ISNN:2181-1296

SHAROF RASHIDOV NOMIDAGI
SAMARQAND DAVLAT UNIVERSITETI

САМАРКАНДСКИЙ ГОСУДАРСТВЕННЫЙ
УНИВЕРСИТЕТ ИМЕНИ ШАРОФА РАШИДОВА

SAMARKAND STATE UNIVERSITY NAMED
AFTER SHAROF RASHIDOV

ILMIY AXBOROTNOMA

НАУЧНЫЙ ВЕСТНИК
SCIENTIFIC JOURNAL

ANIQ VA TABIIY FANLAR SERIYASI

Matematika, Mexanika, Informatika
Fizika, Kimyo, Biologiya, Geografiya

№ 1/2 (155)
2026

ISSN 2181-1296

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Samarqand viloyat matbuot boshqarmasida ro‘yxatdan o‘tish tartibi 09-25.
Jurnal 1999-yildan chop qilina boshlagan va OAK ro‘yxatiga kiritilgan.

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UDC: 547.945 https://doi.org/10.59251/2181-3973.2025.v1.138.1.3829

FLAVONOIDS OF AERIAL PARTS OF SOPHORA LEHMANNII AND CICER MOGOLTAVICUM

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Received: 20.01.2026
Revised: 30.01.2026
Accepted: 18.02.2026
Published: 30.03.2026

Publisher: Samarkand State University named after Sharof Rashidov.

Abstract. In order to search for new sources of biologically active substances, flavonoids from the aboveground parts of two plant species of the Fabaceae family, Sophora lehmanni and Cicer mogoltavicum, were studied. Compounds 1–5 were isolated from the methanol extract of the aboveground parts of Sophora lehmanni by column chromatography on silica gel, and compounds 1, 6–8 were isolated from the ethanol extract of Cicer mogoltavicum. Based on the study of UV, ^{1}H and ^{13}C NMR spectra, as well as HSQC and HMBC experiments, the isolated compounds were identified with luteolin (1), quercetin (2), cynaroside (3), isoquercetin (4), rutin (5), kaempferol (6), sissotrione (7) and kaempferol 3-O-β-D-glucuronide (8).

Keywords: Sophora lehmanni, Cicer mogoltavicum, Fabaceae, luteolin, quercetin, cynaroside, isoquercetin, rutin, kaempferol, sissotrin, kaempferol 3-O-β-D-glucuronide.

Sophora lehmanni va Cicer mogoltavikum o‘simliklari yer ustki qismilarining flavonoidlari

Annotatsiya. Biologik faol moddalarning yangi manbalarini izlash maqsadida Fabaceae oilasiga mansub ikki o‘simlik turi - Sophora lehmanni va Cicer mogoltavicum o‘simliklarining yer usti qismlarining flavonoidlari o‘rganildi. 1-5 birikmalar Sophora lehmanni o‘simligi yer usti qismlarining metanolli ekstraktidan silikagelidagi ustunli xromatografiya orqali, 1, 6-8 birikmalar esa Cicer mogoltavikum o‘simligining etanolli ekstraktidan ajratib olindi. UV, ^{1}H va ^{13}C NMR spektrlarini o'rganish, shuningdek, HSQC va HMBC tajribalari asosida ajratilgan birikmalar luteolin (1), kvertsetin (2), sinarozid (3), izokvertsetin (4), rutin (5), kaempferol (6), sissotrion (7) va kaempferol 3-O-β-D-glyukuronid (8) bilan aniqlandi.

Kalit so‘zlar: Sophora lehmanni, Cicer mogoltavicum Fabaceae, luteolin, quercetin, cynaroside, isoquercetin, rutin, kaempferol, sissotrin, kaempferol 3-O-b-D-glucuronide.

Флавоноиды надземных частей Sophora lehmanni и Cicer mogoltavicum

Аннотация. С целью поиска новых источников биологически активных веществ изучены флавоноиды надземных частей двух видов растений семейства Fabaceae: Sophora lehmanni и Cicer mogoltavicum. Из метанольного экстракта надземной части Sophora lehmanni методом колоночной хроматографии на силикагеле выделили соединения 1-5, из этанольного извлечения Cicer mogoltavicum - соединения 1, 6-8. На основании изучения данных УФ, ЯМР ^{1}H и ^{13}C спектров, а также экспериментов HSQC и HMBC

выделенные соединения идентифицировали с лютеолином (1), кверцетином (2), цинарозидом (3), изокверцитирином (4), рутином (5), кемпферолом (6), сиссотрином (7) и кемпферол 3-O-β-D-глюкуронидом (8).

