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UPLC-ESI-MS METHOD APPLIED TO CHARACTERIZATION OF TWO MAJOR SAPONINS IN *ALLOCHRUSA GYPSOPHILOIDES*

ПРИМЕНЕНИЕ УЭЖХ-ИЭР-МС МЕТОДА ДЛЯ ХАРАКТЕРИСТИКИ ДВУХ ОСНОВНЫХ САПОНИНОВ *ALLOCHRUSA GYPSOPHILOIDES*

*ALLOCHRUSA GYPSOPHILOIDES*НИНГ ТАРКИБИДАГИ ИККИТА АСОСИЙ САПОНИННИ УССХ-ЭРИ-МС УСЛУБИНИ ҚЎЛЛАШ ОРҚАЛИ ТАВСИФЛАШ

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Аннотация

*Туркистон совун ўти (маҳаллий номи "бих") ёки Allochrusa gypsophiloides Ўрта Осиёда кенг тарқалган эндемик таркибида сапонин сақловчи ўсимликдир. Ультра самарадор суюқлик хроматография –электроспрей ионизацион масс-спектрометрияси (УССХ-ЭРИ-МС) A. Gypso-philoides илдизи таркибидаги моддаларни таҳлил қилишда қўлланилди. УССХ-ЭРИ-МС тадқиқоти натижасида ўсимликнинг метанолли экстрактида 2 та асосий тритерпен сапонини: гипсогенин (**A**) ва квиллаик кислота (**B**) ларнинг бисдесмозидлари аниқланди ва тавсифланди.*

Аннотация

*Туркестанский мыльный корень (местное название "бих") или Allochrusa gypsophiloides широко известное эндемичное сапониноносное растение Средней Азии. Комбинированный метод – ультраэффективная жидкостная хроматография-электрораспылительная ионизационная-масс-спектрометрия (УЭЖХ-ИЭР-МС) – применён для анализа компонентов корней A. gypsophiloides. В результате УЭЖХ-ИЭР-МС исследований метанольного экстракта растения были обнаружены 2 основные тритерпеновые сапонины, которые идентифицированы как бисдесмозиды гипсогенина (**A**) и квиллаевой кислоты (**B**).*

Annotation

*Turkestan soap root (local name Beh, Etmak), or Allochrusa gypsophiloides is the best known endemic saponin-bearing plant of Central Asia. Ultrahigh performance liquid chromatography-electrospray ionization-mass spectrometry (UPLC-ESI-MS) was applied to the analysis of the compounds of the roots of A. gypsophiloides. Through UPLC-ESI-MS studies on the plant methanol extract 2 major triterpene saponins were detected and identified as bisdesmosides of gypsogenin (**A**) and quillaic acid (**B**).*

Таянч сўз ва иборалар: *Allochrusa gypsophiloides, УССХ-ЭРИ-МС, сапонин, тритерпен гликозидлари.*
Ключевые слова и выражения: *Allochrusa gypsophiloides, УЭЖХ-ИЭР-МС, сапонин, тритерпеновые гликозиды.*
Keywords and expressions: *Allochrusa gypsophiloides, UPLC-ESI-MS, saponin, triterpene glycosides.*

Introduction

Since ancient times, plants have been utilised by humans as a fundamental resource for food, spices, medicines, and other applications. Many of the plants that were discovered by our ancestors are still in use until today. Turkestan soap root (local name Beh, Etmak) or *Allochrusa gypsophiloides* (Regel) Ovcz. et Czuk. (syn. *Acanthophyllum gypsophiloides* Rgl. from Caryophyllaceae family), is the best known endemic saponin-bearing plant of Central Asia. This plant is distributed in Kazakhstan, Kyrgyzstan, Tajikistan, and Uzbekistan[1, 32]. Roots of *A. gypsophiloides* contain up to 30 % saponins with a hemolytic index of 1:1000 or 1:2860 and

aboveground parts of the plant produce saponins with an index of 1:240. Many plant metabolites such as polysaccharides, triterpene glycosides have been isolated from underground parts of *A. gypsophiloides*[2, 524; 3, 770; 4, 150]. Acanthophyllosides B, C, and D were isolated from the methanol extract of the roots of *A. gypsophiloides*[4, 150].

