

The Evaluation of Antioxidant and Antimicrobial Effect of *Tussilago farfara* L. and *Cetraria islandica* L.

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Abstract

The aim of this study was to investigate the antioxidant and antimicrobial effect of two selected plants: *Tussilago farfara* L. and *Cetraria islandica* L. The antioxidant activity was evaluated by DPPH and phosphomolybdenum method, total polyphenol content with Folin – Ciocalteu reagent, flavonoids content by aluminium chloride method. The detection of antimicrobial activity was carried out by disc diffusion method and evaluation of minimal inhibition concentration against five species of Gram-negative bacteria: *Escherichia coli* CCM 3988, *Klebsiella pneumoniae* CCM 2318, *Salmonella enterica* subsp. *enterica* CCM 3807, *Shigella sonnei* CCM 1373, *Yersinia enterocolitica* CCM 5671 and five Gram-positive bacteria: *Bacillus thuringiensis* CCM 19, *Clostridium perfringens* CCM 4435, *Haemophilus influenzae* CCM4456, *Listeria monocytogenes* CCM 4699, *Staphylococcus aureus* subsp. *aureus* CCM 2461. *Tussilago farfara* showed stronger antioxidant activity (DPPH method – 7.67 mg TEAC – Trolox equivalent antioxidant capacity per g of sample; phosphomolybdenum method – 111.67 mg TEAC per g of sample), total polyphenol (11.92 mg GAE – galic acid equivalent per g of sample) and flavonid content (47.62 µg QE – quercetin equivalent per g of sample) with compare to *Cetraria islandica*. *Tussilago farfara* also showed the higher antimicrobial activity against Gram-negative bacteria confirmed by both methods – disc diffusion and minimal inhibitory concentration.

Keywords: plants, polyphenols, flavonoids, antioxidants

1. Introduction

Medicinal plants are rich source of biologically active compounds. It has been used for centuries as remedies for human diseases and offers a new source of phytochemicals as antioxidant and antimicrobial agents [1]. Medicinal are widely used also as nutritional supplements. Of special interest is their value as a source of natural antioxidants Due to increasing safety concerns involved with consumption of synthetic antioxidants, exploitation of cheaper and safer sources of antioxidants from natural origins, and

especially from plants, is of interest nowadays [2]. Bioactive compounds found in medicinal plants have been shown to have possible health benefits with antioxidative, anticarcinogenic, antihypertensive, antimutagenic, and angiogenesis inhibitor activities. Interestingly, many medicinal plants are known to contain large amounts of phenolic antioxidants other than well-known vitamin C, vitamin E, and carotenoids [3].

Tussilago farfara L. has long been used as a traditional medicine for the treatment of bronchitis and asthma. It has been reported that *Tussilago farfara* inhibits arachidonic acid metabolism and nitric oxide (NO) synthesis in lipopolysaccharide-stimulated macrophages, which contributes to its antiinflammatory action [4]. It has several known pharmacological activities, namely, antimicrobial

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activity inhibitory activity against nitric oxide synthase and antagonistic activity on platelet-activating factor receptor [5]. *Tussilago* flower contains a number of diverse components including essential oils, sesquiterpenes, flavonoids, phenylpropanoids chromones and pyrrolizidine alkaloids [6].

Centraria islandica L. is one of the most common lichen species, which grows in Europe. This plant is known mainly in Slovak folk medicine and used for treatment of diseases such as bronchitis and tuberculosis. In addition, this lichen species has been used as haemostatic drug [7]. Protolichesterinic acid isolated from *C. islandica* has *in vitro* inhibitory effects on arachidonate 5-lipoxygenase. Protolichesterinic acid, α -methylene- γ -lactone, fumarprotocetric acid and β -orcinol depsidone are considered to be the major biologically active secondary metabolites in this plant [8]. Several lichen metabolites of *C. islandica* exhibited highest antimycobacterial activity Aliphatic α -methylene- γ - lactone isolated from the lichen was found to be potent inhibitors of the DNA polymerase activity of human immunodeficiency virus-1 reverse transcriptase (HIV-1RT) [9].

The aim of present study was to investigate possible antioxidant and antimicrobial effect of *Tussilago farfara* L. and *Centraria islandica* L. extract.

2. Materials and methods

2.1 Biological materials

The plants – *Tussilago farfara* L. flower and *Centraria islandica* L. insole were purchased from local market. Before the analysis samples were pulverized in the mortar.

