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Original Article

HPLC ANALYSIS OF AMINO ACIDS CONTENT IN *CRAMBE CORDIFOLIA* AND *CRAMBE KOKTEBELICA* LEAVES

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ABSTRACT

Objective: The aim of our study was to establish the content of some primary metabolites, such as amino acids in *Crambe cordifolia* and *Crambe koktebelica*. The lack of experimental data induced us to determine these compounds.

Methods: *Crambe cordifolia* and *Crambe koktebelica* leaves were selected as the objects of the study. The amino acids in the raw materials were determined by the HPLC method.

Results: The results of the research revealed that the leaves of *Crambe cordifolia* and *Crambe koktebelica* contain fifteen and sixteen free amino acids respectively. Among the free amino acids L-histidine was presented in *Crambe cordifolia* leaves in the greatest amount, its content was 12.19 µg/mg. The content of free L-arginine, L-valine, L-phenylalanine, L-isoleucine was the greatest in *Crambe koktebelica* leaves, it was 2.23 µg/mg, 2.04 µg/mg, 1.74 µg/mg, 1.50 µg/mg respectively. The content of bound L-glutamic acid, Glycine, L-arginine, L-leucine was the highest in *Crambe cordifolia* and *Crambe koktebelica* leaves.

Conclusion: The results of the study showed that *Crambe cordifolia* and *Crambe koktebelica* can be considered as a source of highly digestible amino acids that can be used to treat some diseases.

Keywords: *Crambe cordifolia*, *Crambe koktebelica*, Amino acids, HPLC, Leaves

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INTRODUCTION

Medicinal plants are the major natural resources found in the world, in which it contains more active phytoconstituents [1]. Nowadays, plants are recognized as a form of folk remedies due to their wide therapeutic potential and, in turn, minor side effects and good toleration with patients regardless of age [2–4]. The use for the benefit of mankind of medicinal plants (MP) in folk and scientific medicine has a centuries-old tradition [5, 6]. Medicinal plants play a main role in the development of traditional medicine, as well as actual pharmaceuticals [7-9]. Since 1981, 38 molecules have been derived from medicinal plants, out of which 1,130 new therapeutic agents have approved as pharmaceutical drugs [10]. Herbal remedies are widely used in the complex treatment of various diseases [11].

Crambe L. (*Brassicaceae*) is the genus of one flowering plants' group with almost 38 species of the Old World, and is the second largest member of the family *Brassiceae* [12]. These species are annual, biennial or perennial and have different uses, for example, as a source of biofuel (seeds contains up to 60% of erucic acid), as vegetables or forage plants, in the paint industry, as oilseed, in the food industry for confectionery, in the chemical industry [13, 14]. The genus *Crambe* L. has a wide area of spread that goes from Macaronesia to western China and northern India [15]. Species of *Crambe* genus are very promising in terms of their biochemical and biological properties [16]. A great amount of work has been done upon different species of *Crambe* in order to evaluate their industrial and pharmaceutical importance [17]. For example, *Crambe abissinica* is the most known species due to the content of oil and amino acids, protein, phenolic compounds [18]. Also, *Crambe abyssinica* seed oil contains erucic acid (similar to 59 % of C22:1) which is an important building material for the oleochemical industry [19]. *Crambe orientalis* can prevent seed germination and shows phytotoxic effects [20]. The other member of this genus, *Crambe cordifolia*, is used as livestock feed [17]. It includes a variety of chemical compounds including quercetin and glycosides of kaempferol [18, 21]. *Crambe cordifolia* is a rich source of polyphenol compounds with antioxidant activity [22]. Normally, it contains quercetin 3-feruloylglucoside-7, 4'-diglucoside and kaempferol 3-(p-coumaroyl) glucoside-7, 4'-diglucoside [17, 18, 23].

But there was a complete gap in the amino acid composition of *Crambe cordifolia*. Other species, in particular *Crambe koktebelica*, which is cultivated in Ukraine, has not been studied in chemical and pharmacological aspects. Thus, the aim of our study was to establish the content of some primary metabolites, such as amino acids in *Crambe cordifolia* and *Crambe koktebelica*. The lack in experimental data induced us to determine these compounds.