Ключевые слова: Sophora lehmanni, Cicer mogoltavicum, Fabaceae, лютеолин, кверцетин, цинарозид, изокверцитирин, рутин, кемпферол, сиссотрин, кемпферол 3-O-β-D-глюкуронид.

Introduction. Flavonoids are extremely widespread in the plant kingdom. They exhibit capillary-strengthening, anti-inflammatory, anti-allergic, antitoxic, immunotropic, and a host of other actions, which have inspired the development of numerous medicinal preparations. Therefore, the search for plants containing flavonoids, the study of their chemical structure, and the development of methods for their production with the goal of creating new effective medicinal products and dietary supplements are pressing issues [1-3].

Fabaceae is one of the largest families of flowering plants, comprising approximately 800 genera and approximately 23000 species. It is the most important group of flowering plants in terms of practical significance for humans. Many of them are used in scientific and folk medicine as medicinal plants [4].

Sophora lehmanni (Bunge) Yakovlev is a subshrub with highly branched stems that form wide, low clumps. The plant is widely distributed in the Kyzylkum Desert on flat, finely hilly, fixed or semi-fixed sands and is a good sand stabilizer [5], containing quinolizidine alkaloids [4]. It has insecticidal properties, and root decoctions are used in folk medicine for rheumatism [4,6].

Cicer mogoltavicum (M.Pop.) A. Korov. is a perennial herbaceous plant with an erect, branched, glandular-pubescent stem. It grows on rocky and gravelly slopes in the lower mountain belt in the Tashkent and Syrdarya regions of Uzbekistan and the Chimkent and Dzhambyl regions of Kazakhstan [7].

To search for new sources of biologically active flavonoids, we studied the flavonoids of the aboveground parts of *Sophora lehmanni* and *Cicer mogoltavicum.* The aboveground part of *Sophora lehmanni* was collected during the flowering period in the Kanimekh district of the Navoi region near the village of Kokcha, the aboveground part of *Cicer mogoltavicum* was collected during the flowering period in the vicinity of the city of Angren in the Tashkent region.

Discussion of the obtained results. Compounds 1–5 were isolated from the ethyl acetate and n-butanol fractions of the methanol extract of the aerial parts of *Sophora lehmanni* by column chromatography on silica gel. Compounds 1, 6–8 were isolated from the ethyl acetate fraction of the ethanol extract of *Cicer mogoltavicum.* The isolated compounds were identified by studying their UV, ^{1}H, and ^{13}C NMR spectra, as well as HSQC and HMBC experiments, followed by comparison with those of literature data for these compounds.

Luteolin (1). mp 227–229 °C, UV spectrum (λ_{max}, EtOH, nm): 257, 265, 356; ^{1}H NMR spectrum (600 MHz, ДМСО-d_6+CCl_4, δ, ppm, J/Hz): 6.11 (1H, d, J=2.0, H-6), 6.34 (1H, d, J=2.0, H-8), 6.47 (1H, s, H-3), 6.85 (1H, d, J=8.3, H-5'), 7.29 (1H, dd, J=8.3, 2.1, H-6'), 7.32 (1H, d, J=2.1, H-2'), 9.13 (1H, s, 3'-OH), 9.48 (1H, s, 4'-OH), 10.41 (1H, s, 7-OH), 12.81 (1H, s, 5-OH). ^{13}C NMR spectrum (150 MHz, ДМСО-d_6+CCl_4, δ, ppm): 163.66 (C-2), 102.67 (C-3), 181.34 (C-4), 161.61 (C-5), 98.65 (C-6), 163.89 (C-7), 93.43 (C-8), 157.18 (C-9), 103.73 (C-10), 121.59 (C-1'), 113.05 (C-2'), 145.49 (C-3'), 149.33 (C-4'), 115.67 (C-5'), 118.27 (C-6').

Based on the study of the spectral data, it was identified with luteolin [8].

Quercetin (2). Yellow powder, mp 276-278 °C. ^{1}H NMR spectrum (600 MHz, ДМСО-d_6+CCl_4, δ, ppm, J/Hz): 12.45 (1H, s, 5-OH), 10.64 (1H, s, 7-OH), 9.60 (1H, s, 4′-OH), 9.28 (1H, s, 3-OH), 6.11 (1H, d, J=2.0, H-6), 6.33 (1H, d, J=2.0, H-8), 7.67 (1H, d, J=2.2, H-2′), 6.83 (1H, d, J=8.5, H-5′), 7.52 (1H, dd, J=8.5; 2.2, H-6′). ^{13}C NMR spectrum (150 MHz, ДМСО-d_6+CCl_4, δ, ppm): 146.37 (C-2), 135.59 (C-3), 175.56 (C-4), 160.76 (C-5), 97.97 (C-6), 163.73 (C-7), 92.99 (C-8), 156.06 (C-9), 102.91 (C-10), 121.97 (C-1′), 114.91 (C-2′), 144.68 (C-3′), 147.34 (C-4′), 115.20 (C-5′), 119.65 (C-6′) [9].

