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Tussilago farfara L.: Antioxidant and antibacterial potential of extracts in *in vitro* conditions

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ABSTRACT

Tussilago farfara L., also known as coltsfoot, is a plant that has been used since ancient times to relieve coughs. Subsequently, the effectiveness of coltsfoot in the treatment of bronchial asthma, pneumonia and other respiratory diseases was established. In this research, the antioxidant and antimicrobial activity of different coltsfoot extracts was analyzed. The antioxidant activity was monitored through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical inhibition efficiency and the extract's reducing ability. Antibacterial activity was tested using the diffusion technique. *Tussilago farfara* L. extracts showed extremely high antioxidant activity in *in vitro* conditions. The highest antioxidant capacity was found in extracts prepared by mixing water and organic solvents. High antibacterial activity was found for ethanolic, acetone and aqueous-ethanolic extracts of coltsfoot.

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1. INTRODUCTION

Many plant species from the Asteraceae family exhibit therapeutic potential. A well-known herb belonging to the Asteraceae family is coltsfoot (*Tussilago farfara* L.). It is a perennial herb native to China, North Africa and Europe. It can also be found in other parts of the world and is cultivated in Austria and Germany. In Europe, coltsfoot leaves are used to treat bronchial infections. This medicinal herb is used in traditional medicine, mainly for the treatment of respiratory diseases, allergies, cough, cardiovascular and gastrointestinal diseases. Coltsfoot has been studied for its pharmacological activities, including antioxidative, antimicrobial, anti-inflammatory, anti-diabetic, anti-cancer and other properties [1,2,3]. Many compounds have been identified in leaves and flower buds of *T. farfara*, including phenolic acids, such as ferulic, p-hydroxybenzoic, caffeic, and caffeotartaric acids, flavonoids, such as quercetin, kaempferol, quercetin-3-arabinoside, kaempferol-3-glucosides, kaempferol-3-arabinosides and quercetin glucoside, sesquiterpenes, triterpenoids, chromones and pyrrolizidine alkaloids. Caffeic acid and its derivatives are among the main phytoconstituents and can be efficiently extracted [4,5,6]. The antioxidant effect of this plant may be attributed to quercetin-glycosides, among other phytoconstituents [5]. Previous studies revealed that coltsfoot leaves contain amino acid arginine in a high concentration, and it is suggested that arginine can affect the immune

system [7]. The sesquiterpenoids are characteristic components of coltsfoot flowers. It is suggested that sesquiterpenoids possess the inhibitory activity on nitric oxide (NO) production in lipopolysaccharide (LPS)-induced in macrophages, and a number of studies have shown that overproduction of NO is responsible for inflammation [8]. Studies of volatile oils from coltsfoot inflorescence buds reported several new sesquiterpenoids and triterpenoids [9,10]. A specific sesquiterpenoid, tussilagone, reportedly suppresses colon cancer cell proliferation [11]. Although this plant is widely used for its medicinal properties, there is an important lack of studies on its chemical composition and biological properties [12].

2. RESEARCH METHOD

2.1. Plant material, chemicals and instruments

Dried aerial parts of *Tussilago farfara* L. were purchased in a local market in Tuzla. The sample was determined in the pharmacognosy laboratory of the Faculty of Pharmacy, University of Tuzla. The plant was ground into powder using an electric mill. Aqueous solutions needed for the analyzes were prepared using demineralized water. All reagents were p.a. purity and were used without further purification. Spectrophotometric measurements were performed on a Perkin Elmer Lambda 25 spectrophotometer, in the wavelength range of 510-765 nm.

2.2. Preparation of extracts

The extract was prepared by mixing 0.5 grams of chopped plant material with 25 mL of solvent, or solvent mixture. Mixing was carried out at 200 rpm for 20 hours, after which the mixture was filtered and immediately subjected to analyses. All extracts were clear after filtration. For easier discussion of the obtained results, the extracts are labeled as indicated in table 1.