MATERIALS AND METHODS

Plant materials

Crambe cordifolia and Crambe koktebelica leaves were selected as the objects of the study. The raw material was provided by the Cultural Flora Department of M. M. Gryshko National Botanic Garden of the National Academy of Sciences of Ukraine. The leaves were collected in summer 2018. The raw material was authenticated by Prof. Dzhamal Rakhmetov. A vouchers specimens of Crambe cordifolia no. 253 and Crambe koktebelica no. 254 are kept at the Department of Pharmacognosy and Medical Botany, TNMU, Ternopil, Ukraine [24]. The study plant material was dried using the conventional method and stored in paper bags in a dry place [25, 26].

Chemicals and standards

Standards of amino acids, including L-glutamic acid (Glu), L-aspartic acid (Asp), L-methionine (Met), L-histidine (His), L-alanine (Ala), L-isoleucine (Ile), L-arginine (Arg), Glycine (Gly), L-valine (Val), L-tyrosine (Tyr), L-serine (Ser), L-cystine (Cys), L-phenylalanine (Phe), L-threonine (Thr), L-lysine (Lys), L-leucine (Leu), L-proline (Pro), obtained from Sigma (Sigma-Aldrich, St. Louis, MO, USA), were of analytical grade (>99 % purity) (fig. 1).

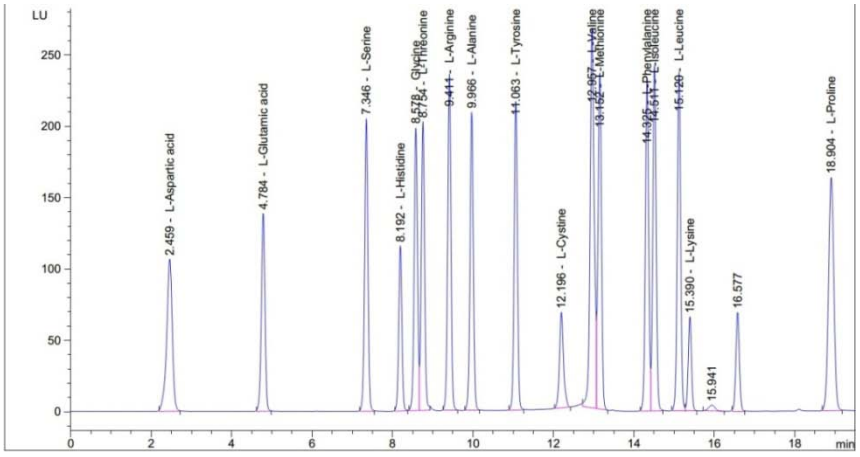

Fig. 1: HPLC chromatogram of amino acids standards

Derivatizing agent's o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) were purchased in Merck. Acetonitrile (ACN) and hydrochloric acid (HCl) were from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

HPLC determination of amino acids

The amino acids composition of *Crambe cordifolia* and *Crambe koktebelica* leaves are determined by HPLC method with a pre-column derivatization FMOC ana OPA.

Reference solutions of free amino acids have been made with distilled water at 0.03 M concentrations of each (weighed with analytical accuracy), stored in the refrigerator and further diluted before use, in every second day.

HPLC analysis of amino acids was conducted using Agilent 1200 (Agilent Technologies, USA). Samples were analyzed using a column length Zorbax AAA–150 mm, inner diameter–4.6 mm, the diameter of sorbent grain 3 μ (Hypersil ODS (prepared by BST, Budapest, Hungary)). Mobile phase A–40 mmol Na_2HPO_4, pH 7.8; mobile phase B–CH_3CN: CH_3OH: H_2O (45:45:10, v/v/v). Gradient separation regime with a constant mobile flow rate of 1.5 ml/min. The temperature of the thermostat column is 40 °C.