2.2 Chemicals

All chemicals were analytical grade and were purchased from Rechem (Slovakia) and Sigma Aldrich (USA).

2.3 Sample preparation

0.1 g of sample was extracted with 20 mL of 80% ethanol for 24 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for measurement (antioxidant activity, polyphenols, flavonoids). Extraction was carried out in triplicate.

2.4 Radical scavenging activity

Radical scavenging activity of extracts was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [10]. The sample (0.4 mL) was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL methanol). Absorbance of the reaction mixture 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; $R^2=0.989$) was used as the standard and the results were expressed in mg/g Trolox equivalents.

2.5 Reducing power

Reducing power of extracts was determined by the phosphomolybdenum method of Prieto et al. [11] with slight modifications. The mixture of 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) was 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.

2.6 Total polyphenol content

Total polyphenol content extracts was measured by the method of Singleton and Rossi [12] using Folin-Ciocalteu reagent. 0.1 mL of each sample was mixed with 0.1 mL of the Folin-Ciocalteu 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 (25-300 mg/L; $R^2=0.998$) was used as the standard and the results were expressed in mg/g gallic acid equivalents.

2.7 Total flavonoid content

Total flavonoids were determined using the modified method of Willett, [13]. 0.5 mL of sample was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 mL of 1 M potassium 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 (0.5-20 mg/L; $R^2=0.989$) was

used as the standard and the results were expressed in $\mu\text{g/g}$ quercetin equivalents.

2.8 Microbial strains

Five strains of microorganisms were tested in this study, Gram-negative bacteria: *Escherichia coli* CCM 3988, *Klebsiella pneumoniae* CCM 2318, *Salmonella enterica* subsp. *enterica* CCM 3807, *Shigella sonnei* CCM 1373, *Yersinia enterocolitica* CCM 5671 and five Gram-positive bacteria: *Bacillus thuringiensis* CCM 19, *Clostridium perfringens* CCM 4435, *Haemophilus influenzae* CCM4456, *Listeria monocytogenes* CCM 4699, *Staphylococcus aureus* subsp. *aureus* CCM 2461. All tested strains were collected from the Czech Collection of microorganisms. The bacterial suspensions were cultured in the nutrient broth (Imuna, Slovakia) at 37 °C.

2.9 Disc diffusion method

Antimicrobial activity of each plant extract was determined by a disc diffusion method. Briefly, 100 μl of the test bacteria were grown in 10 ml of fresh media until they reached a count of approximately 10^5 cells. ml^{-1} . Then 100 μl of the microbial suspension was spread onto Mueller Hinton agar plates. The extracts were tested using 6 mm sterilized filter paper discs. The diameters of the inhibition zones were measured in millimeters. All measurements were to the closest whole millimeter. Each antimicrobial assay was performed in at least triplicate. Filter discs impregnated with 10 μl of distilled water were used as a negative control.

2.10 Microbroth dilution method

MICs were determined by the microbroth dilution method according to the Clinical and Laboratory Standards Institute recommendation [8] in Mueller Hinton broth (Biolife, Italy). Briefly, the DMSO plant extracts solutions were prepared as serial two-fold dilutions obtaining a final concentration ranging between 0.5-512 $\mu\text{g}.\text{ml}^{-1}$. After that each well was inoculated with microbial suspension at the final density of 0.5 McFarland. After 24 h of incubation at 37 °C, the inhibition of microbial growth was evaluated by measuring the well absorbance at 450 nm in an absorbance microplate reader Biotek EL808 with shaker (Biotek Instruments, USA). The 96 microwell plates were measured before and after experiment. Differences between both measurements were evaluated as

growth. Measurement error was established for 0.05 values of absorbance. Wells without plant extracts were used as negative controls of growth. Pure DMSO was used as negative control. This experiment was done in eight-replicates for a higher accuracy of the MICs of used medical plant extracts.

2.11 Statistical analysis

Differences in absorbance between the measurements before and after the analysis were expressed as a set of binary values. These values were assigned to exact concentrations. The following formula was created for this specific experiment: value 1 (inhibitory effect) was assigned to absorbance values lower than 0.05, while value 0 (no effect or stimulant effect) was assigned to absorbance values higher than 0.05. For this assigned to absorbance values higher than 0.05. For this statistical evaluation the probit analysis in Statgraphics software was used.

