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M. M. NYKMUKANOVA[1], B. K. YESKALIYEVA[2], G. SH. BURASHEVA[2]

[1]S. Amanzholov East Kazakhstan State University, Ust-Kamenogorsk, Republic of Kazakhstan,
[2]Al-Farabi Kazakh National University, Almaty, Republic of Kazakhstan.
E-mail: nykmukanova@mail.ru

SEPARATION FLAVONOIDS BY SORBENT RP-18 FROM *VERBASCUM MARSCHALLIANUM*

Abstract. Investigating studies of biologically active substances from the ground parts of the family *Scrophulariaceae* genus of *Verbascummarschallianum* growing in the Altai region of Kazakhstan flavonoids were studied. Raw materials (aboveground part *Verbascummarschallianum*) for research are harvested during the budding period in the territory of East Kazakhstan. For the first time, an effective RP-18 sorbent was used for the isolation flavonoid complex from the raw material and the individual compound (cinorazide) was obtained by high-performance chromatography (HPLC). The structure of the compound is solved by chemical (acid hydrolysis) and spectral: ^{1}H and ^{13}C NMR, UV, IR spectroscopy and mass spectrometry.

Key words: *Verbascummarschallianum*, biologically active substances, RP-18 sorbent, high-performance liquid chromatography, acid hydrolysis, flavonoids, 7-O-β-D-glucopyranoside of luteolin (cynorazide).

In the Republic of Kazakhstan there is considerable scientific and technical potential in the field of development and production of herbal medicines, an extensive resource base and the possibilities for its further strengthening. Namely, the Altai territory has such a variety of zonal and intrazonal landscapes in particular, it could not affect the abundance and species diversity of the plant world. The creation of highly effective domestic production facilities, the proposal of new methods for the isolation of biologically active complexes are a priority and actual task.

The genus *Verbascum L.*, common name mulleins, comprisesabout 370 species of flowering plants in the *Scrophulariaceae* family, predominantly distributed in Asia, Europe and NorthAmerica[1]. The genus *Verbascum*belonging to the *Scrophulariaceae* family is the richestgenus, represented in Turkish flora by 230 species, of which185 are endemic [2]. In Kazakhstan, there are 9 species of mullein [3] and according to the latest data there are 10 species [4]. *Verbascummarshallianum* not completely investigated.

The object of study – the aboveground part of the genus *Verbascummarshallianum* prepared in phase fruiting in August 2017 from the Altai region of Kazakhstan. By general methodology of research I edition of State Pharmacopoeia of the Republic of Kazakhstan in the study raw materials are defined: loss on drying, extractives, total ash and quantitive of biologically active substances [5]. In plant raw materials by quantitative analysis revealed a large number of flavonoids, iridoids and tannins [6].

For the preparation of biologically active substancesdried aerial part (500 g) of plants of the genusVerbascum (family Scrophulariaceae) was crushed to sizeparticles of 2-3 mm, extraction was carried out with 100% methyl alcohol in Soxlet. Received the extract was defended, filtered, concentratedand dried under vacuum. Then the dry extractwas treated with hexane, dichloromethane andn-butanol. The n-butanol extract was concentrateddryness on a rotary evaporator at a temperature of 40-45°C and obtained the butanol extract with flavonoid complexes (26.5 g).

The availability of flavonoids in the extract was detected by a yellow stain using two-dimensional paper chromatography (Watman S2 paper grade, Germany) in the system butanol: acetic acid: water (40: 12.5: 29) and 6% acetic acid as an indicator 1% AIS in aqueous solution and by thin-layer chromatography (Silica gel DC-Alugram 60 UV254, Merck firma) in the system dichloromethane: methanol, cerium sulfate was used as an indicator.

