Agrobiodivers Improv Nutr Health Life Qual, 5, 2021(1): 47–54

Agrobiodiversity for Improving Nutrition, Health and Life Quality

DOI: https://doi.org/10.15414/ainhlq.2021.0006

Research Article OPEN ACCESS

# Antioxidant activity of extracts of wild *Humulus lupulus* L.

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Article Details:
Received: 2021-03-10
Accepted: 2021-03-22
Available online: 2021-05-31

Plants from natural flora (wild-growing) as well as cultural plants demonstrated numerous useful properties that play an important role in human life. They often can be used as medicinal, forage, food, etc. Searching for new sources of valuable capacities of wild plants still is actual. *Humulus lupulus* L. (hop) is well-known species from the small family Cannabaceae that is used as a bittering agent in the brewing industry and as a medicinal plant. *H. lupulus* raw is a valuable source of biologically active compounds and demonstrated different biological activities. This study was aimed to determine the antioxidant activity and accumulation of polyphenol compounds in raw wild plants of *H. lupulus*. Raw (leaves, stems, and female flowers) collected from the natural flora of M.M. Gryshko National Botanical Garden of the NAS of Ukraine. In ethanol extracts of leaves, flowers and stems determined 54.13, 44.69 and 23.76 mg GAE/g (gallic acid equivalent) of polyphenol content (TPC), respectively; 7.24, 4.92, 2.56 mg CAE/g (caffeic acid equivalent) of phenolic acids (TPAC), respectively; 45.48, 29.64 and 18.31 mg QE/g (quercetin equivalent) of flavonoid content (TFC), respectively. Molybdenum reducing power of leaf, flower, and stem extracts was 168.17, 236.45, and 97.57 mg TE/g (Trolox equivalent), respectively; antioxidant activity by DPPH method 8.64, 8.02, and 7.97 mg TE/g, respectively. This study showed that the highest values of polyphenol compounds found in the leaves and the lowest in the stem extracts. The strongest correlation found between TPAC and TFC (r = 0.995), TPC and TPAC (r = 0.978), TPC and TFC (r = 0.952), TFC and DPPH (r = 0.936). This investigation showed the high antioxidant potential of the wild *H. lupulus* plant that can be used in the further pharmacological study.

**Keywords:** *Humulus lupulus*, hop, polyphenol compounds, antioxidant activity, correlation

## Introduction

Genus *Humulus* L. belongs to Cannabaceae Martynov and includes *Humulus lupulus* L., *H. scandens* (Lour.) Merr. and *H. yunnanensis* Hu. Species from this genus originated from China, were spread to America and Europe (Small, 1978; Small, 1980).

*H. lupulus* (hop) is a dioecious perennial plant, which regrows every spring from rhizomes of an underground rootstock. It is vine producing stems annual, slender, climbing, growing up to 6–9 m in length. The leaves are dark green colored, long petiolate, heart-shaped with 3–5 lobs, sharply toothed and they have a very rough surface. The male flowers are long racemes, 7.5–12.5 cm long, while the female inflorescences are cone-like catkins (strobiles), 2.5–5.0 cm long, made up of overlapping membranaceous bracts (Zanoli and Zavatti, 2008).

*H. lupulus* have been used for different medical purposes such as sleep disturbances, insomnia anxiety, excitability, for treating acne, dysmenorrhea, and amenorrhea (Arsene et al., 2015). Specific organoleptic properties of this plant allow to use in the beer industry

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ISSN 2585-8246

(Zanoli and Zavatti, 2008). The genetic diversity of this plant an important direction in selection work in Ukraine (Melnychuk et al., 2008) and abroad (Mafakheri et al., 2020). This species primarily used for medicinal purposes than for beer brewing (Lin et al., 2019).

Different characters such as agronomic traits, diseases and pests resistance, chemical composition have been used for hop improvements (Skomra et al., 2013).