Cynaroside (3). Yellowish crystals with m.p. 256–259°C. ^{1}H NMR spectrum (600 MHz, Py-d_5, δ, ppm, J/Hz): 7.91 (1H, d, J=2.3, H-2'), 7.53 (1H, dd, J=8.3, 2.3, H-6'), 7.28 (1H, d, J=8.3, H-5'), 7.01 (1H, d, J=2.2, H-8), 6.96 (1H, s, H-3), 6.86 (1H, d, J=2.2, H-6), 5.85 (1H, d, J=7.7, H-1″), 4.59 (1H, dd, J= 12.2, 2.3, H-6″b), 4.45 (1H, m, H-3″), 4.43 (1H, m, H-6″a), 4.39 (1H, m, H-4″), 4.38 (1H, m, H-2″), 4.22 (1H, ddd, J= 9.5, 5.3, 2.3, H-5″). ^{13}C NMR spectrum (150 MHz, Py-d_5, δ, ppm): 165.75 (C-2), 104.57 (C-3), 183.29 (C-4), 163.03 (C-5), 101.06 (C-6), 164.43 (C-7), 95.73 (C-8), 158.33 (C-9), 107.03 (C-10), 123.14 (C-1′), 115.15 (C-2′), 148.29 (C-3′), 152.40 (C-4′), 117.32 (C-5′), 120.12 (C-6′), 102.23 (C-1″), 75.26 (C-2″), 78.94 (C-3″), 71.57 (C-4″), 79.71 (C-5″), 62.80 (C-6″) [10].

Isoquercitrin (4). Yellow powder, mp 236–238°C. ^{1}H NMR spectrum (600 MHz, ДМСО-d_6+CCl_4, δ, ppm, J/Hz): 12.47 (1H, s, 5-OH), 10.54 (1H, s, 7-OH), 9.36 (1H, s, 4′-OH), 9.05 (1H, s, 3′-OH), 7.67 (1H, d, *J*=2.2, H-2′), 7.50 (1H, dd, *J*=8.4, 2.2, H-6′), 6.83 (1H, d, *J*=8.4, H-5′), 6.34 (1H, d, *J*=2.0, H-8), 6.14 (1H, d, *J*=2.0, H-6), 5.28 (1H, d, *J*=7.7, H-1″), 5.19 (1H, br.s, OH), 4.93 (1H, br.s, OH), 4.81 (1H, br.s, OH), 3.86 (1H, br.s, OH), 3.57 (1H, dd, *J*=11.8, 2.6, H-6″a), 3.45 (1H, dd, *J*=11.8, 5.0, H-6″b), 3.30 (1H, dd, *J*=7.7, 9.0, H-2″), 3.26 (1H, dd, *J*=9.0, 8.7, H-4″), 3.22 (1H, dd, *J*=9.3, 8.7, H-4″), 3.10 (1H, ddd, *J*=9.3, 5.0, 2.6, H-6″). ^{13}C NMR spectrum (150 MHz, ДМСО-d_6+CCl_4, δ, ppm): 156.47 (C-2), 133.58 (C-3), 177.36 (C-4), 161.30 (C-5), 98.56 (C-6), 164.10 (C-7), 93.27 (C-8), 156.29 (C-9), 103.92 (C-10), 120.97 (C-1′), 116.38 (C-2′), 144.42 (C-3′), 148.28 (C-4′), 115.00 (C-5′), 121.11 (C-6′), 102.03 (C-1″), 73.88 (C-2″), 76.36 (C-3″), 69.45 (C-4″), 76.75 (C-5″), 60.83 (C-6″) [11].

