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O'ZBEKİSTONDA O'SADIGAN MELILOTUS OFFICINALIS VA MELILOTUS ALBUSNING KIMYOVİY TARKIBI

**ХИМИЧЕСКИЙ СОСТАВ MELILOTUS OFFICINALIS И MELILOTUS ALBUS,
ПРОИЗРАСТАЮЩИХ В УЗБЕКИСТАНЕ**

**CHEMICAL COMPONENTS OF MELILOTUS OFFICINALIS AND MELILOTUS ALBUS,
GROWING IN UZBEKISTAN**

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Annotatsiya

Maqola Fabaceae oиласига мансуб Melilotus officinalis (L.) Pall (доривор qashqarbeda) hamda Melilotus albus о'simligining kimyoviy tarkibini оrganishga bag'ishlangan. Melilotus officinalisning yer ustki qismi 75%- linspirtili ekstraktining etilasetatli fraksiyalardan uchta fenol birikmalar ajratildi. UV, ¹H, ¹³C NMR, HSQC, va HMBC spektrlarini оrganish asosida digidrokumarin, melilot kislotasi, E-melilotozid ekanligi aniqlandi.

Melilotus albus о'simligining yer ustki qismi gidrodistillyatsiya yo'li bilan olinigan efir moyining tarkibiy qismi GC-MS orqali оrganildi. Efir moyining asosiy komponenti kumarin 97.92% ekanligi aniqlangan.

Аннотация

Статья посвящена исследованию химических компонентов надземной части лекарственного растения *Melilotus officinalis* (L.) Pall (донник лекарственный) и *Melilotus albus* семейства *Fabaceae*. Из различных фракций 75%-ного спиртового экстракта надземной части донника лекарственного выделены три фенольных соединения. На основании изучения спектров УФ, ¹H, ЯМР ¹³C, HSQC и HMBC выделенные вещества идентифицированы с дигидрокумарином, мелилотовиновой кислотой и Е-мелилотовизидом.

Методом ГХ-МС изучен компонентный состав эфирного масла воздушно-сухой надземной части донника белого, полученного методом гидродистилляции. Основным компонентом эфирного масла является кумарин, содержание которого составляет 97,92%.

Abstract

*The article is devoted to the study of the chemical components of the aboveground part of the medicinal plant *Melilotus officinalis* (L.) Pall (sweet clover) and *Melilotus albus* of the *Fabaceae* family. Three phenolic compounds were isolated from chloroform-ethyl acetate fractions of 75% alcohol extract of the above-ground part of *Melilotus officinalis*. Based on the study of UV, ¹H, ¹³C NMR, HSQC, and HMBC spectra, the isolated substances were identified with dihydrocoumarin, melilotic acid, and E-melilotoside.*

*The component composition of the essential oil of the air-dry aerial part of *Melilotus albus* obtained by hydrodistillation was studied by the GC-MS. The main component of the essential oil is coumarin, which content is 97.92%.*

Kalit so'zlar: *Melilotus officinalis, Melilotus albus, efir moy, digidrocoumarin, melilot kislota, melilotozid.*

Ключевые слова: *Melilotus officinalis, Melilotus albus, эфирное масло, дигидрокумарин, мелилотовиновая кислота, Е-мелилотовизид*

Key words. *Melilotus officinalis, Melilotus albus, essential oil, dihydrocoumarin, melilotic acid, melilotoside.*

INTRODUCTION

Melilotus officinalis (L.) Pall is a biennial herbaceous plant in the *Fabaceae* family that grows in Uzbekistan's Fergana, Namangan, Samarkand, and Kashkadarya regions [1, 2]. Extracts

from the sweet clover herb are used in scientific medicine as an anticonvulsant for angina pectoris, thrombosis of the coronary vessels; the herb is a part of herbal preparations used to treat rheumatism [2-5]. In folk medicine, sweet clover grass is used as an emollient, expectorant, and analgesic for inflammatory diseases of the respiratory organs, menopausal ailments, pain in the bladder and kidneys, migraine, purulent inflammation of the middle ear, headache, hypertension, atherosclerosis, psychosis, and externally with mastitis, articular rheumatism, and malignant tumors [2,5]. An infusion of the herb stimulates urination, improves blood circulation, helps with nervous tension, painful menstruation, insomnia, and heart palpitations, and is also used in the treatment of wounds, cuts and bruises.

