THE STUDY OF THE AMINO ACID COMPOSITION OF THE EXTRACTS OBTAINED FROM CHERRY LEAVES AND SHOOTS, LEAVES OF APRICOT AND PEACH

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The extracts have been obtained from the raw material of cultivated plants from Rosaceae family, and their pharmacological screening has been carried out. It has been found that the extract of cherry shoots shows the diuretic activity that is higher than the activity of the reference drug hypotiazide in the dose of 100 mg/kg, the extracts from cherry and apricot leaves possess a significant anti-inflammatory activity in the dose of 25 mg/kg, and the extract from peach leaves shows the immunotropic activity in the low concentration of 0.4 mg/ml detected in vitro. These extracts are promising for further study and creation of new medicines on their basis. The composition and content of free amino acids in the dry extracts obtained from leaves and shoots of cherry, leaves of apricot and the soft extract from peach leaves have been determined by HPLC. The presence of 21 amino acids has been identified; among them eight acids – threonine, valine, isoleucine, leucine, phenylalanine, histidine, lysine and arginine are essential. Proline, asparagine, \(\gamma\)-aminobutyric and glutamic acids, arginine and alanine prevail. All extracts are free of cystine and methionine, and only extracts from leaves of cherry and peach contain cysteine in small amount. The dry extract of cherry shoots possesses the highest amount of amino acids – 3154.2 mg/L, the lowest content is in the extract from apricot leaves – 605.9 mg/L.

In nature there are a great number of amino acids found in a free state, as well as components of proteins, peptides, enzyme systems, hormones, etc. They can form complexes with other compounds and thus affect their bioavailability and pharmacological effect. Amino acids, their derivatives and products of metabolism are widely used in medicine. For example, the use of arginine amino acid improves the condition of patients after a stroke, \(\gamma\)-aminobutyric acid relieves agitation and has a calming effect, it is used in the complex treatment of epilepsy and hypertension; asparagine is required for normal functioning of the nervous system [1, 4].

In recent years the extracts from apricot and peach leaves, cherry shoots and leaves were obtained at the Department of Chemistry of Natural Compounds of the National University of Pharmacy, and their pharmacological screening was carried out. It has been found that the extract of cherry shoots shows the diuretic activity that is higher than the activity of the reference drug hypotiazide in the dose of 100 mg/kg, the extracts from cherry and apricot leaves possess a significant anti-inflammatory activity in the dose of 25 mg/kg, and the extract from peach leaves shows the immunotropic activity in the low concentration of 0.4 mg/ml detected in vitro [5-10].

The substances obtained are promising for further study and creation of new medicines on their basis.

The aim of our research was a comparative study of the amino acid composition of the extracts obtained from leaves and shoots of cherry, leaves of apricot and peach.

Materials and Methods

The objects of our study were dry extracts from cherry leaves and shoots, apricot leaves and the soft extract from peach leaves. The extracts were obtained by exhaustive extraction with low concentrations of ethyl alcohol.

Determination of the composition and content of free amino acids in the extracts obtained was studied using an Agilent Technologies high performance liquid chromatograph (model 1100) equipped with a continuous-flow vacuum degasifier G1379A, a 4-channel pump of the low pressure gradient G13111A, an automatic injector G1313A, a column oven G13116A, a diode array detector G1316A, a vacuum degasifier G1379A, a 4-channel pump of the low pressure gradient G13111A, an automatic injector G1313A, a column oven G13116A, a diode array detector G1316A. The ZORBAX-XDB-C18 chromatographic column, 4.6×50 mm, filled with the octadecyl silyl sorbent with the grain size of 1.8 µm was used for analysis [2, 3].

Determination was carried out by the following procedure.

In a 10 ml vial place 0.3 g of the extract (accurate weight), and add 3 ml of 0.1 N hydrochloric acid aqueous solution containing 0.2% \(\beta\)-mercaptoethanol. Seal the vial and keep in an ultrasonic bath at 50°C for 2 h. Centrifuge the content of the vial and filter through a Teflon membrane filter with the pore size of 0.45 µm in a vial for analysis.

Take 100 µl of the filtrate into a vial placed in a vacuum desiccator at 40-45°C and the pressure of 1.5 mm Hg to remove hydrochloric acid completely. Then add 200 µl of 0.8 M borate buffer with pH 9.0 and 200 µl of 20 mM solution of 9-fluorenylmethoxycarbonyl chloride in acetonitrile in a vial for analysis consistently with the automatic dispenser and after 10 min exposure add 20 µl of 150 mM solution of amantadine hydrochloride in 50% aqueous acetonitrile to the reaction mixture.