Cons (Liu et al., 2007; Karabín et al., 2016; Kobus-Cisowska et al., 2019) and seeds of hop (Alonso-Esteban et al., 2019) exhibited the antioxidant activity. An antimicrobial activity showed extracts of female inflorescences (Arsene et al., 2015; Bocquet et al., 2019) and seeds (Alonso-Esteban, 2019). Extracts also demonstrated the insecticidal, antiviral (Sotto et al., 2018), antitumor, antiproliferative, anticancer (Van Cleemput et al., 2009), antimutagenic (Wang et al., 2014), anti-inflammatory (Bohr et al., 2005), and detoxication activity. Insecticidal effects of cons extracts of *H. lupulus* caused by xanthohumol, which can act against storage insects (Aydin et al., 2017).

In methanol extracts of *H. lupulus* identified prenylated chalcones, prenylflavanones, 4-hydroxybenzaldehyde, sistosterol-3-O-β-glucopyranoside, humulinone, cohumulinone (Chadwick et al., 2004). The functional components of *H. lupulus* dried cons are α-acids (2–7 %), β-acids (2–10 %), essential oils (0.5–3.0 %), polyphenols (3–6 %), amino acids (0.1 %), protein (15 %) (Lin et al., 2019). Content α- and β-acids increased during vegetation (Kavalier et al., 2011). Among phenolic compounds identified flavan-3-ols, procyanidins, phenolic acids (Kavalier et al., 2011), catechine (13.7 mg/g), epicatechin (3.9 mg/g) (Alonso-Esteban et al., 2019), gallic acid, vanillic acid, caffeic acid, syringic acid, rutin, luteolin, *t*-cinnamic acid, etc. (Keskin et al., 2019). The study of ten hop strains with different content of α-acids showed that the boiling procedure increased the content of polyphenol compounds and total antioxidant activity (Elrod et al., 2019). The main phenolic compounds present in extracts of *H. lupulus* were flavonoids isoquercitrin and quercetin (Almeida et al., 2019).

This study was aimed to determine the antioxidant activity of ethanol extracts of different parts of wild *H. lupulus* plants as a potential source of polyphenol compounds.

## Material and methodology

The plants were grown in 2017 at the experimental fields of the M.M. Gryshko National Botanical Garden of the NAS of Ukraine in the Kyiv city (50°24'55"N, 30°33'45"E).

### Biological material

Plant raw material collected from natural flora of M. M. Gryshko National Botanical Garden of the NAS of Ukraine at the stage of flowering. The leaves, stems, and female inflorescences dried in a ventilated dryer at 60 °C, according to Almaguer et al. (2014).

### Biochemical analysis

The biochemical analysis was done at the Slovak University of Agriculture in Nitra (Slovak Republic). For planned analyses, 0.2 g of milling fraction was extracted with 20 mL of 80 % ethanol for 24 hours. After centrifugation at 4000 g with Rotofix 32 A (Hettich, Germany) for 20 min, the supernatant was used for measurement of the total content of polyphenols.

### Chemicals

All the chemicals used were of analytical grade and were purchased from Sigma-Aldrich (Steinheim, Germany), Merck (Darmstadt, Germany), and CentralChem (Slovakia).

### Phytochemical analyses

#### Total polyphenol content (TPC)

Total polyphenol content extracts were measured by the method of Singleton and Rossi, (1965) using Folin-Chiocalteu reagent. 0.1 mL of each sample extract was mixed with 0.1 mL of the Folin-Chiocalteu reagent, 1 mL of 20 % (w/v) sodium carbonate, and 8.8 mL of distilled water. After 30 min. in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25–250 mg/L; $R^2$ = 0.996) was used as the standard and the results were expressed in mg/g gallic acid equivalents.

#### Total flavonoid content (TFC)

Determination of total flavonoid content was conducted according to a procedure which was described by Shafii et al. (2017). 0.5 mL of sample extract was mixed with 0.1 mL of 10 % (w/v) ethanolic solution of aluminum chloride, 0.1 mL of 1 M sodium acetate, and 4.3 mL of distilled water. After 30 min. in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.01–0.5 mg/l; $R^2$ = 0.997) was used as the standard and the results were expressed in μg/g quercetin equivalents.