The pre-column derivatization was conducted with a help of automatic programmable regulations using OPA reagent and FMOC reagent. Identification of derivatized amino acids was done by a fluorescence detector [27-29]. For the extraction of free amino acids of powdered the raw material (to the 131 mg of *Crambe cordifolia*; to the 133 mg of *Crambe koktebelica*), put in a test flask, 0.1 mol/l water solution of hydrochloric acid was added. The extraction was performed in the ultrasonic water bath at 50 °C for 3 h. Extraction of bound and free amino acids was performed by adding 2 ml of a water solution of 6 M hydrochloric acid to the powdered of the raw material (to the 132 mg of *Crambe cordifolia*; to the 134 mg of *Crambe koktebelica*). Hydrolysis was conducted for 24 h in a thermostat at 110 °C.

0.5 ml of centrifuged extract was vaporized on a rotary evaporator and then rinse three times with purified water to eliminate hydrochloric acid. The product received was resuspended in 0.5 ml water and filtered through membrane filters from restored cellulose with pores of 0.2 μm. Before recording the samples into the chromatographic column in the automatic software mode, fluorescence derivative amino acids were obtained.

Identification of amino acids was performed according to their hold-up time (using standards as a reference) at 265 nm. The quantitative content of amino acids is calculated from the value of the peak area of the amino acids.

The number of amino acids in μg/mg was calculated according to the following equation:

$$X = \frac{C \times V}{m},$$

where: C – concentration, obtained from the chromatogram by calculating the reference solution and the test solution;

V – the volume of solvent for extraction;

m – is a mass of plant material [30, 31].

Table 1: The amino acid composition content of *Crambe cordifolia* and *Crambe koktebelica* leaves

Amino acid name	Amino acid content, μg/mg $x^- \pm \Delta x^-$, n=3, P<0.05			
	Crambe cordifolia		*Crambe koktebelica*	
	Free	Bound	Free	Bound
L-aspartic acid (Asp)	0.99±0.03	6.88±0.04	1.32±0.02	3.49±0.05
L-glutamic acid (Glu)	0.75±0.02	14.93±0.13	0.76±0.01	7.86±0.08
L-serine (Ser)	1.12±0.05	7.14±0.04	0.69±0.02	3.20±0.03
L-histidine (His)*	12.19±0.08	1.92±0.02	1.25±0.04	2.57±0.05
Glycine (Gly)	0	14.75±0.09	0.35±0.01	6.57±0.09
L-threonine (Thr)*	0.83±0.02	4.79±0.05	1.06±0.01	2.20±0.03
L-arginine (Arg)**	0.89±0.03	11.38±0.11	2.23±0.03	6.30±0.12
L-alanine (Ala)	2.16±0.04	8.94±0.07	1.15±0.02	3.42±0.05
L-tyrosine (Tyr)	0.79±0.01	3.89±0.06	1.01±0.01	2.04±0.02
L-valine (Val)*	1.28±0.05	5.60±0.10	2.04±0.04	1.87±0.03
L-methionine (Met)*	0.08±0.01	1.34±0.03	0.14±0.01	0.58±0.01
L-phenylalanine (Phe)*	1.08±0.02	5.40±0.11	1.74±0.03	1.89±0.02
L-isoleucine (Ile)*	1.28±0.04	4.88±0.08	1.50±0.02	2.16±0.04
L-leucine (Leu)*	1.13±0.03	10.73±0.12	0.96±0.01	5.08±0.08
L-lysine (Lys)**	0.96±0.01	3.93±0.09	1.13±0.05	2.63±0.02
L-proline (Pro)	1.51±0.02	0.15±0.01	1.30±0.03	0.10±0.01

Note: Values are mean±SEM (n=3), *essential amino acid; **semi-essential amino acid.

Statistical analysis

All analyzes were performed three times. The results were expressed as mean values and standard deviation. Values were determined using Statistica v 10.0 (StatSoft Inc.) program. The level of significance was set at *p<0.05 for all statistical analyses [32, 33].

RESULTS AND DISCUSSION

The amino acid composition of *Crambe cordifolia* and *Crambe koktebelica* leaves is in (table 1).

The HPLC method identified in *Crambe cordifolia* and *Crambe koktebelica* leaves fifteen and sixteen free amino acids respectively (fig. 2, 3).