The extract from the aerial part of the experiment has anti-exudative, anti-proliferative and anti-hypoxic properties; the anti-ischemic effect is manifested in ischemia of the brain and heart [4,6]. The extract is active against the influenza virus and has antioxidant activity. In animal experiments and clinical observations, it has been established that the coumarins contained in sweet clover have a depressing effect on the central nervous system and have anticonvulsant and narcotic effects. It is recognized that the main medicinal properties of sweet clover herb are due to the high content of coumarins [6-10], which, apparently, explains the effectiveness of the use of the plant in folk medicine for increased excitability, insomnia, convulsions, angina pectoris and coronary thrombosis [3-5].

A wide range of pharmacological activity is due to the content of a complex of biologically active substances in this plant. According to chemical studies, the herb *Melilotus officinalis* contains phenolcarboxylic acids, coumarins, essential oil, flavonoids (kaempferol glucosides, quercetin, luteolin, cynaroside), polysaccharides, protein, saponins, allantoin, monoterpenoids, sesquiterpenoids, triterpene compounds, amino acids, tannins, vitamins C and E, carotenoids, fatty acids, and other macro- and microelements [1,3, 6-9, 11-13].

In order to rationally use plant materials and search for biologically active substances, we are systematically studying the components of *Melilotus officinalis* growing in Uzbekistan. Previously, the component composition and antimicrobial activity of the essential oil of fresh and dried plants were studied [14], coumarin, diisoctyl phthalate and (+)-D-pinitol [15], p-hydroxybenzoic and p-hydroxycinnamic acids were isolated and identified, androsin, daukosterol, kaempferol 3-O-β-D-galactopyranoside, quercetin 7-O-α-L-rhamnopyranoside [16,17].

Melilotus albus is an annual and biennial herbaceous plant. The roots are used in folk medicine for thrombosis. An infusion or decoction made from the plant's aerial part is used as a lactogen, anti-febrile, for ascites, and for headaches. Ointment from leaves and flowers as a wound healing agent [1,5]. The plant has anticoagulant and fibrinolytic effects. The aerial part of the plant contains coumarin, melilotic acid, choline, tannin, resinous substances, essential oil, ascorbic acid, tocopherol [1,5,18].

We also studied the component composition of the essential oil of the air-dry aerial part of *Melilotus albus*.

Methods and materials of study. The aerial part of *Melilotus officinalis* used in this work was harvested on the territory of the Namangan region (Kamchik Pass) of the Republic of Uzbekistan during the flowering period (June 2021).

The above-ground part of *Melilotus albus* was collected on the territory of the Tashkent region (August 2022) during the flowering period.

The qualitative and quantitative composition of the essential oil was determined on an Agilent 5975C inert MSD/7890A GC chromatography-mass spectrometer. The separation of the components of the mixture was carried out on an Agilent HP-INNOWax quartz capillary column (30 m × 250 microns × 0.25 microns). The components were identified based on a comparison of the characteristics of mass spectra with data from electronic libraries (Wiley Registry of Mass Spectral Data-9th Ed. NIST Mass Spectral Library, 2011) and a comparison of the retention indices (IU) of compounds determined with respect to the retention time of n-alkanes (C₉–C₃₀), as well as the study of their mass spectral fragmentation with those described in the literature [5].

Isolation of phenolic compounds from *Melilotus officinalis*. Continuing the study of *Melilotus officinalis* components, 13 mg of compound 1, 17 mg of compound 2 and 10 mg of compound 3 were isolated from the chloroform and ethyl acetate fractions of a 75% alcohol extract

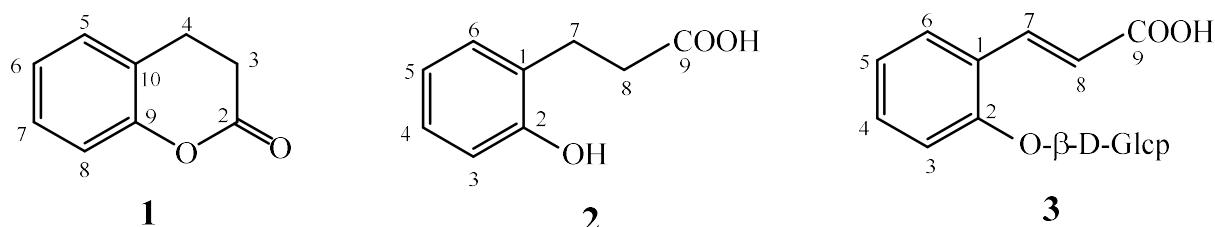
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of the aboveground part by column chromatography on silica gel in the chloroform-methanol gradient system and by rechromatography of the obtained eluates on sephadex LH-20 in the 80% ethanol.

