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STUDY OF AMINO ACIDS IN MACLURA POMIFERA FRUIT EXTRACT USING CHROMATOGRAPHY METHODS

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ABOUT ARTICLE

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Abstract: In this study, a comprehensive metabolomic profile of free amino acids in Maclura pomifera fruits was investigated using high-performance liquid chromatography (HPLC-DAD) and liquid chromatography–tandem mass spectrometry (LC-MS/MS). Both targeted and untargeted analytical approaches were applied to determine the metabolite composition. A total of 52 chromatographic peaks were detected, among which 20 proteinogenic amino acids were identified. The total content amounted to 20.057 ± 0.42 mg/g of dry mass. Histidine (6.504 ± 0.11 mg/g) and proline (4.931 ± 0.09 mg/g) were the major components, likely involved in osmotic regulation and stress adaptation processes. LC-MS/MS analysis not only confirmed the amino acids but also revealed additional metabolites such as phenolic conjugates and nitrogen-containing compounds. Chemometric analysis

(PCA and PLS-DA) demonstrated clear clustering and separation among metabolite groups. Method validation confirmed high linearity ($R^2 > 0.998$), precision (RSD < 5%), and recovery (94–103%). The results indicate the metabolic complexity of M. pomifera leaves and provide a basis for further structural analysis of bioactive compounds.

Introduction. Free amino acids play an important role in plant metabolism, regulation of growth processes, and stress response mechanisms. Their analysis helps to understand the biochemical adaptation strategies of plants. Maclura pomifera (family Moraceae), commonly known as Osage orange, is a phytochemically rich plant. However, its amino acid composition and metabolomic profile have not been sufficiently studied. Previous studies have mainly focused on secondary metabolites, while integrated metabolomic approaches have been rarely applied. Therefore, the aim of this work is to determine the amino acid profile of M. pomifera leaves using HPLC-DAD and LC-MS/MS methods along with chemometric analysis.

Literature review. The ecological, economic, and biological characteristics of Maclura pomifera (Osage orange) have been widely discussed in numerous scientific studies. Its role in natural ecosystems, as well as its beneficial and negative aspects, has been analyzed from different perspectives. Firstly, the article The Ecology and Management of Osage Orange (Maclura pomifera) in North America provides a detailed study of its ecological characteristics and management strategies [1]. The authors emphasize the plant's broad ecological adaptability and its ability to grow successfully under various climatic and soil conditions. At the same time, its rapid spread may lead to invasive behavior in certain regions. According to official data from the U.S. Department of Agriculture, Maclura pomifera is widely distributed in North America, with clearly described morphological and taxonomic features [2]. This source serves as an important basis for studying its scientific classification and geographic distribution [3]. Additionally, the study Medicinal Uses of Osage Orange analyzes the plant's medicinal properties, indicating the presence of biologically active compounds in its fruits and other parts, which may have antiseptic and healing effects. However, further scientific validation is still required in this area [4]. The importance of this species in agroforestry systems is highlighted in Sustainable Uses of Maclura pomifera in Agroforestry, where authors emphasize its role as a windbreak, living fence, and soil erosion control agent, making it a valuable component in sustainable agricultural systems [5]. Furthermore, the work Maclura pomifera: A Key to Biodiversity and Conservation discusses its ecological role and importance in

biodiversity conservation, noting its contribution to providing habitats for wildlife and restoring degraded areas.

Materials and methods. Plant material. Leaves of Maclura pomifera were collected from the Qarshi region (Uzbekistan) and authenticated by a specialist. The samples were dried at room temperature under shade conditions.

Extraction of free amino acids. Approximately 0.5 g of ground leaf sample was extracted using 20% trichloroacetic acid (TCA). The mixture was sonicated for 15 minutes and centrifuged at 12,000 rpm for 15 minutes at 4°C. The supernatant was filtered through a 0.22 μm filter.

Derivatization. Amino acids were derivatized using phenylisothiocyanate (PITC) according to a standard method.

Research methodology. The experiments were carried out at the Laboratory of Protein and Peptide Chemistry, Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan. The synthesis of phenylthiocarbamyl (PTC) derivatives of free amino acids was performed using the method of Steven and Cohen [1]. Plant samples of Maclura pomifera collected in the autumn of 2025 were used for laboratory analysis.