A. gypsophiloides is widely used in traditional medicine and different branches of industry. In traditional medicine the decoction of the roots of this plant has been used as an expectorant for bronchitis, similar to other drugs with saponins. An infusion of the roots is further used as a choleretic, diuretic, and laxative. The root is brewed in a tea and drunk to treat gastrointestinal, skin, and venereal diseases, spleen, liver and kidney disorders, as well as metabolic dysfunction. An infusion of the aboveground parts is used as an expectorant and laxative. Pure saponins from this species are employed in veterinary medicine to prepare vaccines against anthrax and brucellosis. Treatment with saponins antagonized the narcotic effect of chloral hydrate, potentiated the convulsive effect of strychnine, decreased the convulsive and toxic effect of corazole, and increased diuresis in mice. The *A. gypsophiloides*saponins are utilized in manufacturing sweets or natural washing aids[1, 32].

Although, there are some reports about glycosides (triterpene saponins) in this plant[3, 770; 4, 150].UPLC-ESI-MSinvestigations have not yet been conducted on *A. gypsophiloides*. Therefore, the objectives of this study were to study major saponins of the methanol extract of *A. gypsophiloides*using UPLC-ESI-MS.Herein, we report the two known saponins in the methanol extract from*A. gypsophiloides* roots.

Results and discussion

Mass spectrometry, particularly MS/MS provides high sensitivity and selectivity, even for complex biological matrices, e.g. saponin-containing extracts comprising a number of target analytes of analogous structures [5, 93]. Roots from *A. gypsophiloides* have been shown to accumulate triterpenoid saponins [3, 764; 4, 150]. They belong to the group of glucuronide quillaic- and gypsogenin type triterpenoid carboxylic acid 3,28-O-bidesmosidessaponins. Compounds from this group have a glucuronic acid moiety at C-3 hydroxyl of the aglycone and are among the highest glycosylated bidesmosides [6, 568]. Negative ion ESI-MS of saponins offers high sensitivity and more diagnostic fragment ions compared to positive ion MS.

This investigation was designed to characterize the major compounds by electrospray (ESI) and LC-MS/MS on a Q-TOF mass spectrometer. In this study, the total ion chromatogram (TIC) of the of the methanol extract of *A. gypsophiloides* roots showed four major peaks (Figure 1). The chromatographic peaks **A** and **B** of *A. gypsophiloides* were identified by detailed analyses of their MS and MS/MS data, and by comparison with literature data.

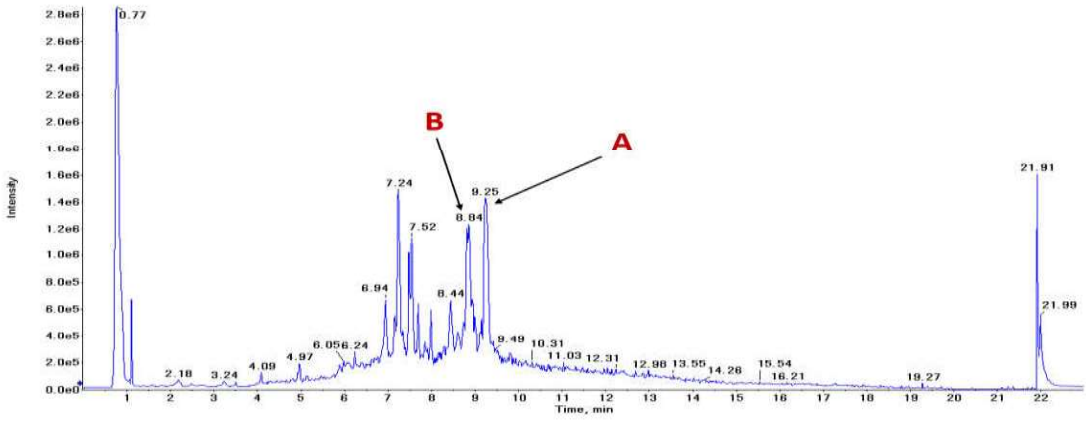

Figure 1. LC-ESI-MS total ion chromatogram (TIC) of the methanol extract of *A.gypsophiloides*roots showing major peaks **A** and **B** detected in the negative ion mode

КИМЁ

Our UPLC-ESI-MS examination of the methanol extract of *A. gypsophiloides* and comparison of the data with literature reports indicate that peak **A** with Rt (retention time) of 9.25 min (Figure 1) was assigned to 3-O-[β-D-galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucurono pyranosyl] gypsogenin 28-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[6-deoxy-β-D-gluco pyranosyl-(1→4)]-β-D-fucopyranosyl ester with an $[M-H]^-$ ion at m/z 1641.7177 (Figure 2).