Extraction of essential oil from the aboveground part of *Melilotus albus*. The extraction of essential oil from 200 g of crushed air-dry ground part of *Melilotus albus* was carried out by hydrodistillation at atmospheric pressure, the distillate was taken for 3 hours. Essential oil was isolated from the distillate by liquid extraction with dichloromethane. The solvent was distilled, essential oil dried with anhydrous sodium sulfate. Light yellow oil with a characteristic odor with a yield of 0.31% was obtained from the air-dry plant. EM was stored in the refrigerator at -4 °C before use.

The results obtained and their discussions. The identification of the isolated compounds was carried out by studying their spectral data of UV, ¹H and ¹³C NMR, as well as HSQC and HMBC experiments, followed by comparison with literature data.

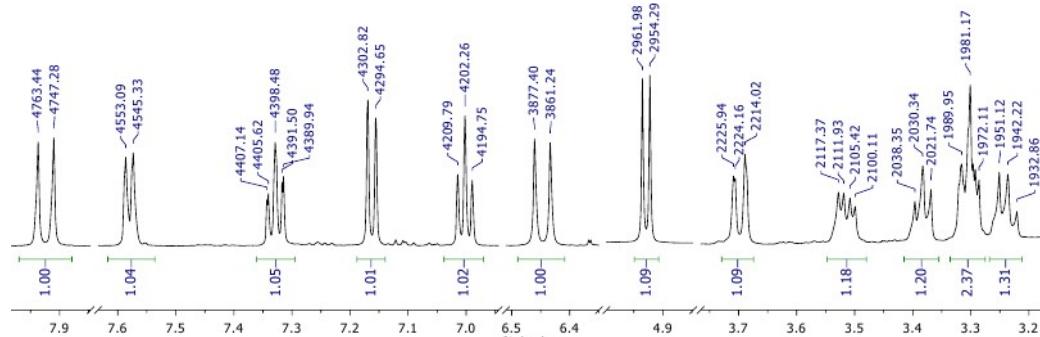
Compound 1. ¹H NMR (600 МГц, CDCl₃, δ, м.д., J/Гц): 2.78 (2H, уш. т, J=7.2, H-4), 2.99 (2H, уш. т, J=7.2, H-3), 7.15-7.04 (2H, м, H-6, H-8), 7.27-7.18 (2H, м, H-5, H-7); ¹³C NMR (150 МГц, CDCl₃, δ, м.д.): 169.43 (C-2), 29.33 (C-3), 23.71 (C-4), 128.43 (C-5), 124.65 (C-6), 126.94 (C-7), 117.08 (C-8), 152.01 (C-9), 128.11 (C-10). Based on the study of ¹H, ¹³C NMR, HSQC, and HMBC spectra, compound 1 was identified with dihydrocoumarin [19].



