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Coltsfoot Leaves (*Tussilago farfara* L.) – A Promising Source of Essential Amino Acids

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ABSTRACT

Objective: Free and bounded amino acids are one of the valuable biologically active substances (BAS) containing in medicinal plants with other BAS groups. The research aims to determine the profile and content of free and bounded amino acids in coltsfoot leaves infusion by HPLC-UV, as well as suggest alternative ways to use it in medicine.

Materials and methods: Coltsfoot leaves (crushed herbal drug) were used as plant material for preparing infusion and analysis. Amino acids profile and content were analyzed by RP-HPLC-UV after pre-column derivatization with o-phthalaldehyde and 3-mercaptopropionic acid (OPA) (derivatization of primary amines) and with fluorenylmethyloxycarbonyl (FMOC) (secondary amines). The sources of scientific literature concerning the amino acid composition of crushed herbal drugs were also studied.

Results: 10 free and 13 bounded amino acids were identified in coltsfoot leaves infusion. Total content was 44.04±2.20 mg/g (Arg > Tyr > Gln > Ala > Asn > Met > Ile > Val > Lys > Leu) and 57.79±2.89 mg/g (Arg > Gln > Lys > Tyr > Gly > Asn > Val > Thr > Ala > Ile > Nva > Leu > Met) respectively. Content of essential amino acids – 0,89±0,04 mg/g (5 free); 9,58±0,48 mg/g (5 bounded).

Conclusions: Since the coltsfoot leaves contain a significant amount of arginine, studies involving the prevention and adjunctive treatment of viral diseases, including SARS-CoV-2 virus infection (COVID-19), are promising.

Keywords: *Tussilago farfara* L., coltsfoot leaves, amino acids, HPLC method, immune system stimulation, SARS-CoV-2 virus, COVID-19 infection

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INTRODUCTION

Coltsfoot (*Tussilago farfara* L.) leaves are known as a crude herbal drug with expectorant and antitussive activities. Coltsfoot leaves (*Tussilaginis farfarae* folia) are used in Russian and European medicine in respiratory diseases, for asthma, bronchitis, and cough. Coltsfoot leaves (Figure 1) 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 upper respiratory tract infections with difficulties in expectorations and cough [1, 2].

Figure 1: Coltsfoot leaves crude herbal drugs: whole (1), crushed (2).

The coltsfoot leaves contain different groups of biologically active substances. Among them are polysaccharides, in particular, mucus (up to 7-10%); they determine the main pharmacological effects. During hydrolysis, polysaccharides break down to form fructose (30%), galactose (24%), arabinose (21%), glucose (15%), xylose (10%) and uronic acids (6%). Inulin is also accumulated in the coltsfoot leaves; it is a characteristic polysaccharide of the *Asteraceae* family plants. Bitter glycosides (2.6%), essential oil (trace amounts), saponins, carotenoids, ascorbic acid, gallic acid, flavonoids, tannins, organic acids (malic, tartaric) are found in raw materials. Pyrrolizidine alkaloid senkirkin in small amounts (about 0.01%) may be present in coltsfoot leaves. Flavonoid profile is presented by kaempferol and quercetin glycosides. Kaempferol derivatives: kaempferol, 3-O-β-glucopyranoside (astragalin), and 3-O-α-rhamnopyranosyl(1→6)-β-glucopyranoside (nicotiflorin). Quercetin derivatives: 3-O-β-D-glucopyranoside (isoquercitrin), 3-O-α-L-Rhamnopyranosyl(1→6)-β-D-glucopyranoside (rutin), 3-O-β-D-galactopyranoside (hyperoside), 3-O-β-L-arabinopyranoside (guajaverin). Three phenolic acids, p-hydroxybenzoic, cis-, trans- p-coumaric, and cis-, trans-chlorogenic acids, are detected [3-8].