For the separation of substances from the butanol extract used adsorption chromatography using silica gel sorbent. Elution of polarity to dichloromethane: methanol solution resulted in 213 fractions. Using a TLC method using specific developers, similar fractions were combined and obtained VB-1 (1.8 g), VB-2 (4.4 g), VB-3 (3.5 g), VB-4 (5.5 g) flavonoid complexes. From the VB-1 fractions, re-chromatography on RP-18 (LiChrospher® 100, Merck firm) (methanol: water 1: 9, 3: 7, 1: 1) yielded VB-A fractions. FractionsVB-A by using NP-HPLC (preparative recycling JAI-LC-908 HPLC, Japan Analytical Industry Co., Tokyo, Japonia) on a Sil-D-60-10 silica gel column (250 × 20 nm × 5 μm) (eluent chloroform: methanol 9.5-0.5) substances **1** (25 mg) were isolated.

According to UV spectra and a result of acid hydrolysis, glycosidic bonds, flavonoid and carbohydrate structures, which are identified with taps [7, 8].

The obtaining 4 polyphenolic complex fractions (figure 1) of the butanol extract were detected in a yellow shade in paper and thin-layer chromatography, then the VB-1 fraction was chromatographed on a RP-18 column, resulting in 6 compounds (figure 2), of which two were similar flavonoids (R_f value).

The spots in the UV light were dark brown. Using of developer of cerium sulfate, the spotswere stained from light yellow to dark brown. To obtain 1 substance,was usedHPLC with sorbent silica gel (Sil-D-60-10). As a result of three times recrystallization in methanole and an unchanged spot in TLC, it proves that substance 1 is pure (figure 3). According to the Bryant Method, substance 1 refers to glycosides [9].

Luteolin-7-O-β-glucopyranoside - light yellow crystals,$C_{21}H_{20}O_{11}$, ESI-MS, *m/z*: 471 $[M+Na]^+$ and*m/z*: 270 $[M]^+$. $T_{melting}$ = 259-263^{0}C. The UV spectrum of this compound has a maximum absorption at a wavelength of 254, 338 nm, which is typical for flavones. When sodium acetate is added to the solution of the substance, the shifts do not occur (254, 338 nm), so the 7-OH group is replaced. With sodium hydroxide, we observe a bathochromic shift of band I at 44 nm, band II at 8 nm, hence the molecule contains free phenolic hydroxyl groups.

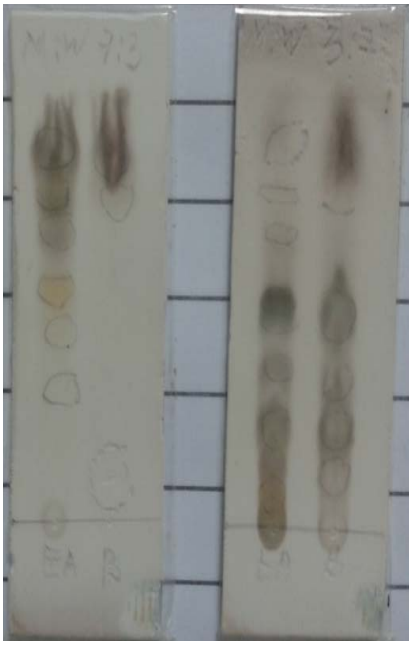


Figure 1

Figure 2

Figure 3

In the IR spectrum, there are bands of vibrations of hydroxyl groups - 3515-3600 sm^{-1}, carbonylγ-pyrone1655, aromatic C=C bonds1528, 1360, C-O glycoside oscillations1080, β-coupling between aglycone and sugar890, and1060,1040, 1030sm^{-1} sugar in glycoside in the form of pyranose, and so according to IR and UV data refers to flavones.