ISSN 2585-8246

### Total phenolic acid content (TPAC)

Determination of total phenolic acid content of extracts was carried out using a method of Farmakopea Polska (1999). 0.5 mL of sample extract was mixed with 0.5 mL of 0.5 M hydrochloric acid, 0.5 mL Arnova reagent, 0.5 mL of 1 M sodium hydroxide (w/v), and 0.5 mL of distilled water. Absorbance at 490 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Caffeic acid (1–200 mg/L, $R^2$ = 0.999) was used as a standard and the results were expressed in mg/g caffeic acid equivalents.

### DPPH radical scavenging assay (DPPH)

The radical scavenging activity of samples was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sánchéz-Moreno et al., 1998). The extracts (0.5 mL) were mixed with 3.6 mL of radical solution (0.025 g of DPPH in 100 mL ethanol). The absorbance of the sample extract was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10–100 mg/L; $R^2$ = 0.988) was used as the standard and the results were expressed in mg/g Trolox equivalents.

### Molybdenum reducing power of extracts (MRP)

The reducing power of extracts was determined by the phosphomolybdenum method of Prieto et al. (1999) with slight modifications. The mixture of the sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M), and distilled water (0.8 mL) incubated at 90 °C for 120 min, then rapidly cooled and detected by monitoring absorbance at 700 nm using the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10–1000 mg/L; $R^2$ = 0.998) was used as the standard and the results were expressed in mg/g Trolox equivalents.

### Statistical analysis

Basic statistical analyses were performed using PAST 2.17. Data were analyzed with ANOVA test and differences between means compared through the Tukey-Kramer test ($p < 0.05$). The variability of all these parameters was evaluated using descriptive statistics.

## Results and discussion

Wild plants had importance in human history from old times and use as a potential source of biologically active compounds and individual components. They are used not as medicinal plants only but as food and forage also (Pinela et al., 2017). Last year's reports showed that searching for natural plant antioxidants has some advantages along with obtaining synthesized antioxidants (Antal, 2010).

The most common polyphenol compounds from *H. lupulus* are catechins, phenolic acids, flavonol glycosides, and procyanidins (Kavalier et al., 2011).

The content of polyphenol compounds at the flowering stage in *H. lupulus* ethanol extracts was from 23.76 to 54.13 mg GAE/g depending on the plant part (Figure 1). As shown from Figure 1, the maximal level of TPC determined in leaves, minimal in stems.

Inflorescence extracts showed 151.42 mg GAE/g of TPC that was 3.3 times more than in our experiment (Arsene et al., 2015). Research of *H. lupulus* cons found 3.5 mg/100 g of caffeic acid equivalent and 4.8 mg/100 g of the chlorogenic acid equivalent of TPC (Bubueanu et al., 2015). Almeida et al. (2019) found that TPC in extracts of *H. lupulus* was from 27.31 to 33.93 mg GAE/g. According to Sotto et al. (2018), the TPC of inflorescence extracts was 7.1 µg/g of tannic acid equivalent. As reported Keskin et al. (2019), methanol extracts of cons contained 7.12 mg GAE/g and leaf's 6.86 mg GAE/g of TPC. Rheay et al.


**Figure 1** Content of total polyphenol content of *Humulus lupulus* L. extracts at the stage of flowering:
GAE – gallic acid equivalent; different superscripts in each column indicate the significant differences in the mean at $p < 0.05$

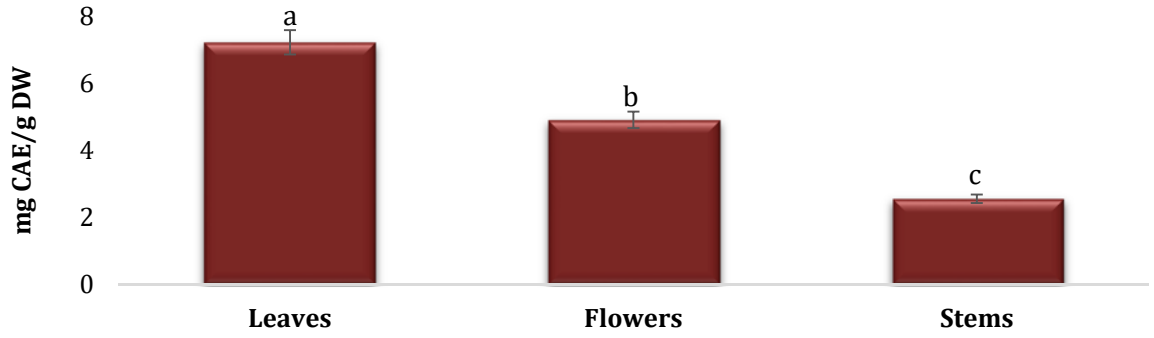


**Figure 2** Content of phenolic acids of *Humulus lupulus* L. extracts at the stage of flowering:
CAE – caffeic acid equivalent; different superscripts in each column indicate the significant differences in the mean at p <0.05