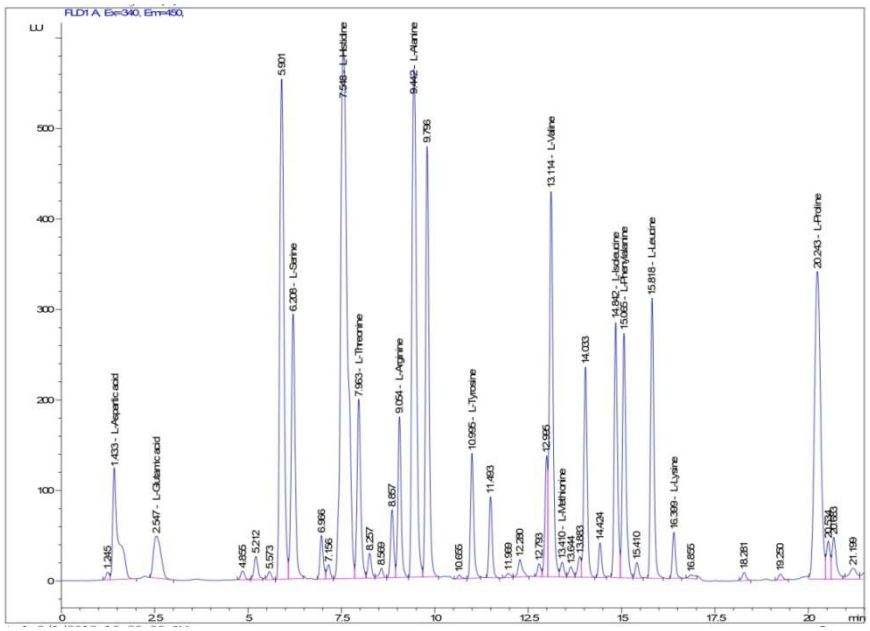

Fig. 2: HPLC chromatogram of *Crambe cordifolia* free amino acids

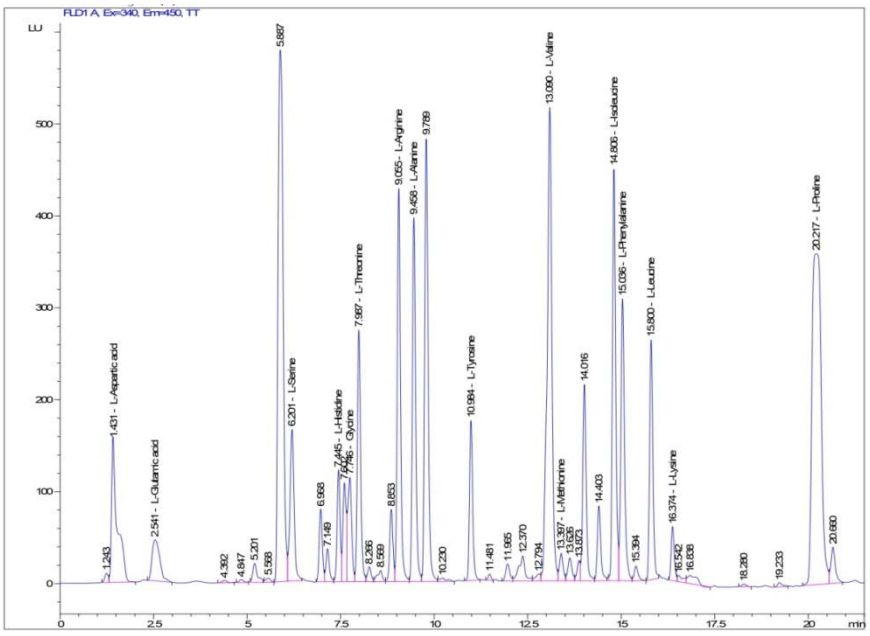

Fig. 3: HPLC chromatogram of *Crambe koktebelica* free amino acids

L-histidine was presented in *Crambe cordifolia* leaves in the greatest amount among the free amino acids, its content was 12.19 μg/mg. The amino acid L-histidine plays a key role in the cultivation and development of plants [34]. Histidine is an essential amino acid needed in infant nutrition [35]. In addition, this amino acid is a histamine precursor and is effective as a component of solutions used to protect the myocardium and preserve organs [36]. This acid is also a precursor to hormones such as thyrotropin-releasing and metabolites that affect gastric secretion, neurotransmission, renal function, and the immune system [37]. Histidine supplements are effective in plasma lipids, insulin resistance, and inflammatory markers, they delay the development of atherosclerosis in metabolic syndrome and diabetes [38].