Table 1. Labels of the samples and color of the extracts after the extraction

Sample	Labels	Color
Methanolic extract	MeOH	dark green
Ethanol extract	EtOH	dark green
Acetone extract	Ace	dark green
Aqueous extract	Water	orange
Water-methanolic extract	Water:MeOH	yellow
Water-ethanol extract	Water:EtOH	olive green
Water-acetone extract	Water:Ace	olive green

2.3. Determination of total phenolic content (TPC)

Total phenolic compounds present in the extracts of coltsfoot were quantified spectrophotometrically through the Folin-Ciocalteu test [13]. 200 µL of extracts was mixed with 2540 µL of 10% Folin-Ciocalteu reagent. After 5 min 420 µL of 10% sodium carbonate was added. The mixture was incubated for 1 hour. 910 µL distilled water was added to each sample prior to measuring. The absorbance of the resulting blue-coloured solution was measured at 765 nm. Quantitative measurements were performed, based on a standard calibration curve of gallic acid (equation 1.).

$$y = 0{,}0042x + 0{,}0076;\ R^2 = 0{,}9998 \quad (1)$$

2.4. Determination of total flavonoid content (TFC)

Total flavonoid content in the extracts was determined by the previously described method, with some modification [14]. 1000 µL of extract solution were mixed with 300 µL of 5% sodium nitrite. 300 µL of 10% aluminium chloride was added after 5 minutes. After 6 minutes incubation at room temperature, 2000 µL of 1 M sodium hydroxide was added to the reaction mixture. Immediately the final volume was make upto 10 mL with distilled water. Absorbance of sample was measured against the blank at 510 nm. The results were derived from the calibration curve of quercetin (equation 2.).

$$y = 3{,}024x - 0{,}0034;\ R^2 = 0{,}9984 \quad (2)$$

2.5. Ferric-reducing antioxidant power (FRAP) Assay

The FRAP (Ferric-Reducing Antioxidant Power) method is based on the ability of the extract to reduce Fe(III) to Fe(II) ions. The test was conducted according to a published protocol [15]. 3000 µL of prepared FRAP reagent (mixture of acetate buffer, iron(III) chloride hexahydrate and 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) reagent in a ratio of 10:1:1 *v*/*v*) was mixed with 100 µL of extracts. Absorbance at 593 nm was recorded after incubation for 30 minutes at 37° C. The FRAP value was calculated from the calibration curve of ferrous sulfate heptahydrate (equation 3.).

$$y = 0{,}001x + 0{,}0698;\ R^2 = 0{,}9997 \quad (3)$$

2.6. DPPH radical scavenging activity

The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method was carried out according to the previously described method [16]. 20, 40 and 60 µL of each extract with a concentration of 3.33 mg/mL was transferred to test tubes and supplemented with methanol up to 2000 µL. Then 500 µL of 0.5 mM DPPH solution was added and the samples were left to incubate for 30 minutes in a dark room at room temperature. Absorbance was measured at 517 nm with methanol as a blank. The radical scavenging effect (%) or DPPH radical inhibition percentage was calculated according to the equation:

$$I = \frac{A_C - A_S}{A_C} \times 100\ [\%] \quad (4)$$

where As is the absorbance of the solution containing the sample at 517 nm and Ac is the absorbance of the control.

2.7. Evaluation of antibacterial activity

Extracts for testing the antibacterial effect of the coltsfoot were prepared by mixing 5 grams of crushed material with 100 mL of solvent, or a mixture of solvents. The mixture was stirred for 20 hours at 200 rpm and then filtered. The filtrate was evaporated to a dry residue and dissolved in dimethyl sulfoxide. The concentration of the extracts for analysis was 100 mg/mL. Antibacterial activity were investigated by diffusion method on reference bacterial strains *E. coli*, *E. faecalis*, *S. aureus*, *B. subtilis*, *S. enterica* and *P. aeruginosa*. From the microorganisms strains of overnight cultures, suspensions of 0.5 McFarland turbidity were prepared. The strains were then placed on the surface of the nutrient substrate Mueller-Hinton agar, dispersed in sterile Petri dishes. Substrate thickness was 4 mm. In the agar sterile drill-shaped holes were made ("wells"), into which 100 µL of extract solutions in concentration of 100 mg/mL were added. After the plates were left at room temperature for 15 minutes, the substance was diffused into agar, incubated at 37°C/24 h. After the incuba-tion period, the size of the inhibitory zone was measured.

3. RESULTS AND DISCUSSIONS

3.1. Content of polyphenols and flavonoids

The content of polyphenols and flavonoids in coltsfoot extracts is shown in Table 2. The highest content of the mentioned compounds was obtained for extracts in which a mixture of water and organic solvents was used as an extraction agent. The most effective mixture in this respect was a water-acetone solvent mixture. Of pure solvents, the highest extraction efficiency of bioactive components was confirmed with methanol, and the weakest with water and acetone. The content of polyphenols and flavonoids in the extracts decreases in a sequence, as follows: Water:Ace > Water:MeOH > Water:EtOH > MeOH > EtOH > Ace > Water. These results confirm previous research in which mixtures of organic solvents with water proved to be significantly more effective in the extraction of bioactive components from plant material [17-20].