The amino acid composition has not been studied in detail. In work [9] the movement of amino acids (alanine, aspartic acid, glutamic acid, glutamine and serine, glycine, serine, cysteine, and methionine) was studied in plant tissues. Amino acids play an important role in different organs and systems function according to biochemical studies. As protein constituents, they take part in all vital processes with carbohydrates, nucleic acids, and lipids. Tyrosine is the primary metabolite serving as precursors for flavonoids in plants. Glutamic and aspartic acids lie at the core of nitrogen fixation, transport, and elimination of biologically active forms of maintaining nitrogen balance. Methionine can improve damaged heart function. Glycine and his derivatives act as hypolipidemic agents; some amino acids stimulate insulin secretion by pancreatic b-cells [10-12]. Essential amino acids are important nutrients and their deficiency leads to different metabolic disorders; coltsfoot leaves contain free and bounded amino acids. Since the main dosage form of coltsfoot leaves is an infusion, it seems to determine the amino acids in it.

The research aims to determine the profile and content of free and bounded amino acids in coltsfoot leaves infusion by HPLC-UV, as well as suggest alternative ways to use it in medicine.

MATERIALS AND METHODS

2.1. Plant material

As the object of analysis, we used samples of the herbal medicine "Coltsfoot leaves" purchased in the pharmacy network of the city of Moscow. The quality of the raw materials met the requirements of the State Pharmacopoeia of the Russian Federation XIV edition (SPRF XIV) [2].

2.2. Infusion preparation

The infusion was prepared following the drug package leaflet for the medical use of the drug. About 10 g (2 tablespoons) of the coltsfoot leaves are placed in an enamel bowl, 200 ml (1 cup) of boiling water is poured, covered with a lid and heated in a boiling water bath for 15 minutes, cooled at room temperature for 45 minutes, filter, the remaining raw materials are squeezed. The volume of the resulting infusion is adjusted with boiled water up to 200 ml [13].

2.3. HPLC-UV conditions

Amino acid content and composition were analyzed by RP-HPLC after pre-column derivatization with o-phthalaldehyde and 3-mercaptopropionic acid (OPA) (derivatization of primary amines), or with fluorenylmethyloxycarbonyl (FMOC) (secondary amines) [14]. Chromatograph Agilent 1260 (solvent supplying system and degassing system for 4 solvents; diode array detector, column thermostat; autosampler). Software – ChemStation (ver. A.09.03). Chromatographic column – XBridge BEH300 C18 Sugar-Pak (Waters, USA), column length – 250 mm, internal diameter – 4.6 mm, pore size – 5 μm. Standard amino acids solution 79248-5X2MI (USA) was purchased from Sigma-Aldrich. Autosampler was programmed to perform precolumn derivatization: 5 μl of borate buffer, 1 μl of OPA reagent were loaded into sample loop, mixed, then 1 μl of FMOC reagent was loaded, mixed again, 8 μl were injected. Eluent flow-rate – 0.8 ml/min. Detection by UV at 338 nm (reference wavelength – 380 nm).

2.4. Preparation of solutions

2.4.1. Preparation of OPA and FMOC reagents

50 mg of o-phthalaldehyde, OPA, were dissolved in 1 ml of methanol, 40 ml of 3-mercaptopropionic acid (CAS 107-96-0, 63768 Sigma (> 99,0%)) were then added, made up to the mark of 10 ml with 0.4 N sodium borate aqueous solution (pH = 10.4). 50 mg of fluorenylmethyloxycarbonyl, FMOC, (CAS 28920-43-6, Sigma 23186 (> 99.0%)) were dissolved in 10 ml of acetonitrile.