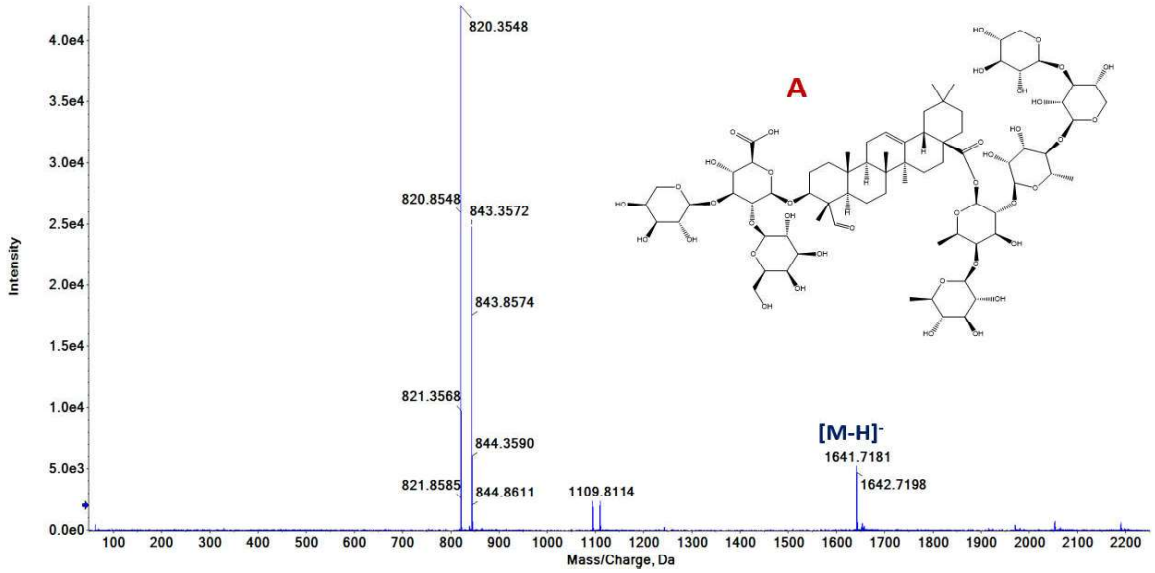

Figure 2. LC-ESI-MS of peak **A** obtained in the negative ion mode

Peak **B** with Rt of 8.84 min (Figure 1 and 3) with an $[M-H]^-$ ion at m/z 1657.7127 was suggested to be 3-O-[β-D-galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucurono pyranosyl] quillaic acid 28-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[6-deoxy-β-D-glucopyranosyl-(1→4)]-β-D-fucopyranosyl ester. The HR-ESI-MS spectra of the compounds **A** and **B** were measured previously in positive mode [3, 771].