(2020) determined TPC from 50.08 to 92.41 mg GAE/g depending on *H. lupulus* cultivar.

Phenolic acids are secondary metabolites from plants that are divided into two major groups – hydroxycinnamic and hydroxybenzoic acids (Tanase et al., 2019). This group of polyphenol compounds well-known antioxidants that also exhibited cardioprotective, antidiabetic, antiulcer, anticancer, anti-inflammatory, neuroprotective, hepatoprotective activities (Saibabu et al., 2015). Leaves of vegetables contain phenolic acids in the highest concentration, as reported Kumar and Goel (2019). Among phenolic acids of *H. lupulus*, the most widespread is ferulic acid (Ahmed et al., 2019).

The total content of phenolic acids in *H. lupulus* extracts was from 2.56 to 7.24 mg CAE/g DW (Figure 2).

It should be noted that the least content of phenolic acids found in the stems and the most in the flowers. The same results were found in different organ extracts of *Scutellaria baicalensis* and *Galega* spp., where the lowest content of phenolic acids found in stems at the flowering (Vergun et al., 2019, 2020).

One of the most widespread polyphenols are flavonoids. This a group of natural substances found in fruits, vegetables, grains, herbs, stems, roots, flowers, etc. The main groups of flavonoids are flavones, flavanones, catechins, and anthocyanins that act as antioxidants (Nijveldt et al., 2001). Flavonoids exhibited numerous health-promoting effects and biological activities such as antioxidant, antiviral (Tapas et al., 2008), anti-inflammatory, anti-cancerogenic, anti-mutagenic. They found abundantly in plant raw that makes plants valuable source of these compounds (Panche et al., 2016). Flavonoids also demonstrated antimicrobial and antifungal activity (Saleem et al., 2017). The total content of flavonoids in the ethanol extracts was from 18.31 to 45.48 mg QE/g DW (Figure 3).

TFC of inflorescence extracts, according to Arsene et al. (2015), was 26.46 mg RE/g. According to Sotto et al. (2018), the TFC of inflorescence extracts was 3.8 µg QE/g. Almeida et al. (2019) found that TFC in *H. lupulus* extracts was from 52.94 to 54.47 mg QE/g. Ahmed et al. (2019) found in the ethanol leaf extracts 56 mg QE/g of TFC.

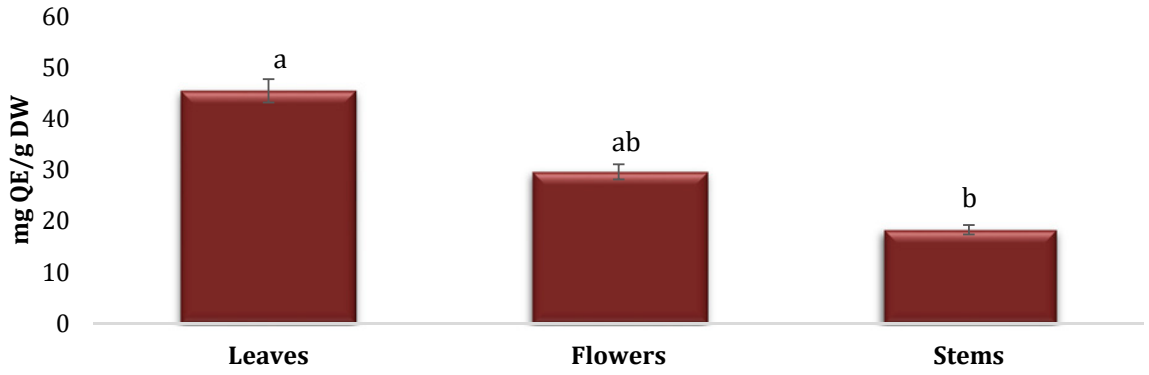


**Figure 3** Content of flavonoids of *Humulus lupulus* L. extracts at the stage of flowering:
QE – quercetin equivalent; different superscripts in each column indicate the significant differences in the mean at p <0.05

