

# Melilotus Officinalis (L.) Pall. of the Medicinal Plant Analyzed by Chromato-Mass Spectrometry

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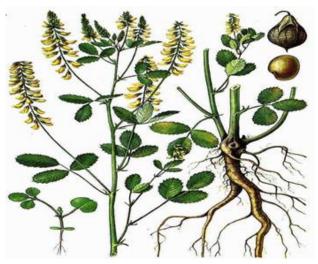
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Currently, the creation and production of drugs based on natural compounds is one of the main tasks of the fields of bioorganic chemistry and medicine. Because drugs produced on the basis of natural substances differ sharply from synthetic drugs in terms of their solubility, non-toxicity and wide range of effects. In this regard, the work carried out in the field of production of biologically active substances extracted from plants and their modification is widely developed in Uzbekistan and recognized by foreign scientists. Among them, several works are being carried out on the chemical study of the composition of plants growing freely in the territory of Uzbekistan. Melilotus officinalis (medicinal sedum) is one of these plants.

For research, the above-ground parts of Melilotus officinalis "Qashqarbeda", a medicinal plant growing in the mountain and sub-mountain areas of Namangan region, were selected and its content of phenolic compounds, essential oils, flavanoids, macro and microelements, as well as a number of biologically active substances were obtained. It was found that the chemicals found in the "Qashqarbeda" plant can be used as a blood thinner for human health and in medicine. Since Melilotus officinalis growing in the conditions of Uzbekistan has not been studied, this scientific work was focused on this direction, and as a result of the conducted research, several flavonoids with biological activity were isolated and presented to relevant groups for studying their biological activity. done.

Scientific classification: Section: Flowering plants. Class: Dicotyledons. Order: Angular flowers. Family: Corner cousins (Legumens). Category: Yellow Species: Melilotus officinlis (Qashqarbeda).



In folk medicine, experience in the treatment of various diseases with medicinal plants has been accumulated for many years. The drug is actively used in medicinal Latin beda (kashqarbeda). Melilotus officinalis (Kashqarbeda) this plant can be found in temperate latitudes, in different regions, in fields, meadows, and highways. Kashgarbeda has a lot of useful properties due to its chemical composition. There are 19 species of kashqarbeda growing on earth. In Uzbekistan, you can find 4 types. Kashgarbeda has many medicinal properties, lowers blood pressure, has an antispasmodic effect in patients with angina and atherosclerosis. It has a calming effect on cardiospasm, excitability, insomnia, headache, menopause. It is used as a mucolytic agent for cough and bronchitis.

# Chemical composition of Melilotus officinalis plant

The chemical composition of melilotus officinalis is very rich and includes: coumarins and derivatives, protein (17.6%), vitamin C (up to 389 mg), vitamin E (more than 45 mg), carotene (up to 84 mg), lactone, aldophosphamide glycoside, flavonoids (robinin, fluvin, kaempferol), essential oil (0.01%), polysaccharides (mucilage), saponins, allantoin, hydroxycinnamic, coumaric, melodic acids, phenolic triterpene compounds, carbohydrate compounds, nitrogenous bases, amino acids, tannins, oil-like substances (up to 4.3%), macro- and microelements (collects molybdenum, selenium), fatty acids (available in seeds). Preliminary phytochemical analysis showed that Melilotus officinalis contains coumarins, melilotin, phenolic acids, flavonoids, steroids, saponins, volatile oils, oils, triterpenes, carbohydrates, sugars, anthraquinone glycosides, mucilage, tannin, hydrocoumarins includes.

The dominant components in total lipophilic compounds of Melilotus officinalis chloroform extract are 1,3-di-o-methylmyo-inositol (75.503%), acetal (5.874%), palmitic acid (2.252%) and linoleic acid (1.958%).