Analysis was performed under the following conditions: the chromatographic linear gradient, the operating pressure of the eluent – 220-275 kPa, the thermostat
The amino acids composition of the extracts obtained from cherry leaves and shoots, apricot and peach leaves

| Amino acids                | Content, (mg/L) |
|----------------------------|-----------------|
|                            | Cherry leaves   | Cherry shoots | Peach leaves | Apricot leaves |
| Aspartic acid              | 34.9            | 19.8          | 55.4         | 18.9           |
| Glutamic acid              | 250.6           | 56.3          | 41.9         | 10.4           |
| 4-Hydroxyproline           | 20.5            | 30.1          | 20.6         | 15.8           |
| Asparagine                 | 105.5           | 138.9         | 195.5        | 114.0          |
| Glutamine                  | 87.3            | 41.3          | 7.6          | 13.1           |
| Serine                     | 33.0            | 30.2          | 33.9         | 14.9           |
| Arginine                   | 64.7            | 887.6         | 71.4         | 37.2           |
| Glycine                    | 27.2            | 47.3          | 14.4         | 13.0           |
| Threonine                  | 26.1            | 83.7          | 17.2         | 14.6           |
| Alanine                    | 83.8            | 178.1         | 169.1        | 36.7           |
| Proline                    | 361.2           | 880.7         | 1439.0       | 141.3          |
| γ-Aminobutyric acid        | 212.1           | 404.7         | 185.3        | 69.3           |
| Valine                     | 23.7            | 48.2          | 41.2         | 10.5           |
| Methionine                 | 0.0             | 0.0           | 0.0          | 0.0            |
| Isoleucine                 | 37.7            | 53.3          | 39.8         | 5.6            |
| Leucine                    | 14.1            | 61.9          | 21.2         | 9.2            |
| Phenylalanine              | 15.9            | 48.6          | 68.4         | 10.5           |
| Monooethanolamine          | 45.2            | 54.5          | 19.1         | 24.1           |
| Cystine                    | 0.0             | 0.0           | 0.0          | 0.0            |
| Histidine                  | 17.9            | 29.3          | 7.0          | 22.3           |
| Lysin                      | 7.4             | 53.2          | 10.0         | 15.6           |
| Cysteine                   | 5.4             | 0.0           | 1.9          | 0.0            |
| Tyrosine                   | 24.0            | 6.9           | 0.0          | 8.8            |
| Total                      | 1498.4          | 3154.2        | 2460.0       | 605.9          |

Fig. The chromatograms obtained when determining the content of free amino acids in the extracts from: A – cherry leaves, B – cherry shoots, C – peach leaves, D – apricot leaves.
ВИВЧЕННЯ АМИНОКИСЛОТНОГО СКЛАДУ ЕКСТРАКТІВ, ОТРИМАНИХ З ЛИСТЬЯ ТА ПАГОНІВ ВИШНІ, ЛИСТЬЯ АБРИКОСА ТА ПЕРСИКА
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Ключові слова: амінокислоти; вишня; персик; абрикос; ВЕРХ

Вивчено амінокислотний склад екстрактів, отриманих з листів та побігів вишні, листів абрикоса та персика. Екстракти вишні були оцінені за гіпотензивну активність в дозі 100 мг/кг, знижує кровяне тиску. Екстракти абрикоса і персика мали антиоксидантні та антотоксичні властивості. Методом ВЕРХ виявлено 15 амінокислот в екстрактах, серед яких вісім є незамінними – треонин, валін, ізолейцин, лейцин, фенілаланін, гістидин, лізин та аргінін. Превалюють пролін, аспарагін, γ-аміномасляна та глутамінова кислоти, аргінін, аланин.

ИЗУЧЕНИЕ АМИНОКИСЛОТНОГО СОСТАВА ЭКСТРАКТОВ, ПОЛУЧЕННЫХ ИЗ ЛИСТЬЕВ И ПОБЕГОВ ВИШНИ, ЛИСТЬЕВ АБРИКОСА И ПЕРСИКА
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Ключевые слова: аминокислоты; вишня; персик; абрикос; ВЭЖХ

Из сырыя культивируемых растений Rosaceae были получены экстракты и проведен фармакологический скрининг. Оказалось, что экстракт побегов вишни проявлял дуриетическую активность в дозе 100 мг/кг, что выше чем у препарата сравнения гипотензивный, противовоспалительная активность экстрактов из листьев вишни и абрикоса в дозе 25 мг/кг была значимой, экстракт из листьев персика имел иммунотропную активность, установленную in vitro в низкой концентрации 0,4 мг/мл. Полученные субстанции являются перспективными для дальнейшего изучения и создания на их основе новых лекарственных средств. Методом ВЭЖХ исследован состав и содержание свободных аминокислот в сухих экстрактах, полученных из листьев и побегов вишни, листьев абрикоса и в густом экстракте из листьев персика. Установлено наличие 21 аминокислот, среди которых восемь являются незаменимыми – треонин, вален, изолейцин, лейцин, фенилаланин, гистидин, лизин и аргинин. Превалируют пролин, аспарагин, γ-аминомасляная и глутаминовая кислоты, аргинин, аланин. Во всех экстрактах не обнаружены цистин и метионин, а цистеин содержался только в экстрактах из листьев вишни и персика в незначительном количестве. Самым высоким было содержание аминокислот в сухом экстракте из побегов вишни – 3154.2 мг/л, а наименьшим – в экстракте из листьев абрикоса – 605.9 мг/л.