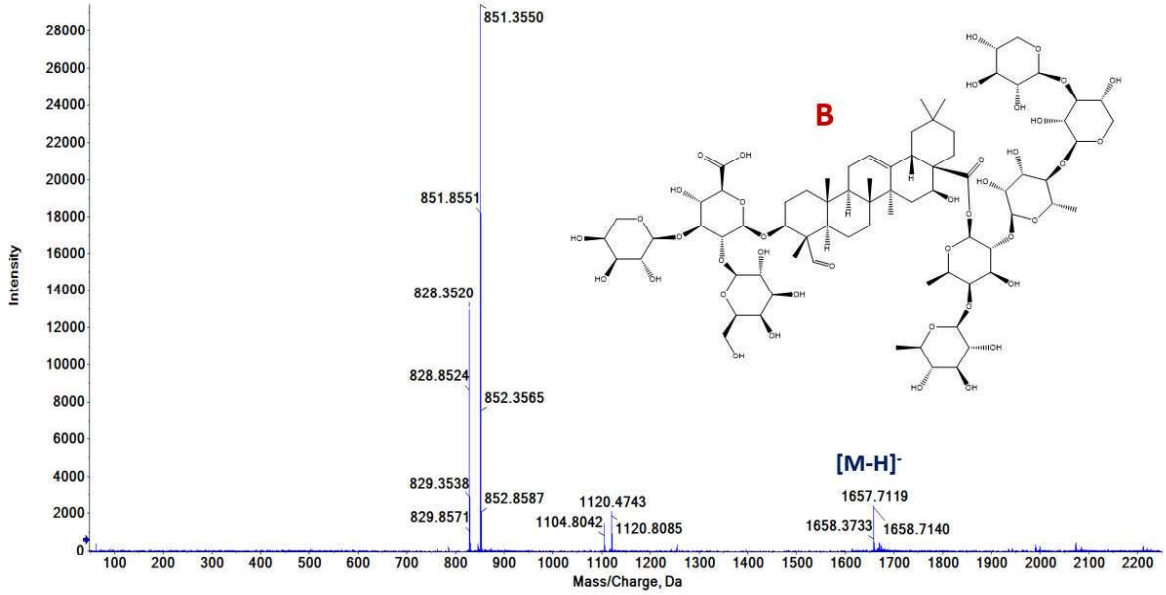

Figure 3. LC-ESI-MS of peak **B** obtained in the negative ion mode

EXPERIMENTAL

Chemicals

The methanol used for sample preparation was purchased from Merck (LC-grade; Darmstadt,Germany). HPLC grade acetonitrile (Merck), ultrapure water (Milli-Q system; Millipore, Bedford,MA, USA) and formic acid (Merck) were used for mobile phase preparation in the LC-ESI-MSanalysis. All other reagents used in this study were of analytical or HPLC grade.

Plant material

The roots of *A. gypsophiloides*were collected from Tashkent regions of Uzbekistan. Voucher specimens (QDPI 20192051) were identified by Dr. R.N. Muminova and deposited at the Department of Botany (Kokand State Pedagogical Institute, Uzbekistan).

Sample preparation for LC-ESI-MS study

Dried root material (10 mg) was extracted with methanol (5 ml) using sonication for 15 min at room temperature. Theextract was filtered through a 0.45 mm membrane filter (Millipore). A 10 µl sample of the extract wasinjected onto the analytical column for analysis.

LC-ESI-MSanalysis

UPLC-ESI-MS was performed using UPLC-TripleTOF mass spectrometer with an Acquity UPLC System equipped with Nucleoshell RP 18 column (150×2.0 mm^2, particle size 2.7 µm; Macherey Nagel) was used in this measurement with the elution binary gradient. The mobile phase consisted of water containing 0.3 mM ammonium formate acid (A) and acetonitrile (B) at a flow rate of 0.4 ml min^{-1}. The mobile phase was prepared daily, filtered through a 0.45 mm membrane filter (Millipore), and sonicated before use. The samples were measured in the negative mode.

***3-O-[β-D-Galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucurono pyranosyl] gypsogenin 28-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[6-deoxy-β-D-glucopyranosyl-(1→4)]-β-D-fucopyranosyl ester*(A).** $C_{75}H_{118}O_{39}$, Mr = 1642.73 g/mol. HR-ESI-MS: for [M-H]- found 1641.7177, calc. 1641.7172.

***3-O-[β-D-Galactopyranosyl-(1→2)-[α-L-arabinopyranosyl-(1→3)]-β-D-glucurono pyranosyl] quillaic acid 28-β-D-xylopyranosyl-(1→3)-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-[6-deoxy-β-D-glucopyranosyl-(1→4)]-β-D-fucopyranosyl ester* (B).** $C_{75}H_{118}O_{40}$, Mr = 1658.72 g/mol. HR-ESI-MS: for [M-H]- found 1657.7127, calc. 1657.7121.

Conclusion

This is the first study to examine the chemical composition of *A. gypsophiloides* roots, determined by ultra-high performance liquid chromatography-electrospray ionization-mass spectrometry (UPLC-ESI-MS). The combination of higher selective UPLC with HR-MS detection enlarges the capabilities for recognition of saponins in plants. On the basis of exact masses and comparison with literatures, two saponins were identified in the *A. gypsophiloides* roots.

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