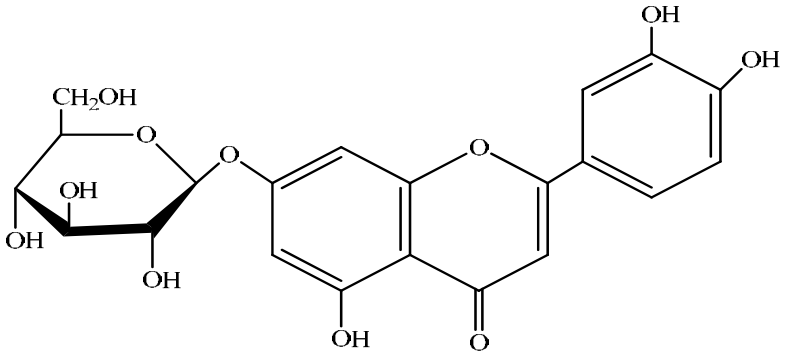
In the ^{1}H-NMR and DEPT-135 spin system, the lower field is located an anomeric glucose protonδ 5.05(1H, d, J = 7.0 Hz) shows that the glycoside in the β-configuration. Chemical shifts of H-6 and H-8 (respectively 0.2 + 0.3 ppm) shows, that C-7 is bonded with sugar.

^{1}H NMR spectra (600 MHz, Piridin, δppm) shows signals of 5,7,3',4'-tetrasubstitutedflavon and sugar: 6.72 (1H, s, H-3), 6.55 (1H, s, H-6), 6.77 (1H, s, H-8), 7.37 (1H, s, H-2'), 6.81 (1H,d,*J* = 8.5Hz, H-5′), 6.75 (1H,d, *J* = 8.8 Hz, H-6′), 5.10 (1H, d, J=7.0,H-1′′), 3.19-3.45 (1H, t, H-2′′), 3.19-3.45 (1H, t, H-3′′), 3.31 (1H, t, J=8.8, H-4′′), 3.11 (1H, m, H-5′′), 3.76 (1H, d, J=12.0, H-6′′a), 3.55 (1H, d, J=12.8, H-6′′б).

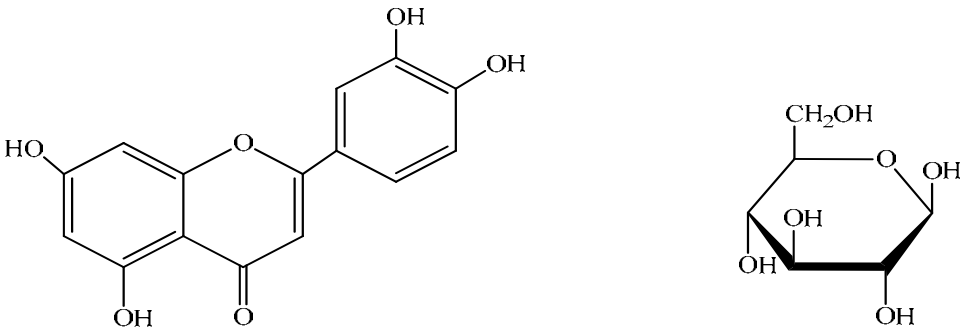
^{13}CNMR (100 MHz, Piridin, δppm): 165.2 (C-2), 104.3 (C-3), 182.2 (C-4), 159.5 (C-5), 102.5 (C-6), 164.3 (C-7), 95.2 (C-8), 161.8 (C-9), 105.8 (C-10), 122.4 (C-1'), 115.5 (C-2'), 147.6 (C-3'), 151.2 (C-4'), 117.1 (C-5'), 120.0 (C-6'), 100.6 (C-1"), 74.2 (C-2"), 75.9 (C-3"), 70.2 (C-4"), 77.5 (C-5"), 61.5 (C-6")

A complete acid hydrolysis of the substance was carried out with a mixture of 5 ml of 5% hydrochloric acid and ethanol (1: 1) for 2 hours, and the resulting aglicon was alkaline hydrolysed with 50% potassium hydroxide, using nitrogen

for 20 minutes. As a result, luteolin and glucose were obtained. The hydrolysis products were identified using the TLC method. Based on physicochemical methods of analysis and comparison with the literature data, substance 1 is identified as luteolin-7-O-β-glucopyranoside [10, 11].



