Biologically active compounds from *Tussilago farfara* L.

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Abstract. The modern interest in the chemical component of the coltsfoot is due to the fact that many of the ingredients in the tea have physiological activity and can be used in the treatment and prevention of many diseases. The results from this study showed, that the main phenolic acids was caffeic acid (1867.34±3.12 µg/g), ferulic acid was in approximately lower concentration, triple lower than caffeic acid (545.23±4.36 µg/g), while chlorogenic acid was not detected. Ascorbic, malic and citric acids were determined by HPLC analysis as the main organic compounds in the leaves of coltsfoot. The most abundant organic acids were ascorbic acid (67.43±0.95 mg/g) and malic acid (45.25±1.15 mg/g). The 17 amino acids were determined in our study. The highest quantities of essential amino acids were phenylalanine (6.23 g/100 g protein) and valine (5.56 g/100 g protein). Other essential amino acid such as threonine, leucine, isoleucine and lysine were found at 4.90 g/100 g protein, 4.64 g/100 g protein, 3.76 g/100 g protein, and 2.45 g/100 g protein respectively.

Key words: coltsfoot, HPLC, amino acids, phenolic acids, organic acids.

1. Introduction

According to the World Health Organization (WHO), 5% of the earth’s inhabitants predominately relied on plant-based traditional medicines for their primary healthcare. *Tussilago farfara* L., known as coltsfoot, is a perennial herbaceous plant from Asteraceae family, widespread in Europe, Asia and in Bulgaria. The root and leaves of coltsfoot have medical benefits in chronic bronchitis, asthma, influenza, chest complaints and inflammations [1]. Coltsfoot leaves are used in official and traditional medicine as a component of various herbal drugs (herbal teas/mixture herbal products, tablets, syrups, extracts) for treatment of respiratory tract infections with difficulties in expectorations [2,3]. The leaf extracts and phenolic components in the coltsfoot are effective against several Gram-negative bacteria displaying an anti-microbial effect [4]. Fresh juice from the leaves is inhaled into the nostrils to eliminate sinus colds. The juice of leaves is also mixed with powdered sugar to treat tuberculosis [5]. The widespread use of coltsfoot leaves in folk medicine in the past and today is due to the biologically active substances contained in the plant.

Bioactive compounds are present in small quantities in foods, mainly in fruits, vegetables and medicinal plants and provide health benefits beyond the basic nutritional value. Most of the bioactive compounds have antioxidant, anticarcinogenic, antiinflammatory and antimicrobial properties [6].
The phenolic acids contained in the various parts of plants are biological compounds known with their benefit to human health, and their importance in a number of commercial industries. Phenolic acids are present in almost all edible plants, and the dietary intake by humans is reported to be approximately 200 mg per day. The most widely studied phenolic acids are caffeic and ferulic acids because of their widespread presence in the human diet [7]. Caffeic and ferulic acids have been reported to possess have anticancer, anti-inflammatory, strong antioxidant, antimicrobial and antiviral activities [8,9]. Ferulic acid is widely distributed in the plant kingdom and has a wide range of potential therapeutic effects useful in the treatments of cancer, diabetes, lung and cardiovascular diseases, as well as hepatic, neuro- and photoprotective effects, antimicrobial and anti-inflammatory activities [10].

Plants are a rich source of amino acids and their individual abundance in plants is of the great significance especially for our health. They are the building blocks of proteins and necessary elements of a healthy diet. Amino acids are necessary for metabolic processes as well as for the transport and storage of all the nutrients such as carbohydrates, proteins, vitamins, minerals, water and fats [11]. Amino acids are classified into three groups: essential amino acids, non-essential amino acids and the conditional amino acids. Essential amino acids cannot be made by the body and as a result, they must come from food. They include: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. The non-essential amino acids mean that our bodies produce them, if we do not get them from food. They comprise of alanine, aspartic and glutamic acids. Conditional amino acids are usually not essential except in times of illness and stress [12,13]. More than 60% of the proteins required by humans for the growth and development come from plant resources [14].