3. Results and discussion

3.1 Radical scavenging activity and reducing power

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution [14].

Both evaluated plants had effect to eliminate radical (Tab. 1) with higher activity in *Tussilago farfara* L. – 7.67 \pm 0.16 mg TEAC/g. Dobravalskyte et al. [15] also determined antioxidant activity of *Tussilago farfara* L. flower, leaves and root. Acetone extract showed the best activity in all observed parts with the best results in roots. Kim et al. [16] isolated two flavonoids from *Tussilago* flower quercetin 3-O- β -L arabinopyranoside and quercetin 3-O- β -D glucopyranoside. These two sugar conjugates of quercetin exhibited higher antioxidative activity than their aglycone, quercetin by NBT superoxide scavenging assay.

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electro

n donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [17]. Reducing power results are presented in Table 1. Similar like DPPH method, both plants showed strong reducing power effect with the higher value in sample of *Tussilago* – 111.67 ±0.72 mg TEAC/g.

Table 1. Antioxidant activity of selected plants

Sample	DPPH method [mg TEAC/g]	Reducing power [mg TEAC/g]
<i>Tussilago farfara</i> L.	7.67 ±0.16	111.67 ±0.72
<i>Centraria islandica</i> L.	5.61 ±0.21	3.51 ±0.64

3.2 Total polyphenol and flavonoid content

Polyphenols are ubiquitous secondary metabolites in plants. They are known to have antioxidant activity and it is likely that the activity of these extracts is due to these compounds [18]. Table 2 shows total polyphenol content in evaluated plants. *Tussilago* extract showed higher content of these compounds.

Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants [19]. *Tussilago* flower extract is rich for these compounds (Tab. 2). Wu et al. [20] isolated seven flavonoids from *Tussilago* flower: quercetin, quercetin-3-O-beta-D-glucopyranoside, quercetin-4'-O-beta-D glucopyranoside, hyperoside, rutin, kaempferol and kaempferol-3 O-alpha-L-rhamnopyranosyl-(1-6)-beta-D glucopyranoside.

Table 2. Total polyphenols and flavonoid content of selected plants

Sample	Total polyphenol [mg QE/g]	Total flavonoid [µg QE/g]
<i>Tussilago farfara</i> L.	11.92 ±0.176	47.62 ±1.37
<i>Centraria islandica</i> L.	0.37 ±0.01	2.08 ±0.07

3.3 Antibacterial activity

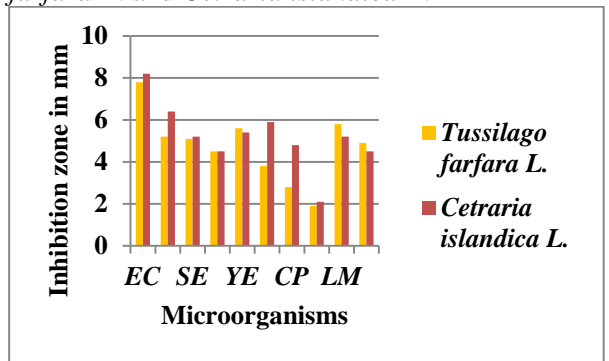
In our study were tested 2 medicinal plants *Tussilago farfara* L. and *Centraria islandica* L.

against 10 different strains of G⁺ and G⁻ bacteria with two different methods.

The results of disc diffusion method showed that coltsfoot exhibited the highest antibacterial activity with 7.8 mm zone of inhibition and iceland moss exhibited the highest antibacterial activity with 8.2 mm zone of inhibition against *Escherichia coli* CCM 3988 (Figure 1).

Minimal inhibition concentration of *Tussilago farfara* L. ranged from 34.13 resp. 38.10 to 102.58 resp. 163.38 µg/ml and MIC of *Centraria islandica* L. from 34.13 resp. 38.10 to 102.58 resp. 163.38 µg/ml. The best antimicrobial activity with MIC was found against *Escherichia coli* CCM 3988 (Tab. 3).