Table 2. Results of the content of total polyphenols and flavonoids in the extracts of coltsfoot

Extract	Total Phenolic Content/TPC (mg GAE/g)	Total flavonoid content/TFC (mg QE/g)
MeOH	42.79	0.057

Extract	Total Phenolic Content/TPC (mg GAE/g)	Total flavonoid content/TFC (mg QE/g)
EtOH	25.41	0.041
Ace	22.62	0.035
Water	21.69	0.027
Water:MeOH	55.30	0.070
Water:EtOH	53.08	0.066
Water:Ace	57.51	0.076

In the past few years, studies of the antioxidant activity and content of bioactive components of coltsfoot extracts have been conducted. Barragan Ferrer et al. [21] analyzed the content of bioactive components in methanolic, ethanolic and acetone extracts of coltsfoot leaves and roots. Extracts were obtained by Soxhlet extraction. Results vary depending on the origin of the plant's leaves. The ethanolic extract of the coltsfoot leaf collected in Lithuania had the highest content of polyphenols, while the lowest content of these compounds was recorded in the acetone extract. Coltsfoot leaf extracts collected in France show different results. In the case of these extracts, the highest polyphenol content was reported for the methanolic extract. In this research, the extraction technique and the choice of a suitable solvent did not affect the extraction efficiency, but the geographical origin did. The presented results in this study indicate a higher content of polyphenols and flavonoids compared to our research, and the reason, in addition to the different geographical origin of the plant, could be the extraction technique used and the evaporation of solvents after extraction, which was avoided in this study [21].

Dobravalskyte et al. [22] analyzed acetone, methanol and ethanol extracts of coltsfoot. The authors subjected the sample to hydrodistillation before extraction in order to remove volatile components. Their results indicate a higher content of polyphenols and flavonoids compared to our study, and acetone proved to be the most effective solvent, and ethanol the weakest. The differences in the results can be attributed to the different geographical origin of the plant and to the different treatment of the plant material before and during the extraction [22].

3.2. Antioxidant activity

The antioxidant activity was tested by monitoring the inhibition of DPPH radicals at concentrations of 0.033, 0.066 and 0.099 mg/mL, and the reduction of Fe(III) ions to Fe(II) ions. The results of the reduction capacity are shown in table 3, and the graphic comparison of DPPH radical inhibition is shown in figure 1. The antioxidant potential is fully correlated with the content of polyphenols and flavonoids. Ascorbic acid, which was used as a positive control, shows a significantly higher reduction potential than the extract of the coltsfoot. In the inhibition of the DPPH radical, no great difference was observed in the efficiency of ascorbic acid compared to water-acetone, water-methanol and water-ethanol extracts, which proved to be the most effective in this regard.

Table 3. The reducing ability of the extract of the coltsfoot

Extract	FRAP value (μmol/g)
MeOH	623.47
EtOH	475.00
Ace	287.75
Water	244.39
Water:MeOH	808.67
Water:EtOH	689.79
Water:Ace	832.65
Ascorbic acid (control)	14 250

In comparison with the results of other studies previously described, the high antioxidant potential of the extracts of the coltsfoot was confirmed. The reducing capacity of a compound can be a significant indicator of its potential antioxidant activity. The antioxidant activity of phenolic compounds is mainly the result of their redox properties, which can play an important role in the adsorption and neutralization of free radicals, the quenching of singlet and triplet oxygen or the decomposition of peroxides. Fe(III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant activity. The antioxidant capacities of the extracts have

a strong relationship with the solvent used, mainly due to the different antioxidant potential of compounds with different polarities [18].