Plants possess a unique richness and diversity of metabolites including organic acids. Organic acids (malic, citric, tartaric, oxalic, acetic, formic and ascorbic acids) make up a large group of biologically active substances and play an important role in plant and human metabolism. Organic acids have a wide range of biological effects on the human body in addition to vitamin properties, they have a choleric effect that normalizes the activity of the digestive system [15].

Over the last decades, medicinal plants have paid attention to their potential applications in the food and nutraceutical fields and, thereby making a huge impact on both national and international health agendas and, on global trade.

In our continuous efforts to study bioactive natural products from herbs and food plants, we have studied some biological active compounds in leaves of *T. farfara*, based on their traditional usage for the treatment of various ailments.

To the best of our knowledge, there is no investigation for the chemical composition from Bulgarian populations. Therefore, the purpose of the present study was to determine the variability of the contents of the some bioactive compounds (organic, amino and phenolic acids) in leaves of *T. farfara* from Bulgaria.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and solvents used in the study were analytical grade. Acetonitrile, acetic acid, potassium dihydrogen phosphate and ethanol were HPLC grade and were purchased from Merck (Darmstadt, Germany). The standards of organic and phenolic acids were obtained from Sigma Aldrich (Germany). The mix of amino acid standard, buffer and derivatizing reagent were from Waters (Corporation, USA).

2.2. Plant materials

The leaves of *T. farfara* were purchased from pharmacy, harvest 2019.

2.3. Amino acids analysis

About 30 mg of the sample was weighed into glass ampoules. Three milliliters of 6 M HCl were added into the ampoule put in an oven pre-set at 105°C for 24 h. The ampoule was allowed to cool before
broken open at the tip and the filtrate evaporated to dryness at 90°C under vacuum in a rotary evaporator. The residue was dissolved with 2 ml 20 mM HCl and filtered through 0.45 μm.

From the hydrolyzed and filtered sample 10 μl were derivatized with AccQ-Fluor reagent kit (WATO52880-Waters) and AccQ-Fluor borate buffer (70 μl) was added in the sample tube with micropipette and vortexed. Thereafter, 20 μl of AccQ-Fluor reagent was added and immediately vortexed for 30 s and the vials were heated for 1 min. in a water bath at 55°C before injection in HPLC.

The AccQ-Fluor amino acid derivatives were separated on ELITE LaChrom HPLC system (VWR™ Hitachi, Tokyo, Japan). Sample of 20 μl was injected into an HPLC with reversed phase AccQ-Tag™ silica-bonded amino acid column –C18, 3.9 mm×150 mm (Waters). The elution of the amino acids was performed by gradient system with mobile phase, eluent A, buffer WAT052890 (Waters) and mobile phase, eluent B, 60% acetonitrile (Merck), in a separation gradient with a flow rate of 1.0 ml/min. The amino acids were detected using a diode array detector (DAD) at 254 nm with the column condition set at 37°C for 40 min. For qualitative and quantitative determination of amino acids were used the retention time and calibration curves for each amino acids calibration curves with linearity range of 50-500 μg/ml.

The determination of the total protein content was according to the AOAC Method 976.06 [16], using an UDK 152 System (Velp Scientifica, Italy).

2.4. Phenolic acids analysis
Leaves of T. farfara (0.5 g) were extracted three times with 10 ml 70% ethanol (v/v) under reflux-heat at 70 °C for 20 min. The residue of leaves material was removed through filter paper filtration and the combined extracts were evaporated to dryness under vacuum. The dried extracts were stored in refrigerator at 4 °C in dark and used for the next analyses.

Qualitative and quantitative determination of phenolic acids was performed by using Elite LaChrome (Hitachi) HPLC system equipped with DAD. Separation of the phenolic acids was performed by Supelco Discovery HS C18 column (5 μm, 25 cm × 4.6 mm), operated at 30°C under gradient conditions with mobile phase consists of 2% (v/v) acetic acid (solvent A) and acetonitrile (solvent B).

Caffeic, 3,4 –hydroxybenzoic and ferulic acids were used for creation of standard calibration curves with linearity range of 10-100 μg/ml. The detection of compounds was carried out at 280 and 320 nm and the flow rate was 0.8 ml/min. The results were expressed in μg per g of dry weight (dw) sample.