Figure 1. Antibacterial activity of *Tussilago farfara* L. and *Centraria islandica* L. in mm



EC-*Escherichia coli* CCM 3988, KP-*Klebsiella pneumoniae* CCM 2318, SE-*Salmonella enterica* subsp. *enterica* CCM 3807, SS-*Shigella sonnei* CCM 1373, YE-*Yersinia enterocolitica* CCM 5671, BT-*Bacillus thuringiensis* CCM 19, CP-*Clostridium perfringens* CCM 4435, HI-*Haemophilus influenzae* CCM4456, LM-*Listeria monocytogenes* CCM 4699, SA-*Staphylococcus aureus* subsp. *aureus* CCM 2461

In the study Kačaniová et al. [21] highest antibacterial activity of *Tussilago farfara* L. ethanolic extract was measured in Grampositive bacteria *Lactobacillus rhamnosus* (6.67±1.53 mm) and lower in yeast *Saccharomyces cerevisiae* (1.67±0.58 mm) with disc diffusion method used. The ethanolic extract present an important activity against *Saccharomyces cerevisiae* (MIC50=24 µg/ml; MIC90=25.69 µg/ml) and *Serratia rubidaea* (MIC=48.01 µg/ml; MIC90=51.26 µg/ml) with microbroth dilution technique used. In our study the best antimicrobial effect was found against Gramnegative bacteria.

The results of Janovská et al. [22] study showed that the extracts from *Sanguisorba officinalis*, *Tussilago farfara* (aerial part; rhizome), *Chelidonium majus* (root), *Tribulus terrestris*

(aerial part) and *Schisandra chinensis* (leaves) possessed antimicrobial activity. Although the plants differ significantly in their activities against the microorganisms tested, more of the extracts showed antimicrobial activity against *B. cereus* and *S. aureus* than against *E. coli*, *P. aeruginosa* and *C. albicans*.

In the study Hleba et al. [23] the organism *S. cerevisiae* CCM 8191 was found to be more susceptible to the *T. farfara* extract with a MIC50 value of 24 µg/mL. *S. rubidaea* CCM 4684 was less susceptible to *T. farfara* with MIC50 value of 48.01 µg/mL. The organisms *E. coli* CCM 3988, *S. epidermis* CCM 4418, *L. rhamnosus* CCM 1828, *P. aeruginosa* CCM 1960 and *E. raffinosus* CCM 4216 were less susceptible to the *T. farfara* extract with higher MIC50 values (MIC₅₀≥64 µg/mL).

Table 3. Minimal inhibition concentration in µg/ml

Tested microorganisms	<i>T. farfara</i>		<i>C. islandica</i>	
	MIC 50	MIC 90	MIC 50	MIC 90
<i>E. coli</i>	34.13	38.10	34.13	38.10
<i>K. pneumoniae</i>	68.27	76.20	68.27	76.20
<i>S. enterica</i>	68.27	76.20	68.27	76.20
<i>S. sonnei</i>	66.79	134.90	66.79	134.90
<i>Y. enterocolitica</i>	67.53	123.42	67.53	123.42
<i>B. thuringiensis</i>	97.08	171.51	97.08	171.51
<i>C. perfringens</i>	102.58	163.38	102.58	163.38
<i>H. influenza</i>	102.58	163.38	102.58	163.38
<i>L. monocytogenes</i>	102.58	163.38	102.58	163.38
<i>S. aureus</i>	68.27	76.20	68.27	76.20

In the study Türka et al. [24] found that the *Cetraria aculeata* extracts showed antimicrobial activity against most of the tested bacteria, except the ones *P. syringae*, *K. pneumoniae*, *Y. enterocolitica*. Zone diameters were found roughly the same for each extract of *C. aculeata*. In addition there was no antimicrobial activity of the extracts against all fungi tested.

In study of Srivastava et al. [25] only methanol extract was showing activity against *Enterococcus faecalis* with a 13.5±0.8 mm zone. MIC value noted against *Staphylococcus aureus* and *Enterococcus faecalis* was 6.25 µg/ml and 25 µg/ml, whereas against *Bacillus cereus* and *Pseudomonas aeruginosa*, MIC calculated were 3.125 µg/ml and 200 µg/ml, respectively. In conclusion present study demonstrates the relatively higher activity of this

lichen against not only gram (+) but significantly also against gram (–) bacteria. This indicates that this lichen might be a rich source of effective antimicrobial agents.

4. Conclusions

In conclusion, we observed that *Tussilago* and *Centraria* extracts could dose-dependently and significantly inhibit free DPPH radical and can act as reductions. Tested plants showed very good antimicrobial activity against gram-positive and gram-negative bacteria. The results show that these plants can be used as possible applications as food supplement for human health care.

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