**Table 1** Antioxidant activity of *Humulus lupulus* L. extracts at the stage of flowering

| Plant extract | Molybdenum reducing power of extract (mg TE/g DW) | DPPH radical scavenging assay (mg TE/g DW) |
|---|---|---|
| **Leaves** | 168.17 ±9.17ab | 8.64 ±0.03a |
| **Flowers** | 236.45 ±10.72a | 8.02 ±0.05a |
| **Stems** | 97.57 ±3.28b | 7.97 ±0.27b |

Note: TE – trolox equivalent; different superscripts in each column indicate the significant differences in the mean at p <0.05

**Table 2** Pearson's coefficients between antioxidant parameters of *Humulus lupulus* L. extracts at the stage of flowering

| Parameter | TPC | TPAC | TFC | MRP | DPPH |
|---|---|---|---|---|---|
| **TPAC** | 0.978** | 1 | | | |
| **TFC** | 0.952** | 0.995** | 1 | | |
| **MRP** | 0.680* | 0.513* | 0.424* | 1 | |
| **DPPH** | 0.783* | 0.895** | 0.936** | 0.077* | 1 |

Note: TPC – total polyphenol content, TPAC – total phenolic acids content, TFC – total flavonoid content, MRP – molybdenum reducing power, DPPH – antioxidant activity by DPPH method; ** – correlation is significant at the level of 0.01; * – correlation is significant at the level of 0.05

In this study, the antioxidant activity was determined by both the phosphomolybdenum and DPPH methods. MRP of investigated extracts decreased in the following order: flowers > leaves > stems (Table 1). Concerning the antioxidant activity by the DPPH method, results decreased by the following order: leaves > flowers > stems.

There are not enough data about antioxidant activity by the phosphomolybdenum and DPPH methods but some authors confirmed exhibiting the antioxidant activity by different extracts of plants from Cannabaceae and found a correlation between TPC and reducing power of extracts (Mkpenie et al., 2012; Niknejad et al., 2014).

The correlation analysis showed a very strong relations between TPAC and TFC (r = 0.995), TPAC and TPC (r = 0.978), TPC and TFC (r = 0.952), antioxidant activity by DPPH method and TFC (r = 0.936) and TPAC (r = 0.895) (Table 2). Strong correlation found between total content of polyphenols and antioxidant activity by DPPH method (r = 0.783) and molybdenum reducing power (r = 0.680).

According to Gorjanović et al. (2013), the TPC of *H. lupulus* extracts correlated with antioxidant activity by the DPPH method (r = 0.986). In our experiment, this parameter was lower. Our results of antioxidant parameters relations showed that TPC was positively correlated with all investigated parameters.

## Conclusions

This study demonstrated that ethanol extracts of wild plants of *H. lupulus* are a valuable source of polyphenol compounds with high antioxidant activity. The most content of polyphenols, flavonoids, and phenolic acids found in the leaf's extracts, the least content detected in the stem extracts. Values of correlation between investigated groups of polyphenol compounds and antioxidant activity by DPPH method was higher than with antioxidant activity by phosphomolybdenum method but all groups of polyphenols showed a strong correlation with antioxidant activities (by two methods). The results of this study could be used in further pharmacological studies.

## Acknowledgments

This work was supported by the Bilateral Scholarship of the Ministry of Education, Science, Research and Sport (Slovak Republic), SAIA, and Visegrad Fund. Experimental activities were realized in the laboratories of the Centre of Excellence for the Conservation and Use of Agrobiodiversity at the Faculty of Agrobiology and Food Resources, Slovak Agricultural University in Nitra. This work was supported by the Bilateral Scholarship of the Ministry of Education, Science, Research and Sport (Slovak Republic), SAIA, and Visegrad Fund.

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