2.5. Organic acids analysis
The organic acids were determined by performed using a HPLC (Elite LaChrome, Hitachi) system with DAD. Separation was achieved by Discovery® SH C18 column (5 μm, 250 mm × 4.6 mm) with reversed phase and detection by 244 nm for ascorbic acid and 210 nm for malic and citric acids. The elution was performed with 25 mM potassium dihydrogen phosphate (the pH is adjusted to 2.4 with phosphoric acid). The flow rate was 0.5 ml/min. The organic acids found were quantified by comparison of the area of their peaks recorded with calibration curves obtained from commercial standards of each compound.

3. Results and discussion
3.1. Amino acids composition
A total of seventeen amino acids are determined and their corresponding values obtained from the sample are compared to other medicinal plants like C. olitorius L. and T. occidentalis L., because there is not enough information in the literature about the amino acids composition in the leaves of T. farfara. Table 1 shows the content of the essential amino acids composition in our sample expressed in g/100 g protein.

The main essential amino acids in this study are phenylalanine, valine, threonine, and leucine. The highest value obtained is that of phenylalanine (6.23 g/100 g protein). Its content in leaves of T. farfara is higher than the value of other medical plant (T. occidentalis) - 4.57 g/100 g protein [17]. This amino
acid is beneficial for healthy nervous system, boosts memory and learning it elevates mood and alertness and helps by depression [18].

Valine was found to be the second essential amino acids (5.56 g/100 g protein). The valine is need for muscle metabolism and coordination: it supplies energy to the muscle tissue to promote muscle growth and tissue repair [19].

Table 1. Essential amino acid composition in leaves of *T. farfara*.

| Amino acids       | Content, g/100 g protein |
|-------------------|--------------------------|
| Valine            | 5.56±0.25                |
| Leucine           | 4.64±0.39                |
| Isoleucine        | 3.76±0.56                |
| Threonine         | 4.90±0.45                |
| Methionine        | 1.89±0.23                |
| Phenylalanine     | 6.23±0.43                |
| Histidine         | 1.95±0.32                |
| Lysine            | 2.45±0.39                |

* All data are presented as mean value ± standard deviation (n=3).

The third predominant acid is threonine (4.90 g/100 g protein), followed of leucine (4.64 g/100 g protein). The higher leucine content was reported in leaves of *C. olitorius*-7.35 g/100 g protein [17]. Isoleucine and lysine have values of 3.76 and 2.45 g/100 g protein, respectively. Isoleucine is needed for the healing and repair the muscle tissue, skin cells and bones. The lysine and the threonine help to maintain intestinal integrity and health, while the leucine helps to lower blood sugar [20].

The non-essential amino acids identified on the course of analysis are shown in Table 2. Non-essential amino acids are those amino acids that the human body produces or synthesizes [21].

Table 2. Non-essential amino acid composition in leaves of *T. farfara*.

| Amino acids | Content, g/100 g protein |
|-------------|--------------------------|
| Alanine     | 8.36±0.23                |
| Glutamic acid | 7.54±0.56              |
| Aspartic acid | 6.67±0.12              |
| Arginine    | 12.09±0.22               |
| Cysteine    | 0.97±0.36                |
| Tyrosine    | 7.54±0.24                |
| Glycine     | 5.56±0.18                |
| Proline     | 6.34±0.45                |
| Serine      | 8.24±0.29                |

* All data are presented as mean value ± standard deviation (n=3).
The highest value obtained was that of arginine (12.09 g/100 g protein) followed by alanine (8.36 g/100 g protein), serine (8.24 g/100 g protein) and glutamic acid (7.54 g/100 protein). In our study the arginine has the highest value of 12.09 g/100 g protein, while T. occidentalis and C. litorius contain 4.00 and 3.81 g/100 g protein, respectively [17]. The results for alanine and glutamic acid are quite close to the reported results for leaves of C. litorius and T. occidentalis [17]. The alanine helps preserve balanced levels of nitrogen and glucose in the body. The glutamic acid is important in the metabolism of sugars and fats.

The results of the content of amino acids in the leaves of T. farfara were close to results reported for other medical plants such as: C. olitorius and T. occidentalis. This shows that the leaves of T. farfara are good sources of amino acids and explain the medicinal properties of the plant.