The quantitative content of other amino acids was much smaller. Glycine was not found. The content of free L-arginine, L-valine, L-

phenylalanine, L-isoleucine was the highest in *Crambe koktebelica* leaves, which was 2.23μg/mg, 2.04 μg/mg, 1.74 μg/mg and 1.50 μg/mg respectively. L-valine maintains a branched-chain amino acid balance, while alanine is involved in hepatic autophagy, transamination, and gluconeogenesis [28].

L-arginine is a semi-essential amino acid that plays an important role in the cardiovascular system. It is the only substrate for the production of nitric oxide, from which L-arginine has an effect on the cardiovascular system [39]. It is of great importance as an intermediate in the synthesis of urea [40]. The amino acid L-arginine is used by the body in tissue recovery, protein synthesis, and immune cell function [41]. Also, it is extremely needed by young people [28].

L-phenylalanine is essential for humans and can be converted *in vivo* to tyrosine, which afterwards becomes converted into catecholamine neurotransmitters. Therefore, supplements with this amino acid have antidepressant action [35, 42]. L-phenylalanine is widely used in pharmaceutically active compounds, such as central nervous system neuropeptides, anti-inflammatory remedies, and HIV protease inhibitors [43]. In addition, it is used in food additives, nutraceuticals, aroma, and flavor enhancers, as a building material for medicines, dietary supplements, and ingredients in cosmetics. In particular, L-phenylalanine is used in the production of sweetener aspartame, which has a growing global demand [44].

The amino acid L-isoleucine is of interest as a dietary and nutritional supplement, as well as for enteral and parenteral protein nutrition. It affects the replenishment of the lack of proteins, amino acids, carbohydrates, has an antitoxic effect [45].

Sixteen bound amino acids were found in the raw material of studied species (fig. 4, 5). The quantitative content of all amino acids, except L-histidine, L-valine and L-proline, showed a tendency to increase significantly after hydrolysis.