Rutin (5). Yellow powder, mp. 189–191°C. ^{1}H NMR spectrum (600 MHz, ДМСО-d_6+CCl_4, δ, ppm, J/Hz): 12.44 (1H, s, 5-OH), 10.52 (1H, s, 7-OH), 9.34 (1H, s, 4′-OH), 8.91 (1H, s, 3′-OH), 7.57 (1H, d, *J*=2.2, H-2′), 7.54 (1H, dd, *J*=8.4, 2.2, H-6′), 6.83 (1H, d, *J*=8.4, H-5′), 6.35 (1H, d, *J*=2.1, H-8), 6.14 (1H, d, *J*=2.1, H-6), 5.28 (1H, d, *J*=7.5, H-1″), 4.41 (1H, d, *J*=1.5, H-1‴), 3.71 (1H, dd, *J*=11.6, 1.5, H-6″a), 3.49 (1H, m, H-2‴), 3.34 (1H, dd, *J*=11.6, 5.9, H-6″b), 3.39-2.96 (7H, m, glucose and rhamnose protons), 1.05 (3H, d, *J*=6.2, H-6‴). ^{13}C NMR spectrum (150 MHz, ДМСО-d_6+CCl_4, δ, ppm): 156.76 (C-2), 133.49 (C-3), 177.28 (C-4), 161.26 (C-5), 98.54 (C-6), 164.03 (C-7), 93.32 (C-8), 156.37 (C-9), 103.91 (C-10), 121.02 (C-1′), 116.27 (C-2′), 144.41 (C-3′), 148.19 (C-4′), 114.98 (C-5′), 121.44 (C-6′), 102.12 (C-1″), 73.83 (C-2″), 76.44 (C-3″), 69.39 (C-4″), 75.61 (C-5″), 66.49 (C-6″), 100.53 (C-1‴), 70.14 (C-2‴), 70.56 (C-3‴), 71.95 (C-4‴), 67.85 (C-5‴), 17.50 (C-6‴) [12].

Kaempferol (6). Yellow powder, mp. 305-306 °C. ^{1}H NMR spectrum (600 MHz, ДМСО-d_6+CCl_4, δ, ppm, J/Hz): 12.33 (1H, s, 5-OH), 10.35 (1H, s, 7-OH), 9.71 (1H, s, 4′-OH), 8.84 (1H, s, 3-OH), 6.11 (1H, d, J=2.0, H-6), 6.32 (1H, d, J=2.0, H-8), 8.02 (2H, d, J=9.0, H-2′,6′), 6.86 (2H, d, J=9.0, H-3′,5′). ^{13}C NMR spectrum (150 MHz, ДМСО-d_6+CCl_4, δ, ppm): 146.28 (C-2), 135.51 (C-3), 175.58 (C-4), 160.75 (C-5), 97.95 (C-6), 163.71 (C-7), 92.99 (C-8), 156.05 (C-9), 102.90 (C-10), 121.58 (C-1′), 129.03 (C-2′,6′), 115.00 (C-3′,5′), 158.95 (C-4′) [13].

Sissotrin (biochanin A 7-O-β-D-glucopyranoside, 7). M.p. 180-181°C, λ_{max} (EtOH) 264, 336 nm. ^{1}H NMR spectrum (400 MHz, Py-d_5, δ, ppm, J/Hz): 3,73 (3H, s, -OCH_3), 4,00-4,60 (6H, m, protons of glucose residue), 5,68 (1H, d, J=6,5, H-1′), 6,60 (1H, d, J=2,0, H-6), 6,67 (1H, d, J=2,0, H-8), 6,98 (2H, d, J=9,0, H-3',5'), 7,66 (2H, d, J=9,0, H-2',6'), 8,05 (1H, s, H-2), 13,43 м.д. (1H, br.s, 5-OH).

Acid hydrolysis of compound 7 yielded biochanin A (5,7-dihydroxy-4'-methoxyiso- flavone) with a mp of 213-214 °C and D-glucose. Spectral results and comparison with literature data allowed us to identify compound 7 with sissotrione (biochanin A 7-O-β-D-glucopyranoside) [14].

Kaempferol 3-O-β-D-glucuronide (8). mp 108-110 °C, λ_{max} (EtOH) 266, 282, 347 nm. The ^{1}H NMR spectrum of the compound contains proton signals characteristic of kaempferol and the glucuronide residue. ^{1}H NMR spectrum (400 MHz, ДМСО-d_6+CCl_4, δ, ppm, J/Hz): 3.32–3.78 (4H, m, protons of the carbohydrate part), 5.38 (1H, d, J=7.5, H-1′), 6.22 (1H, d, J=2.2, H-6), 6.42 (d, J=2.2, H-8), 6.89 (2H, d, J=8.8, H-3',5'), 8.10 (2H, d, J=8.8, H-2',6'), 12.62 (1H, s, 5-OH).

Acid hydrolysis of substance 8 yielded kepferol and D-glucuronic acid. Spectral results and comparison with literature data allowed us to identify substance 8 as kaempferol 3-O-β-D-glucuronide [15].