2.4.2. Preparation of mobile phases and buffer

1.36 g of sodium acetate trihydrate was put into 1000 ml measuring cup, then 500 ml of distilled water, and 90 μl of triethylamine were added, then mixed. 1-2% acetic acid was added to pH = 7.2, then 1.5 ml of tetrahydrofurane was added, then mixed. The resulting solution was filtered with a fluoroplastic membrane filter of 0.45 μm pore diameter (phases A). 1.36 g of sodium acetate trihydrate was put into 500 ml measuring cup, then 100 ml of distilled water was added, then mixed until complete dissolution. 1-2% acetic acid was added to pH = 7.2, then 200 ml of methanol and 200 ml of acetonitrile were added, then mixed. The resulting solution was filtered with a fluoroplastic membrane filter of 0.45 μm pore diameter (phase B). 76 g of sodium tetraborate decahydrate were dissolved in 1000 ml of distilled water, then 1 M sodium hydroxide was added to pH = 10.4. The resulting solution was filtered using fluoroplastic membrane filter with 0.45 μm pore (borate buffer).

2.5. HPLC-UV analysis of amino acids

The infusion was filtered with glass filter (POR-40), the filtrate was evaporated to dryness. The dry extract was

dissolved in 10 ml of water. 50 µl was subjected to amino acid analysis (free amino acids). Hydrolysis of samples was performed according to Manual on Quality and Safety Control of Biological Food Additives R 4.1.1672-03 "Amino acids composition analysis. Testing" with 6M hydrochloric acid [15]. The hydrolysate was filtered. Dry residue on the filter was washed with hot water. Filtrates were evaporated on a water bath. 20 ml of water and 50 µl of resulting solutions were added to residues and then chromatographed (bounded amino acids). The resulting hydrolysate was used for analyzing. Amino acids were identified by retention time. Each peak area was calculated automatically and the quantitative content was determined.

RESULTS AND DISCUSSION

3.1. Amino acids in coltsfoot leaves infusion

Chromatography data are shown in Figure 2. Results of qualitative analysis of detected amino acids are presented in Table 1. As it is seen from tables and chromatograms, 10 free and 13 bounded amino acids were identified in coltsfoot leaves infusion (total content was 44.04±2.20 mg/g and 57.79±2.89 mg/g respectively). Content of essential amino acids – 0.89±0,04 mg/g (5 free); 9.58±0,48 mg/g (5 bounded). Free amino acids can be distributed as follows – Arg > Tyr > Gln > Ala > Asn > Met > Ile > Val > Lys > Leu; and bounded – Arg > Gln > Lys > Tyr > Gly > Asn > Val > Thr > Ala > Ile > Nva > Leu > Met. From the presented data, we can conclude that arginine is the major amino acid in the infusion of coltsfoot leaves.

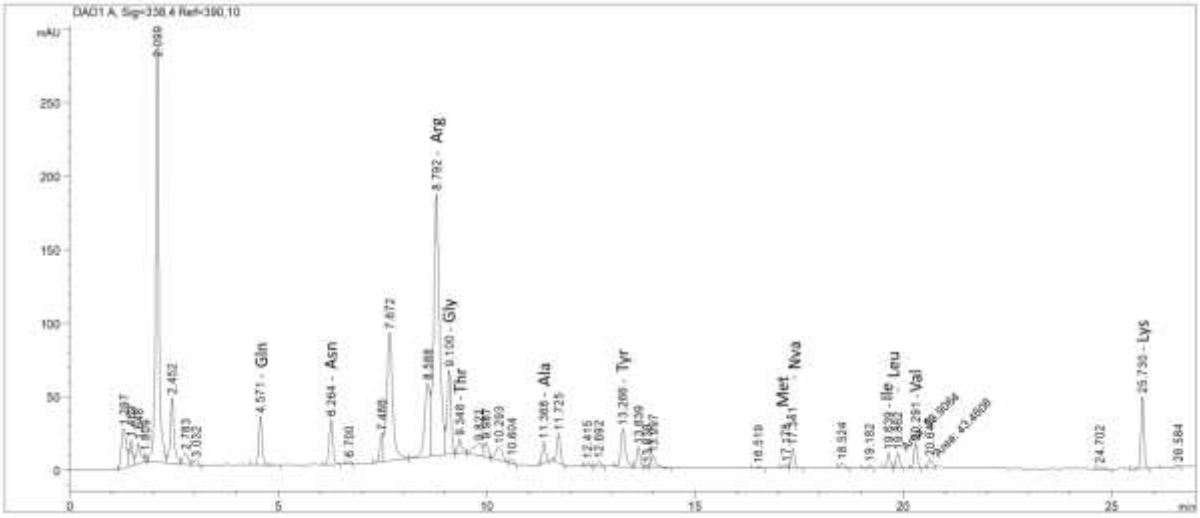

Figure 2: Chromatogram of amino acids profile of coltsfoot leaves infusion after hydrolysis.

