ;  $p=0.024$ ) and these results correlated to each other significantly taking into account the same solvent applied for extraction ( $r=0.499$ ;  $p=0.008$ ).

## DISCUSSION

*H. lupulus* L. has been used as an ingredient in herbal preparations with sedative, estrogenic, anti-inflammatory, anti-viral, antibacterial and anticancer properties [2, 19]. High antioxidant potential of this plant has been proved by others [2, 11, 20-23]. The most often analyzed raw material of hop are cones. There are few reports on antioxidant potential and the content of biologically active ingredients in other parts of the plant, such as leaves, stems or

roots. Choi *et al.* compared the antioxidant activity of extracts from leaves, stems and roots of Japanese hop (*H. japonicus* Siebold & Zucc) using DPPH and FRAP methods. They proved that hop leaves are a more valuable source of antioxidants as compared to other evaluated raw materials. The highest pro-health polyphenols content was also found in leaves [24]. The high antioxidant activity of hop leaves extracts was also confirmed in our study. The antioxidant potential evaluated with DPPH method was the highest in case of extracts prepared in concentrated methanol (60 minutes), in the first as well as in the second year of collection ( $3.67 \pm 0.13$  and  $3.72 \pm 0.00$  mg Trolox/g raw material).

Considering antiradical properties evaluated with FRAP method, higher potential was found for methanolic extracts in undiluted extractant – in 2017:  $7.44 \pm 0.04$  and in 2018:  $8.55 \pm 0.02$  mg  $\text{FeSO}_4/\text{g}$  raw material (tab. 2). Önder *et al.* found the antioxidant potential of hop cone extracts, to be  $14.95 \mu\text{g}$  Trolox/100 g extract when assessed by DPPH and  $1.56 \text{ mmol Fe}^{2+}/\text{g}$  extract, in the case of FRAP analysis [23]. Yumaguchi *et al.* also confirmed the high antioxidant activity of hop cone extracts, determined by the ORAC (oxygen radical absorbance capacity) method [11].

The ability to scavenge free radicals by plant extracts depends on the content of some compounds such as polyphenols [2, 25-27]. These compounds, due to their antioxidant properties, may also show



among others anti-cancer or anti-inflammatory effects [26]. Researchers found higher polyphenols content in leaves extracts than in other raw materials, such as flowers, fruit or roots of the same plant [24, 28, 29]. In our study, polyphenols content in hop leaves ranged between  $0.51 \pm 0.11$  and  $6.60 \pm 0.26$  mg GA/g raw material in 2017 and from  $0.02 \pm 0.01$  to  $6.22 \pm 0.15$  mg GA/g raw material in 2018 was found (tab. 2). Similarly to previous methods, the higher total polyphenol content was found in methanolic extracts prepared during one hour ultrasound-assisted extraction. The total polyphenol content varied between the extracts. It may be partly due to the different extraction conditions. The studied extracts were prepared in three different alcohols. Moreover, three concentrations of aqueous solution of each alcohol were used to obtain the extracts. Taking into account that polyphenols solubility varied between solvents, the differences between total polyphenol content in examined extracts could depend, among others, on solvent and extraction time. The results obtained in our study were confirmed by others. Proestos *et al.* determined total polyphenol content in hop leaves at 2.9 mg GA/g dry mass of raw material [30]. Choi *et al.* compared the content of polyphenols in different parts of hop and found the highest concentration in leaves (3089.9 mg/kg fresh weight), as compared to stem (1313.9 mg/kg fresh weight) or roots (655.2 mg/kg fresh weight) [24]. On the other hand, cones tested by Önder *et al.* showed the content of these compounds at a level of 8343–9079 mg ferrulic acid/100 g extract [23]. Moreover, the interesting source of valuable biologically active substances seems to be young shoots, in which Maietti *et al.* found the flavonoids content, from 517 to 2698  $\mu\text{g/g}$  fresh weight, depending on the plant variety [21].

The solvent type is an important factor in the extraction process, having a major impact on the antioxidant activity of obtained plant extracts [31, 32]. In our study, three polar alcohols (methanol, ethanol and isopropanol) differing, *inter alia*, in the length of the molecule carbon chain were used as solvents. Regardless the measurement method applied, the higher antioxidant potential was found in extracts made using concentrated methanol, while the lowest, in most cases, extracts in 70% (*v/v*) isopropanol. Also, the prolongation of the extraction time had a positive effect on the activity of obtained extracts (tab. 2). Methyl alcohol is often used to prepare plant extracts including also *H. lupulus* L. raw material [21, 30]. Zielonka-Brzezicka *et al.* reported that the most effective extractant to pineapple parts

(*Ananas comosus*) extraction, taking into account radical scavenging activity [%] of obtained extracts, seemed to be concentrated methanol. This alcohol showed better extraction capabilities against ingredients of plant antioxidants than the other applied polar alcohols, such as 70% and 96% (*v/v*) ethanol [33]. Also, Önder *et al.* confirmed the methanol and ethanol efficacy in the extraction of polyphenols from *H. lupulus* L., evaluated by the Folin-Ciocalteu method [23]. Nowak *et al.* observed higher antioxidant concentrations in *Ginkgo biloba* leaves extracts prepared in 40% and 70% (*v/v*) ethanol as compared to those in concentrated methanol [27]. According to Pawlak and Sielicka, except the concentration of the applied solvent for extraction, also its polarity seems to be crucial. These authors showed the highest content of phenolic compounds in extracts of chokeberry (*Aronia melanocarpa*) made with a 50% aqueous solution of acetone [34].

The method and time of extraction are also important factors which may affect the activity of the obtained plant extracts. In our study, the highest antioxidant potential, including the total polyphenol content, was found in extracts prepared using an ultrasonic bath for 60 minutes, while the lowest was obtained during the 15-min. extraction. These results confirm the results of studies on other plants, in which the extension of ultrasound-assisted extraction time led to increase of antioxidant activity of prepared extracts [28, 33, 35]. It is worth mentioned, that ultrasound-assisted extraction is considered as so-called green extraction technique. Wang *et al.* suggested that application of ultrasounds for plant material extraction is beneficial mainly due to the lower use of solvents than in classical techniques, and as a consequence to less adverse environmental impact [36]. Taking into account the influence of various factors on antioxidant activity of extracts, it should be mentioned that there are no universal extraction method, and therefore, the choice of extraction procedure should be individually selected for particular plant raw material [37].

The results of presented study show that the date of plant material harvesting may have a significant impact on their antioxidant activity, as well as the technique of extraction. In our study hop leaves were collected from the same place during the same vegetation stage, but in different years. The statistically significant differences estimated with the Wilcoxon test were found between the activity of extracts from different harvesting years (except for the FRAP method results). One of important factor responsible for these differences seems to be different

climatic conditions in particular years, which could be one of the main reasons for the content of biologically active substances in plants diversity [38]. Variable weather conditions can cause environmental stress, having a significant impact on the plant secondary metabolites profile [39].

## CONCLUSION

1. The extracts of young hop leaf harvested at the beginning of vegetation, showed high antioxidant activity.
2. The antioxidant potential of *H. lupulus* leaves extracts was influenced by several factors, such as type of solvent or time of extraction. The most effective process seems to be one hour ultrasound-assisted extraction in undiluted methanol.
3. Antioxidants content differed depending on the plant material harvesting time. This parameter may be also influenced by environmental factors, such as climatic conditions.
4. Hop leaves extracts seems to be a valuable source of natural antioxidants to be applied in different industry branches.

*Conflict of interest: Authors declare no conflict of interest.*

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