Luteolin-7-O-β-glucopyranoside (Substance 1.)



Luteolin β-D-glucose

For the first time from plant of genus *Verbascum marschallianum* growing in Altai region of Kazakhstan was studied chemical investigation and for separation flavonoid complexes aneffective sorbent is proposed RP-18 and individual compounds are obtained usinghigh performance liquid chromatography. Luteolin-7-O-β-glucopyranoside(cinorazide) first time obtained from genus of *Verbascum.* The structure of the compound is solved bychemical (acidic, alkaline hydrolysis) and spectral: 1D(13C-NMR, 1H-NMR), 2D (HMBC, HSQC, COSY, NOESY), IR, UV spectroscopyand mass spectrometry (EIMS, ESI-MS, FAB-MS).

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Резюме

М. М. Ныкмуканова, Б. К. Ескалиева, Г. Ш. Бурашева

VERBASCUM MARSCHALLIANUM ӨСІМДІГІНЕН
RP-18 СОРБЕНТІ КӨМЕГІМЕН ФЛАВОНОИДТЫ БӨЛУ

Қазақстанның Алтай өңірінде өсетін *Scrophulariaceae* (сабынкөкгүлділер) тұқымдас құрамындағы биологиялық белсенді заттарды зерттеуді жалғастыра отырып, *Verbascum marschallianum* (Маршалла аюқұлағы) өсімдігінен флавоноидтар кешені алынды. Зерттеуге арналған шикізат (*Verbascum marschallianum*жер үсті бөлігі) Шығыс Қазақстан аймағынан жеміс беру кезінде жиналған. Алғаш рет аталған шикізаттан флавоноидтар кешенін бөлуде тиімді сорбент ретінде RP-18 пайдаланып, жеке зат – лютеолиннің 7-O-β-D-глюкопиранозиді (циноразид) жоғары эффективті сұйықтық хроматография (HPLC) көмегімен бөлінді. Жеке заттың құрылысы химиялық (қышқылдық гидролиз) және спектралды (^{1}H, ^{13}C ЯМР, УК-, ИҚ-спектроскопия және масс-спектрометрия) әдістермен дәлелденді.

Түйін сөздер: *Verbascum marschallianum*, биологиялық белсенді заттар, RP-18 сорбенті, жоғары эффективті сұйықтық хроматографиясы, қышқылдық гидролиз, флавоноидтар, лютеолиннің 7-O-β-D-глюкопиранозиді (циноразид).

Резюме

М. М. Ныкмуканова, Б. К. Ескалиева, Г. Ш. Бурашева

ВЫДЕЛЕНИЕ ФЛАВОНОИДА С ПОМОЩЬЮ СОРБЕНТА RP-18 ИЗ РАСТЕНИЙ *VERBASCUM MARSCHALLIANUM*

Продолжая исследования биологически активных веществ у представителей семейства *Scrophulariaceae*(Норичниковые), изучены флавоноиды надземных частей *Verbascum marschallianum* (коровяк Маршалла),собранного из Алтайского региона Казахстана в период бутонизации. Для получения флавоноидного комплекса из надземных частей *Verbascum marschallianum* впервые использован эффективный сорбент RP-18 и с помощью высокоэффективной хроматографии (HPLC) выделено индивидуальное соединение (циноразид). Структура выделенного вещества доказана химическими (кислотный гидролиз, щелочное расщепление) и спектральными: (^{1}H и^{13}C ЯМР, УФ-, ИК- и масс-спектрометрия) методами.

Ключевые слова: *Verbascum marschallianum*, биологически активные вещества, сорбент RP-18, высокоэффективная жидкостная хроматография, кислотный гидролиз, флавоноиды, 7-O-β-D-глюкопиранозид лютеолина (циноразид).