Twenty-six constituents were identified in Melilotus officinalis essential oil, with hexahydrofarnesylacetone (16.64%), p-eudesmol (11.49%) and globulol (8.65%) representing the major constituents. At the same time, the identified compounds and their percentages: para-hydroxybenzaldehyde 1.96, camphor 3.15, terpinen-4-ol 4.17, 2-methylbenzaldehyde 2.70, aromadendrene 1.38, geranylacetone 1.21, 2, 6,10-farend8-methyl-6-methyl-8-methyl-6, 6,10-6, 8-methyl. ionone 0.93, p -ionone-5,6-epoxide 2.08, epi-globulol 3.05, isophytol 1.47, spathulenol 4.27, globulol 8.65, viridiflorol 2.98, epi-eudesmol 2, 50, Y- eudesmol 2.50, Y- eudesmol 3.05, y- eudesmol 3.05, y- eudesmol 3.05, isophytol 1.47, y- eudesmol 3.05, y- eudesmol 3.05, isophytol 1.47, y- eudesmol 2.50, y- eudesmol 3.05, isophytol 1.47, y- eudesmol 3.05, y- eudesmol 3.05, isophytol 1.47, y- eudesmol 2.50, y- eudesmol 3.05, isophytol 1.47, hexahydrofarnesileceton 16 .64, methyl palmitate 2.68, methyl linoleate 1.77, methyl linoleate 5.19, phytol 4.52, ethyl linoleate 0.96 and phytyl acetate 1.12%. The main compounds identified in the volatile oils of the methanol extract

of Melilotus officinalis leaves were n-docosane (39.82%), hydrocoumarin (15.39%) and methyl 3-(2-hydroxyphenyl) propionate) (14.29%). The main compounds identified in the volatile oils of the hexane extract of kashqarbeda leaves growing in the region of Syria are: palatinol (17.77%), 9,12,15-octadecatrienoic acid, methyl ether (12.85%), 1-(dimethylamino)-5-[ (4'-ethynylphenyl)ethynyl]naphthalene (12.59%), 2,4-dioctylphenol (9.73%), hexadecanoic acid, methyl ether (8.99%) and ecosane (8.53%).

## Analysis of essential oils of Melilotus officinalis

50 grams of the dry mass of the Melilotus officinalis plant were taken and its ethers were extracted by paradistillation. The following results were obtained when the collected ether was examined by the chromatographic mass spectroscopic method.

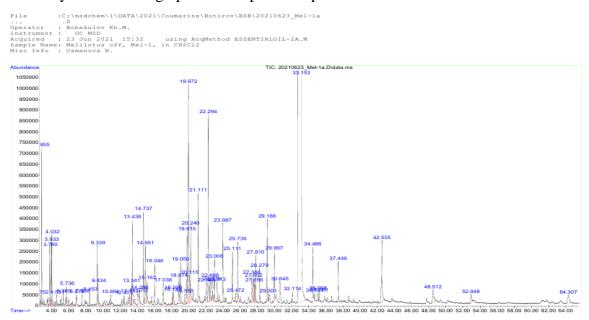
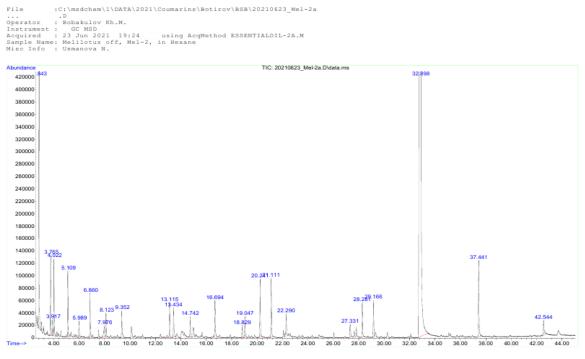


Figure 2

The results obtained using GC MSD liquid chromato-mass spectrometer of the above-ground part of Melilotus officinalis plant.





#### 2.2 Discussion of the analysis of the gasoline aggregate.

Ultraviolet analysis is the process of looking at the signs of a substance in UV spectroscopy. In this case, the fraction taken on the silifol paper was examined through the system of the spots placed with the help of a capillary from the sample. A 6:1 ratio of hexane and ethyl acetate was used for gasoline fractions. According to the literature, it was known that coumarin and chlorophyll were isolated from the spots shown above. The important thing for us was to separate the coumarin substance from the precipitate, which is usually thrown away individually, and the result was achieved. IR analysis of obtained coumarin substance was considered. According to him, it was found that it exhibits signs characteristic of coumarin substance and it was isolated in a pure state for NMR spectroscopy.