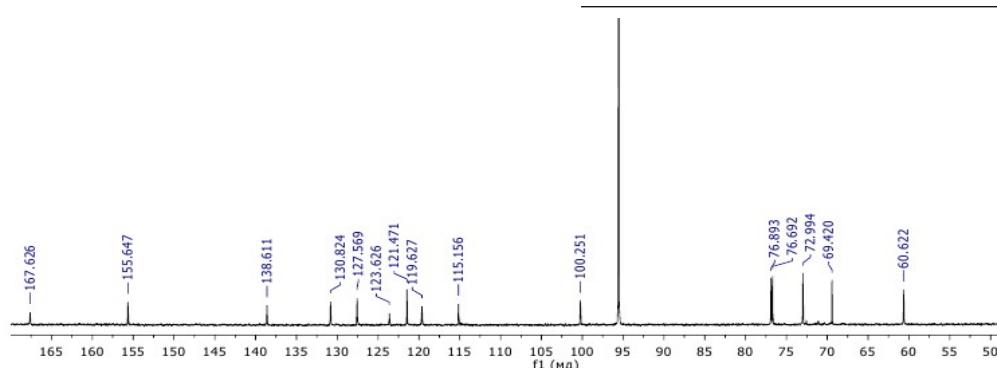
Compound 2. ¹H NMR (600 МГц, CDCl₃, δ, м.д., J/Гц): 2.73 (2H, уш. т, J=6.9, H-7), 2.90 (2H, уш. т, J=6.9, H-8), 6.81 (дд, J=8.4, 1.8, H-3), 6.86 (дт, J=7.4, 1.8, H-5), 7.08-7.10 (2H, м, H-4, H-6); ¹³C NMR (150 МГц, CDCl₃, δ, м.д.): 126.94 (C-1), 153.92 (C-2), 128.12 (C-3), 130.58 (C-4), 121.11 (C-5), 116.59 (C-6), 24.89 (C-7), 34.68 (C-8), 180.22 (C-9). Based on the study of ¹H, ¹³C NMR, HSQC, and HMBC spectra, compound 2 was identified with melilotic acid [19].

Compound 3. Brown amorphous powder C₁₅H₁₇O₈, UV λ_{max} (MeOH) 275 and 320 (sh) nm; ¹H NMR (DMSO-d₆+CCl₄, δ, ppm, 600 MHz): 3.23 (1H, м, H-4'), 3.30 (2H, м, H-3',5'), 3.38 (1H, м, H-2'), 3.51 (1H, дд, J=12.0, 5.3 H-6'), 3.70 (1H, дд, J=12.0, 1.8 H-6'), 4.93 (1H, д, J=7.7, H-1'), 6.44 (1H, д, J=16.3, H-8), 7.00 (1H, д, J=7.5, H-5), 7.16 (1H, д, J=7.2, H-3), 7.32 (1H, дт, J=7.2, 1.5 H-4), 7.58 (1H, уш.д, J=7.8, H-6), 7.93 (1H, д, J=16.3, H-7); ¹³C NMR (DMSO-d₆+CCl₄, δ, ppm, 150 MHz): 123.63 (C-1), 155.65 (C-2), 115.16 (C-3), 130.82 (C-4), 121.47 (C-5), 127.57 (C-6), 138.61 (C-7), 119.63 (C-8), 167.63 (C-9), 100.25 (C-1'), 72.99 (C-2'), 76.89 (C-3'), 69.42 (C-4'), 76.69 (C-5'), 60.62 (C-6'). Based on the study of ¹H, ¹³C NMR, HSQC, and HMBC spectra, compound 3 was identified with E-melilotoside [20].

a)



b)

Fig.1. ¹H (a) and ¹³C (b) NMR spectra of melilitoside (3).

As a result of pharmacological studies, it was found that extracts and individual compounds isolated from *Melilotus officinalis* possess antioxidant, anticoagulant, and antidiabetic properties.

By the GC-MS method [21, 22], 5 compounds were identified in the composition of essential oil from the air-dry plant *Melilotus albus*, which is 98.93% of the total amount of essential oil. The main component of the essential oil is coumarin, which content is 97.92%. Benzyl alcohol (0.30%), 2-hexenal (0.27%), dihydrocoumarin (0.26%) and phenoxyran (0.08%) were found in the essential oil, except coumarin. Therefore, the aerial part of *Melilotus albus* may serve as a rich source of coumarin.

It should be noted that the composition of the essential oil of the aerial part of *Melilotus albus* growing in Uzbekistan differs significantly from the composition of the essential oil of the plant growing in the Krasnoyarsk region of Russia [18].

Conclusion. Three phenolic compounds were isolated from chloroform ethyl acetate fractions of a 75% alcohol extract of the above-ground part of *Melilotus officinalis*. Based on the study of UV, ¹H, ¹³C NMR, HSQC and HMBC spectra, the isolated substances were identified with dihydrocoumarin, melilotic acid, and E-melilitoside.

The component composition of the essential oil of the air-dry aerial part of *Melilotus albus* obtained by hydrodistillation was studied by the GC-MS. The main component of the essential oil is coumarin, which content is 97.92%. It has been established that the composition of the essential oil of the aerial part of *Melilotus albus* growing in Uzbekistan differs significantly from the composition of the essential oil of the plant growing in Russia.

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