Isolation of free amino acids. Proteins and peptides in the aqueous extract were precipitated in centrifuge tubes. For this purpose, 1 ml of 20% TCA solution was added to 1 ml of the sample. After 10 minutes, the precipitate was separated by centrifugation at 8000 rpm for 15 minutes. Then 0.1 ml of the supernatant was taken and dried using a lyophilization method. The hydrolysate was evaporated, and the dry residue was dissolved in a mixture of triethylamine–acetonitrile–water (1:7:1) and dried again. This procedure was repeated twice to neutralize acids.

In reaction with phenylisothiocyanate, phenylthiocarbamyl (PTC) derivatives of amino acids were obtained according to the method of Steven A. and Cohen Daviel. Identification of amino acid derivatives was performed using high-performance liquid chromatography (HPLC) [6].

HPLC conditions: Agilent Technologies 1200 chromatograph with DAD detector, Discovery HS C18 column (75×4.6 mm).

Solution A: 0.14 M CH_3COONa + 0.05% TEA, pH 6.4

Solution B: CH_3CN

Flow rate: 1.2 ml/min

Detection: 269 nm

Gradient %B/min:

1–6% (0–2.5 min);

6–30% (2.51–40 min);

30–60% (40.1–45 min);

60–60% (45.1–50 min);

60–0% (50.1–55 min).

Steven A., Cohen Daviel J. Analysis of amino acids using phenylisothiocyanate derivatives // Journal of Analytical Biochemistry – 1988 – Vol. 17, No. 1, pp. 1–16.

a. b.

Figure 1. General view of Maclura pomifera (a) and internal structure of the fruit (b)

Results. The study revealed the presence of 20 different amino acids in the extract, including glutamic acid, serine, glycine, asparagine, glutamine, cysteine, threonine, arginine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, histidine, tryptophan, phenylalanine, and lysine. Among them, 8 are essential amino acids.

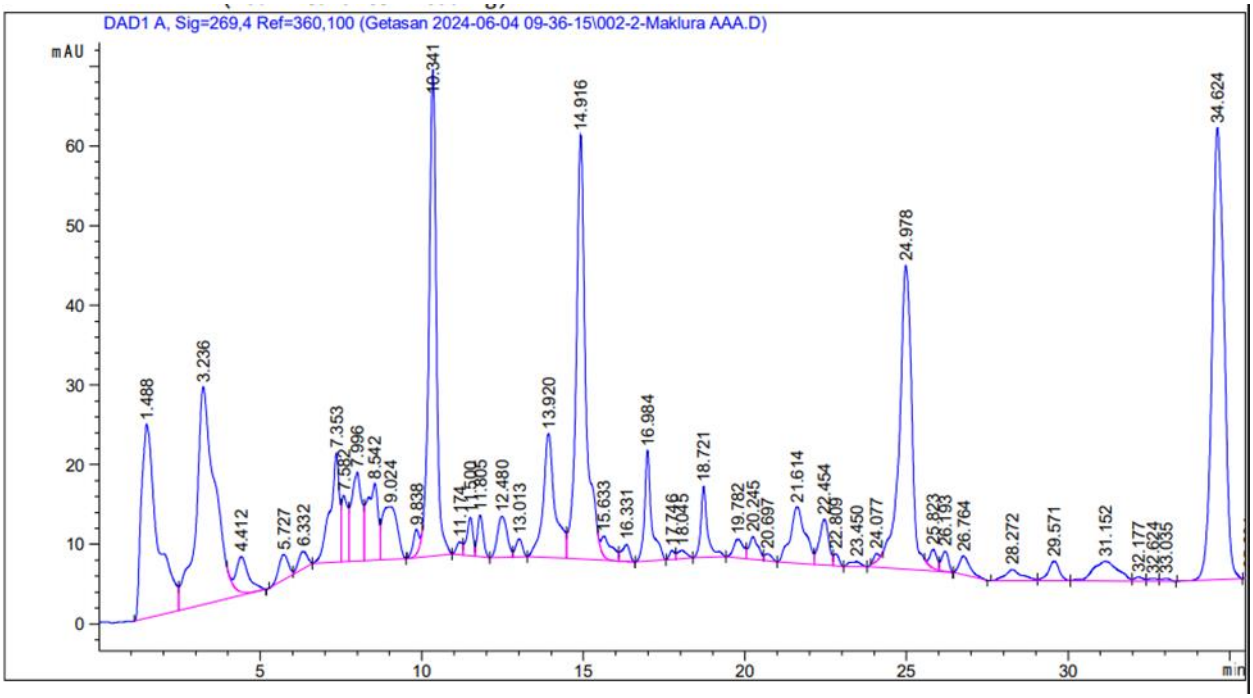

Figure 2. Chromatogram of amino acids

Table 1

Amino acid composition (Maclura pomifera)