#### IR spectrum of coumarin substance:

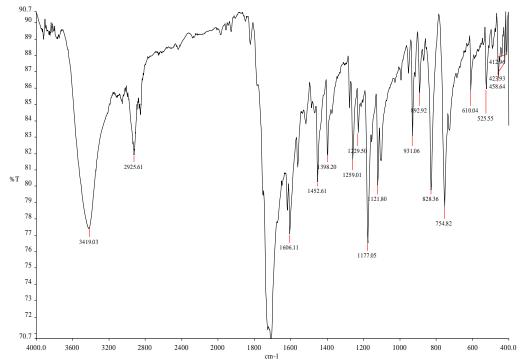
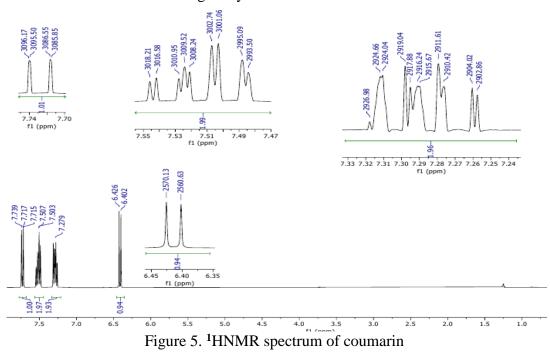


Figure 4 After the isolated substance coumarin was purified, in order to clarify its structural formula, NMR spectroscopy analysis was carried out and the results were as expected. The following analysis results were obtained.



Substance 1. C<sub>9</sub>H<sub>6</sub>O<sub>2</sub>т. пл. 69-70°С. <sup>1</sup>H-YaMR (CDCl<sub>3</sub>, δ, m.d., J/Gst): 6.41 (1H, д, 9.5 Gst, H-3), 7.26 (1H, dd, 7.6 и1.2 Gst, H-8), 7.30 (1H, dt, 7.6 и 1.2 Gts, H-6), 7.50 (1H, dt, 7.7 I 1.7 Gst, H-7), 7.53 (1H, dd, 7.7 и 1.7 Gst, H-5), 7.72 (1H, d, 9.5 Gst, H-4).

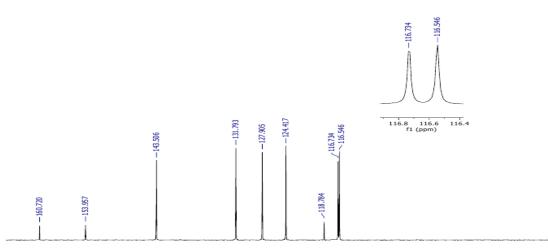


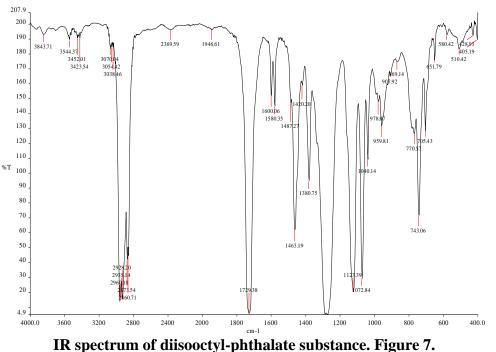
Figure 6. <sup>13</sup>C-NMR spectrum of coumarin

<sup>13</sup>C-YaMR (CDCl<sub>3</sub>, δ, m.d.): 160.7 (C-2), 154.0 (C-9), 143.5 (C-4), 131.8 (C-7), 127.9 (C-5), 124.4 (C-6), 118.8 (C-10) 116.7 (C-3), 116.5 (C-8).

The above pictures show the structural formula and X-ray structure of the coumarin substance obtained. Above is the result of NMR spectroscopy analysis.

#### Analysis of the chloroform sum

After the gasoline fraction was finished, the chloroform fraction process was carried out, which has a higher polarity than gasoline. The separator was washed with 2 L of chloroform solvent during the day in the column. At the end of the process, a total of 50 400 ml containers with a volume of up to 20 l of plant chloroform extract were taken. The fraction in each vessel above was pumped through the rotor and analyzed by NQX (hexane:ethyl acetate 1:1 system). All fractions were combined into one pot because they were almost identical according to the test results. The combined fractions were dried by adding 25 grams of silica gel and the total mass was 37.5 grams when measured on a balance. A separate substance was isolated from the dried sum using column chromatography.



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Pictured above is an IR analysis of diisooctyl phthalate extracted from the chloroform fraction.

The results of NMR and PMR spectroscopic analysis of the isolated diisooctyl phtate substance were obtained using modern devices. According to the results, the structure of the obtained substance was proved by comparison with the standards.