Cynaroside (3) has a hypoazotemic effect, and a medicinal product for the treatment of kidney diseases has been developed based on it at the Institute of Plant Chemistry of the Academy of Sciences of the Republic of Uzbekistan [16]. Rutin is widely used in medical practice as a capillary strengthening agent [17].

Experimental part. Thin-layer chromatography (TLC) was performed on Silufol UV-254 plates. Column chromatography was performed using KSK silica gel (100/200 μm, KSK from Tianjin Sinomed Pharmaceutical, China) and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden). For TLC of flavonoids, the following solvent systems were used: chloroform – methanol – acetic acid – water (9:3:0.5:0.5; 7:3:0.5:0.5). Phenolic compounds were detected in the chromatograms by their characteristic fluorescence under UV light at 254 nm or 365 nm, as well as after treatment with ammonia vapor, 1% aluminum chloride in alcohol, and 1% vanillin in 5% sulfuric acid in alcohol.

1D and 2D NMR spectra were recorded on a JNM-ECZ600R spectrometer (JEOL, Japan) at an operating frequency of 600 MHz for ^{1}H in Py-d_5 and DMSO-d_6+CCl_4 solutions (Cambridge Isotope Laboratories, Inc., USA). TMS (0 ppm) was used as an internal standard in the ^{1}H NMR spectra. The chemical shift of the solvent relative to TMS was used as an internal standard in the ^{13}C NMR spectra.

Isolation of components from the aerial parts of *Sophora lehmanni*. The crushed air-dried aboveground part of *Sophora lehmanni* (1.4 kg), collected during the flowering period (July 2023) in the Kanimekh district of the Navoi region [7], was extracted five times with methanol at room temperature. The combined extracts were concentrated in vacuum to 2.0 L and diluted with water to 4.0 L. The resulting solution was subjected to sequential liquid-liquid extraction with chloroform and ethyl acetate. After distillation of the solvents, 37.0 g of chloroform and 61.0 g of ethyl acetate fractions were obtained. A portion of the ethyl acetate fraction (35.0 g) was chromatographed on a silica gel column in a gradient solvent system of chloroform and methanol. When eluting the column with a chloroform-methanol mixture (98:2), 0.37 g of luteolin (1) was obtained, and upon further washing the column with the above-mentioned solvent mixture in a ratio of 94:6, 1.25 g of quercetin (2) was obtained. When eluting the column with a chloroform-methanol mixture (82:18), 2.36 g of cynaroside crystals (3) were isolated. Elution of the column with a chloroform-methanol mixture in a ratio of 8:2 resulted in the isolation of 0.58 g of isoquercitrin (4). When washing the column with a chloroform-methanol mixture (75:15), 3.02 g of rutin (5) were obtained.

Isolation of components from the aerial parts of *Cicer mogoltavicum.* The crushed air-dried aerial parts of *Cicer mogoltavicum* (1.12 kg), harvested during the flowering period (July 2023) in the vicinity of Angren, Tashkent region, Republic of Uzbekistan [7], were extracted five times with 96% ethanol at room temperature. The combined extracts were concentrated in vacuo to 1.5 l and diluted with water to 3.0 l. The resulting solution was subjected to sequential liquid-liquid extraction with chloroform and ethyl acetate. After distillation of the solvents, 30.1 g of chloroform and 42.3 g of ethyl acetate fractions were obtained. The ethyl acetate fraction was chromatographed on a silica gel column in a chloroform-methanol gradient system. Elution of the column with the mixture at a ratio of 98:2 yielded 0.37 g of luteolin (1). Further washing of the column with the above-mentioned solvent mixture at a ratio of 95:5 yielded 1.87 g of kaempferol (6). Washing with the system at a ratio of 9:1 yielded sissotrin (biochanin A 7-O-glucopyranoside, 7), and from the eluates at a ratio of 85:15, light yellow crystals of 1.25 g of kaempferol-3-O-glucuronide (8) were isolated.

Conclusions. Flavonoids from the aerial parts of two plant species of the Fabaceae family, *Sophora lehmann* and *Cicer mogoltavicum*, were studied. Luteolin, quercetin, cynaroside, isoquercetin, and rutin were isolated from *Sophora lehmann*, and luteolin, kaempferol, sissotrin, and kaempferol 3-O-β-D-glucuronide were isolated from *Cicer mogoltavicum.* The isolated flavonoids were identified based on spectral data and acid hydrolysis results.

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ISSN 2181-1296