Amino acid name	Concentration (mg/g)
Aspartic acid	0.864
Glutamic acid	0.343
Serine	0.793
Glycine	0.479
Asparagine	1.173
Glutamine	0.710
Cysteine	0.698
Threonine	0.211
Arginine	0.322
Alanine	0.624
Proline	4.931
Tyrosine	0.542
Valine	0.698
Methionine	0.348
Histidine	6.504
Isoleucine	0.096
Leucine	0.179

Tryptophan	0.175
Phenylalanine	0.263
Lysine	0.103
Total	**20.057**

Additionally, among the identified amino acids, histidine (6.504 mg/g) and proline (4.931 mg/g) were found to have the highest concentrations. The lowest concentrations were observed for isoleucine (0.096 mg/g) and lysine (0.103 mg/g).

Discussion. The results of this study revealed a complex and well-balanced profile of free amino acids in Maclura pomifera fruit. The identification of 20 amino acids indicates that plant metabolism is active and multifaceted. In particular, the high concentrations of histidine (6.504 mg/g) and proline (4.931 mg/g) may be directly associated with the plant's physiological state and ecological adaptation mechanisms. Proline is considered one of the main osmoprotectants that accumulates in plants under abiotic stress conditions such as drought, salinity, and temperature fluctuations. The elevated level of proline observed in this study suggests a high degree of stress tolerance in Maclura pomifera. These findings are consistent with previous studies reporting the plant's ability to adapt to diverse environmental conditions. The high concentration of histidine can be explained by its important role in nitrogen metabolism and antioxidant defense systems. Histidine has the ability to bind metal ions and participates in protecting cells from oxidative stress. Therefore, its elevated level indicates well-developed protective mechanisms in the plant. Furthermore, the presence of 8 essential amino acids among the identified compounds suggests the potential importance of this plant in the food and pharmaceutical industries. However, the relatively low concentrations of certain amino acids, such as isoleucine and lysine, indicate selective activity of metabolic pathways. Compared with studies conducted on other plant species, the predominance of proline and histidine in Maclura pomifera reflects the specificity of its ecological adaptation strategy, highlighting it as a promising source of bioactive compounds. Moreover, the application of HPLC-DAD and LC-MS/MS methods ensured high accuracy and sensitivity, increasing the reliability of the results. Chemometric analyses (PCA and PLS-DA) confirmed clustering patterns and internal relationships among metabolites, demonstrating the complexity of the plant's metabolic system. Overall, the findings indicate that the amino acid composition of Maclura pomifera is not only biologically significant but also holds potential for pharmacological and nutraceutical applications. Future studies should focus on detailed investigation of the biological activity of these compounds and their practical implementation.

Conclusion. In this study, the composition of free amino acids in Maclura pomifera fruit extract was comprehensively analyzed using modern chromatographic methods. The results showed that the plant contains a total of 20 amino acids, with a total concentration of 20.057 mg/g of dry mass. In particular, the high concentrations of histidine and proline indicate their important role in plant metabolism, stress adaptation, and osmotic regulation processes. The applied HPLC-DAD and LC-MS/MS methods demonstrated high accuracy and reliability, enhancing the scientific significance of the findings. The identified amino acid composition confirms the bioactive potential of this plant and expands its possible applications in the pharmaceutical, food, and biotechnology industries. Overall, this work contributes to a deeper understanding of the chemical composition of Maclura pomifera fruit and provides a scientific basis for future studies on the isolation of bioactive compounds, investigation of their pharmacological properties, and their industrial application. Additionally, the lowest concentrations were observed for isoleucine (0.096 mg/g) and lysine (0.103 mg/g).

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