Table 1: Amino acids content (mg/g) in coltsfoot leaves infusion

No	Amino acids	Three letter code	Content, mg/g	
			Free	Bounded
1.	Glutamine+	Gln	3,83±0,19	10,45±0,52
2.	Asparagine	Asn	0,92±0,05	2,45±0,12
3.	Arginine+	Arg	31,87±1,59	26,19±1,31
4.	Glycine+	Gly	–	2,98±0,15
5.	Threonine*	Thr	–	1,09±0,05
6.	Alanine	Ala	1,79±0,09	0,94±0,05
7.	Thyrosine+	Tyr	4,74±0,24	4,53±0,23
8.	Methionine*	Met	0,39±0,02	0,21±0,01
9.	Norvaline	Nva	–	0,67±0,03
10.	Isoleucine*	Ile	0,23±0,01	0,85±0,04
11.	Leucine*	Leu	0,06±0,003	0,64±0,03
12.	Valine*	Val	0,13±0,01	1,74±0,09
13.	Lysine*	Lys	0,076±0,004	5,05±0,25
Total content			44,04±2,20	57,79±2,89

*Essential amino acids
+Conditional amino acids

3.1. Prospects for use coltsfoot leaves reached in amino acids.

Infusion of coltsfoot leaves can serve as an additional source of essential and conditional amino acids during the period of illness and recovery. The presence of arginine in sufficient quantities is especially valuable. The physiological need of tissues and organs of most mammals for arginine is satisfied due to its endogenous synthesis and/or food intake. However, this amino acid becomes essential at a young age and for adults under stress or illness. Arginine is a necessary precursor for the synthesis of proteins and many biologically important molecules such as ornithine, proline, polyamines, creatine, and agmatine. However, the main role of arginine in the human body is to be a substrate (NO donor) for the synthesis of nitric oxide (NO).

NO plays an important role in the physiology of mammals, having a wide range of bioregulatory effects. NO catalyzes the formation of cyclic guanosine monophosphate (cGMP), which accounts for most of the physiological NO effects. However, other physiological effects of NO are now known, independent of the activation of guanylate cyclase or even NOS, including post-translational modification of proteins, lipids, and other biomolecules. Other potential targets for NO are soluble adenosine diphosphate (ADP)-ribosylating enzyme and transcription factors through which NO can directly affect gene transcription and mRNA translation. One of the physiological actions of NO is the regulation of immune processes (cell-mediated immunity, macrophage activity in the lung tissue, the effect of neutrophilic granulocytes on pathogenic microorganisms, non-specific immune defense) [16-20]. Arginine intake promotes a non-specific immunity with monocyte expansion and increased NO production [21]. These studies are necessary to update the Leaflet for medical use of the "Coltsfoot leaves" drug and standardization procedures [22].

CONCLUSION

Coltsfoot leaves are a pharmacopoeial medicinal plant material. In Russia, official OTC drugs are made on its basis; they are already widely used by the population. The infusion of leaves has an expectorant effect and is used in the treatment of the broncho-pulmonary system, due to the presence of the main group of biologically active substances – polysaccharides. Our study showed that the infusion of coltsfoot leaves also contains a significant amount of amino acids, the main of which is arginine. This amino acid has a number of positive effects on the immune system. Thus, further studies aimed at studying the therapeutic effects of infusion of coltsfoot leaves in the prevention and complex treatment of bronchopulmonary diseases of viral etiology, including the SARS-CoV-2 virus (COVID-19 infection), are very relevant.

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CONFLICTS OF INTEREST

None.

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