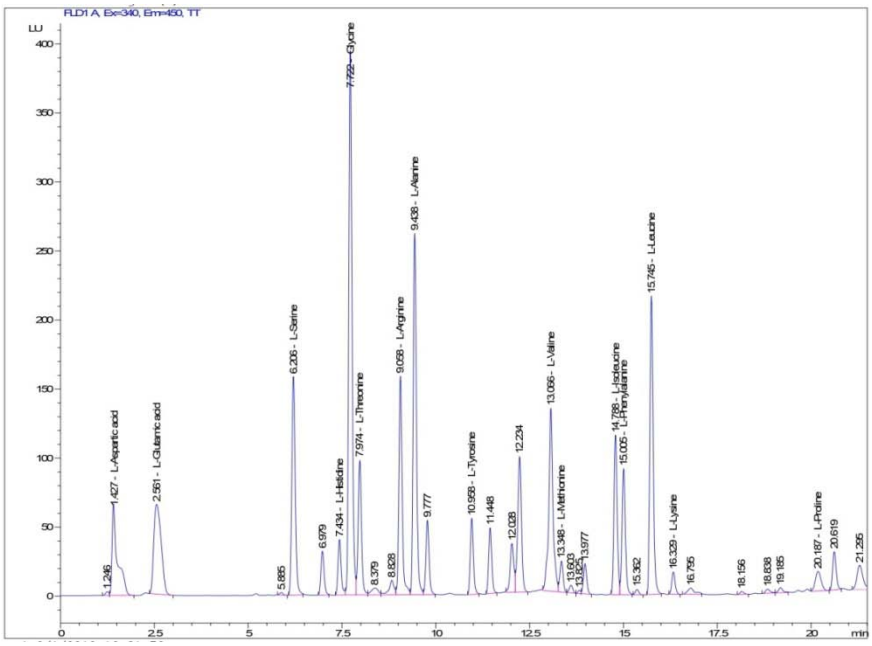

Fig. 4: HPLC chromatogram of *Crambe cordifolia* bound amino acids

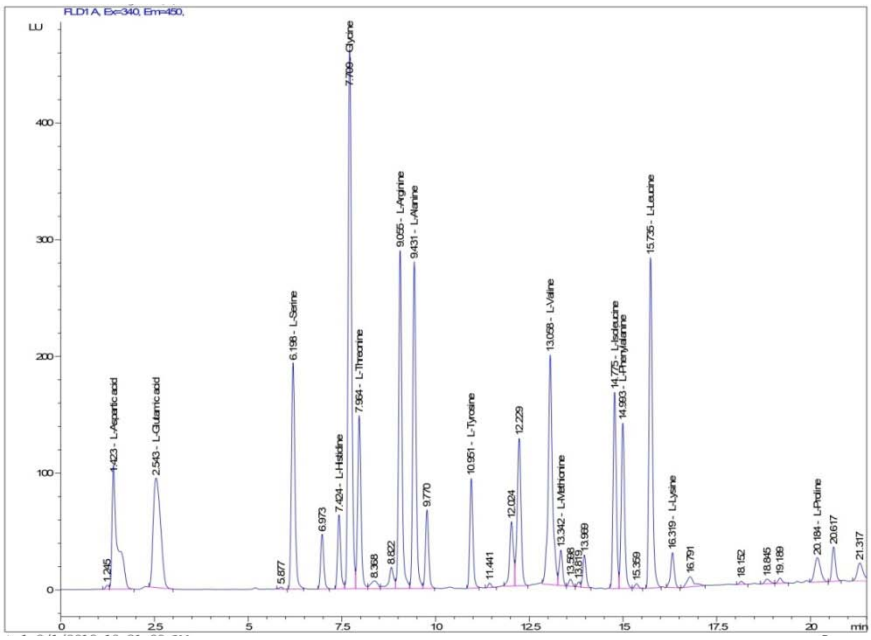

Fig. 5: HPLC chromatogram of *Crambe koktebelica* bound amino acids

The content of bound L-glutamic acid, Glycine, L-arginine, L-leucine in *Crambe cordifolia* and *Crambe koktebelica* leaves was the highest. Glutamic acid is a source of glucose and participates in a great amount of metabolic reactions. It holds the acidity, the normal blood glucose level and acts as a source of fuel for the intestinal epithelium. Glutamic acid conjugate is able to increase the effectiveness of antitumor drugs and reduce their toxicity to normal cells. Synthetic amides of L-glutamic acid are active against Ehrlich ascites carcinoma [31, 46]. Glycine is a component of glycocholic and bile acids. It is an essential substrate for the synthesis of several biologically important compounds and biomolecules, such as porphyrin, glucose, purine nucleotides, creatinine, and neurotransmitters. It is involved in detoxification reactions, protein synthesis of glutathione tripeptide. Glycine has an extensive spectrum of immunomodulatory, cytoprotective, and anti-inflammatory properties [47]. L-leucine collaborates to energy metabolism, that is fatty acid oxidation, glucose uptake, mitochondrial biogenesis [48]. This amino acid has a significant signaling role in adipose tissue and skeletal muscle, breast epithelial cells, and placental cells [31]. Nevertheless, Glycine was detected only in a bound form in *Crambe cordifolia* leaves.

CONCLUSION

This experiment used external standards and high-performance liquid chromatography to determine the amount of amino acids in *Crambe cordifolia* and *Crambe koktebelica* leaves. High concentrations of L-glutamic acid, Glycine, L-arginine, L-leucine were predominant in both types of analyzed plants. Concrete metabolic processes in which these amino acids participate may be related to the medicinal properties of plants as per their use in traditional medicine and, thus can alleviate the understanding of their helpful properties.

The specific metabolic processes, in which these amino acids are involved, may be related to the medicinal properties of plants according to their use in traditional medicine. Therefore, they may facilitate the understanding of their beneficial properties.

Thus, *Crambe cordifolia* and *Crambe koktebelica* can be considered as a source of highly digestible amino acids that can be used to treat some diseases.

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Nil

AUTHOR CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare no conflict of interest.

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