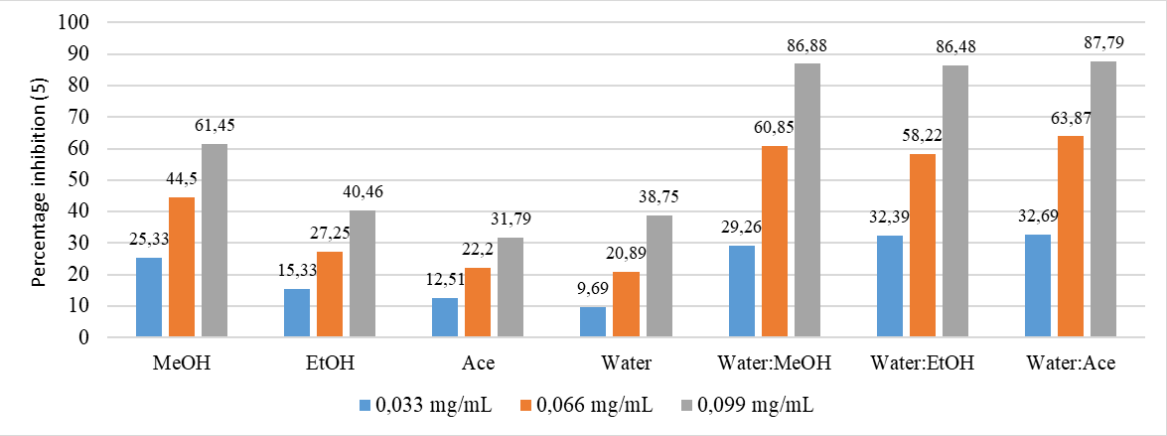

Figure 1. Efficiency of DPPH radical inhibition depending on the concentration of the extract

3.3. Antibacterial activity

The antibacterial activity of the extracts was tested using the diffusion technique on reference bacterial strains from the ATCC collection. The results are shown in Table 4. Complete absence of antibacterial activity was recorded for methanol, water, water-methanol and water-acetone extracts. An extremely high antibacterial potential was recorded for the ethanolic and water-ethanolic extract, as well as for the acetone extract of the coltsfoot. The zones of inhibition are in the range of 15-22 mm for the acetone extract, i.e. greater than 30 mm for the ethanol and water-ethanol extracts. Compared to Ciprofloxacin, which was used as a positive control, the ethanol and water-ethanol extracts showed the same and/or similar antibacterial effect.

Table 4. Antibacterial activity of coltsfoot extracts

Bacterial strain	ATCC	Inhibition zones of coltsfoot extracts (mm)						
		MeOH	EtOH	Ace	Water	Water:MeOH	Water:EtOH	Water:Ace
S. aureus	25923	-	> 30	22	-	-	> 30	-
E. faecalis	51299	-	> 30	-	-	-	> 30	-
E. coli	25922	-	> 30	18	-	-	27	-
B. subtilis	6633	-	> 30	15	-	-	> 30	-
S. enterica	13076	-	> 30	20	-	-	> 30	-
P. aeruginosa	27853	-	> 30	16	-	-	28	-

Janovska et al. [23] prepared an ethanolic extract by mixing different parts of the coltsfoot with 450 mL of 80% ethanol for five days. The filtered extract was evaporated and further used for testing antimicrobial activity. The ethanolic extract of the aerial part of the plant showed activity against *S. aureus* (MIC 62.5 mg/mL), while the inhibitory activity of the extract was not confirmed in the case of *E. coli* and *P. aeruginosa* [23]. Kačaniova et al. [24] also investigated the antimicrobial potential of ethanolic extracts of the coltsfoot. Through this test, antibacterial activity against *E. coli* and *S. enterica* was confirmed, with inhibition zones of 11.33 and 13.33 mm, respectively [20]. Prior to this research, in 2013, Kačaniova et al. [25] published a paper in which they examined the antibacterial potential of an ethanolic extract that was prepared by long-term shaking for two weeks. A weak antibacterial potential of the extract was reported in the case of *E. coli* [25]. The differences in the results of the described studies and our study can be explained by the different geographical origin of the sample as well as the length of the extraction. Through other studies, the extracts were prepared by stirring for a longer period of time, which may affect the stability of the components in the extract that are responsible for the antibacterial potential of this plant.

4. CONCLUSION

Extracts obtained by mixing water and organic solvents showed a high level of extraction of polyphenolic compounds that are responsible for the high antioxidant capacity of coltsfoot. Certain extracts showed extremely high antibacterial activity, with zones of inhibition over 30 mm. This study indicates that coltsfoot extracts have biological activity *in vitro*. This study needs to be expanded and examined for cytotoxic and genotoxic effects, which will provide more detailed information about the safety of these extracts, and their potential antitumor activity.

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