3.2. Phenolic acids
Phenolic compounds are the most abundant plant secondary metabolites that have been shown to possess multiple pharmacological activities [22]. The results of phenolic acids in the leaves of T. farfara are presented in Table 3.

| Phenolic acids                      | Values, µg/g       |
|------------------------------------|--------------------|
| Caffeic acid                       | 1867.34±3.12       |
| Ferulic acid                       | 545.23±4.36        |
| 3,4-Hydroxybenzoic acid            | 98.56±3.18         |

* All data are presented as mean value ± standard deviation (n=3).

In our sample the following phenolic acids were found: 3,4-hydroxybenzoic acid, ferulic acid and caffeic acid. Among the identified phenolic acids, caffeic acid has been identified with the highest amount-1867.34 µg/g. Ferulic acid is in a relatively high concentration (545.23±4.36 µg/g), but in approximately lower concentration, triple lower than caffeic acid. The lowest content has been determined of 3,4-hydroxybenzoic acid-98.56 µg/g.

Hleba et al. [23], found that chlorogenic acid is the main compound in leaves of T farfara - 5899.29 µg/ mg, following of ferulic (896.8 µg/mg), 3,4-hydroxybenzoic (112.6 µg/mg) and gallic (2.8µg/g) acids. The presence of caffeic and ferulic acids in T. farfara extract has been previously reported with 160 µg/g water extract and 168 µg/g methanolic extract content, respectively [24]. The differences in the reported quantities of the phenolic acids in leaves of T. farfara and this finding in our study could be explained with the differences in the climate and geographical conditions, as well as with the type of solvents and methods of extractions.

HPLC analyses of the phenolic profile in the leaves of T. farfara reveals that the caffeic and ferulic acids are the major phenolic compounds. That is of great importance because of their valuable biological activities.

3.3. Organic acids
Organic acids are the biologically active substances which are in plants in the free state, in the form of salts, esters and compounds with other substances. The results of quantitative contents of organic acids in leaves of T. farfara are shown in Table 4. The our results were compared to other medicinal plants of the same family, because there is not enough information in the literature about the organic acids composition in the leaves of T. farfara.
HPLC analysis of the organic profile revealed, that the major organic compound in the leaves of *T. farfara* is ascorbic acid (67.43 mg/g). In leaves of *A. africana* has been reported, that the ascorbic acid content is 6.42 mg/100 g in sample [25]. Other authors found ascorbic acid in the leaves of *A. conyzoides* with 10.01 mg/100 g content [26]. The results showed higher content of ascorbic acid in leaves of *T. farfara* compared to other medical plants of the same family. The ascorbic acid is one of the most active compounds of organic acids and a powerful phytochemical antioxidant. The traditional use of this plant as a remedy for cough and other medicinal applications could be attributed to the content of ascorbic acid (vitamin C) in the leaves of coltsfoot.

The other organic acids found in our sample of leaves of *T. farfara* was malic acid (45.25 mg/g), followed by citric acid (26.23 mg/g). The obtained results in this study for both organic acids are higher than the values found in the research study conducted by Marchyshyn et.al. [27]. They reported that the content of malic and citric acids in the leaves of *C. shortorum* was 2795 mg/kg and 1562 mg/kg respectively.

The differences in organic acid content in coltsfoot and the others medical plants of the same family could be explained with variation in the climatic and agronomic conditions, as well as the type of extraction techniques and with the different reagents for extraction and to equipment for analysis, which are used.

### 4. Conclusions

The obtained results showed that the leaves of coltsfoot were characterized with the relatively high and balanced contents of the main active compounds, especially organic acids, phenolic acids and essential amino acids, which are important for human health and nutrition. In leaves of coltsfoot was found a significant amount of amino acids. Phenylalanine was detected as the main essential amino acid with the highest content - 6.23 g/100 g protein. Moreover, in leaves of *T. farfara* were identified three phenolic acids such as caffeic - 1867.34±3.12 µg/g, ferulic - 545.23±4.36 µg/g and 3,4 - hydroxybenzoic acids and three organic acids such as ascorbic, malic acid and citric acids. The obtained results demonstrated the potential application of the leaves of coltsfoot as a source of essential amino acids and phenolic compounds, with potential antioxidant activity for healthy balanced diet.

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