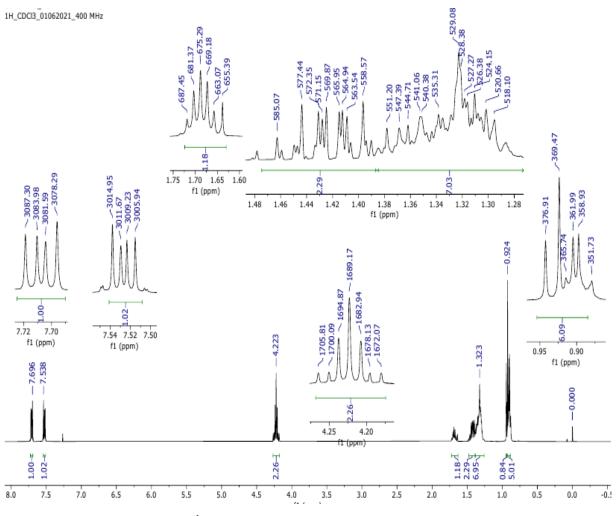


Figure 8. <sup>1</sup>H -NMR spectrum of diisooctyl phthalate

Substance 2 is a syrupy yellow mass of C24H38O4. UV spectrum (EtOH, lmax, nm): 231, 281. In its 1H-NMR spectrum (DMSO-d6, d, ppm, J/Hz), aromatic protons of 1,2-substituted benzene ring and two isooctyl signals. groups 0.84-0.88 (12H, t, 7.2 Hz, 4CH3), 1.22-1.37 (16H, m, 8CH2), 1,61-1,66 (2H, m, 2CH), 4,10 -4,17 (4H, t, 622O) ), 7,66 (2H, dd, 3,6 va 5,6 Hz, H-4,5), 7,73 (2H, dd, 3,6 va 5,6 Hz, H-3, 6)[38,39,40].

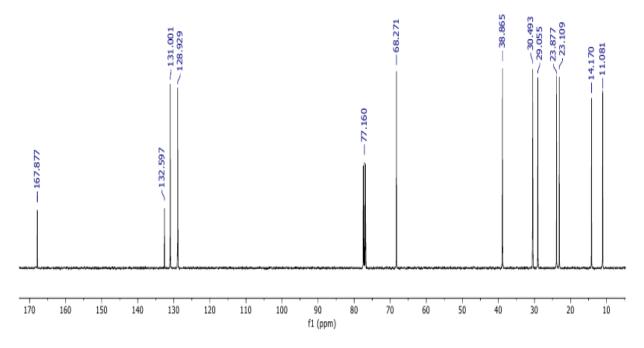


Figure 9. <sup>13</sup>C-NMR spectrum of diisooctyl phthalate

The <sup>13</sup>C-NMR spectrum of the compound (CDCl<sub>3</sub>, d, ppm) includes the signals of carbon atoms of the 1,2-substituted benzene ring, two carbonyl and two isoctyl groups: 167.88 (C-1.8), 132.60 (C -2.7), 131.00 (C-4.5), 128.93 (C-3.6), 68.27 (C-1', 1"), 38.87 (C-2 ', 2"), 30.49 (C-3', 3"), 29.06 (C-4', 4"), 23.88 (C-5',5"), 23, 11 (C- 6', 6"), 14.17 (C-7', 7"), 11.08 (C-8', 8"). Alkaline hydrolysis of compound 2 afforded phthalic acid. Therefore, the test compound is the diisooctyl ester of phthalic acid.

The figure below shows the structural formula of diisooctyl phthalate extracted from the chloroform fraction.

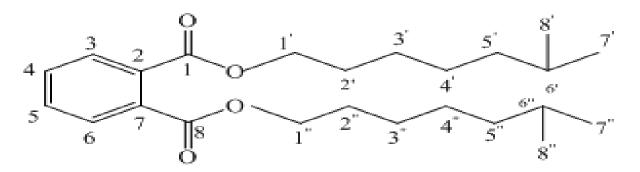


Figure 10. Structural formula of diisooctyl phthalate

Substances  $N_{2}$  1, 2, 3 were isolated using column chromatography of the obtained fractions. Based on UF, IR, 1H 13C NMR spectra, the isolated substances were found to be the above substances that are abundantly stored in this plant. Biological activity was studied by pharmacologists, coumarin was recommended as a blood thinner, and pinitol was used as a folk medicine against diabetes.

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