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OPTIMISATION METHOD FOR OBTAINING A BIOLOGICALLY ACTIVE SUBSTANCES FROM THE PLANT *PETROSIMONIA BRACHIATA*

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Abstract. Kazakhstan is rich in medicinal plants, so it needs to be studied. Ten species of the *Petrosimonia* belonging to the *Chenopodiaceae* family are growing in Kazakhstan. These plants have been adapted to harsh climatic conditions in the country's salty, saline, and desert areas. *Petrosimonia* plants are a new species that has not been fully studied. Chinese researchers studied *Petrosimonia sibirica* species and found that the plant contains alkaloids, steroids, terpenes, flavonoids, and phenolic acids and has antibacterial activity (Wen et al, 2015; Ying et al, 2016). The qualitative and quantitative compositions of biologically active substances in *P. sibirica, P. Glaucescens, P. triandra*, and *P. brachiata* growing in Kazakhstan were studied. To prepare phytopreparations from *P. Sibirica, P. Glaucescens* and *P. triandra* plants were extracted using the classical maceration method, and individual representatives of biologically active compounds were isolated (Toktarbek et al, 2021). In this study, a phytochemical study of *P. brachiata* was conducted. The method for obtaining biologically active complexes with high efficiency was optimised by using supercritical fluid and ultrasonic extraction methods. These methods have a short extraction time, can be carried out at room temperature, are cost effective, and are a modern green chemical approach. Using supercritical fluid CO_2 extraction (180 bar, 2 hours), the plant was purified from lipophilic substances. Further, a biologically active complex was obtained by pouring 70% ethanol-water solvent into the plant raw material and performing ultrasound extraction. The obtained complex was analysed by thin-layer chromatography using different organic solvent

systems. Based on our analysis, steroids, terpenes, phenolic acids, and flavonoid glycosides were found in the extract. Hexane and ethyl acetate fractions were obtained as a result of liquid-liquid extraction of the extract using organic solvents. Stigmasterol 3-O-β-D-galactopyranoside, isovanillic acid, quercetin 3-O-β-D-glucopyranoside, and isorhamnetin 3-O-α-L-rhamnopyranoside were isolated by washing the hexane and ethyl acetate fractions in a silica gel column with an organic solvent system. The isolated compounds will be tested for biological activity.

Keywords: *Chenopodiaceae* family, *Petrosimonia brachiata,* ultrasonic extraction, supercritical fluid extraction, chromatography.

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PETROSIMONIA BRACHIATA ӨСІМДІГІНЕН БИОЛОГИЯЛЫҚ БЕЛСЕНДІ ЗАТТАРДЫ АЛУ ӘДІСІН ОҢТАЙЛАНДЫРУ

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Аннотация. Қазақстан аумағы дәрілік өсімдіктерге өте бай және олар зерттеуді қажет етеді. Еліміз аумағында Алабұта (*Chenopodiaceae*) туысына жататын *Petrosimonia* тұқымдасының 10 түрі өседі. Бұл өсімдіктер еліміздегі тұзды, сортаң және шөлеитті жерлерде қатаң климат жағдайына бейімделген. *Petrosimonia* өсімдіктері толық зерттелмеген жаңа нысан болып табылады. Қытай ғалымдары *Petrosimonia sibirica* түрін зерттеп, өсімдік құрамында алкалоидтар, стероидтар, терпендер, флавоноидтар және фенол қышқылдары қатарлы екіншілік метабилиттердің бар екенін анықтап, бактерияға қарсы белсенділік көрсеткенін дәлелдеген (Wen et al, 2015; Ying et al, 2016). Қазақстанда өсетін *P. Sibirica, P. Glaucescens, P. triandra* және *P. brachiata* түрлерінің құрамындағы биологиялық белсенді заттардың сапалық және сандық құрамы зерттелінген. *P. Sibirica, P. Glaucescens* және *P. triandra* өсімдіктерінен фитопрепарат алу үшін класикалық мацерация экстракцияланып, биологиялық белсенді заттардың жеке өкілдерін бөліп алған (Toktarbek et al, 2021). Бұл зерттеуде *P. brachiata* өсімдігіне фитохимиялық

зерттеу жүргізілді. Жоғары критикалық флюидті және ультрадыбысты экстракция әдістерін пайдалану арқылы тиімділігі жоғары биологиялық белсенді кешен алудың жолы оңтайландырылды. Аталған әдістер экстракция уақыты қысқа, бөлме температура жағдайында жүргізілетін, артық шығынсыз және заманауи жасыл химиялық бағыт болды. Жоғары критикалық флюидті CO_2 экстракцияны (180 bar, 2 сағат) қолданып өсімдік құрамы липофильді заттардадан тазартылды. Ары қарай өсімдік шикізатына 70% этанол-су еріткішін құйып, ультрадыбыспен экстракция жасау арқылы биологиялық белсенді кешен алынды. Алынған кешенге жұқа қабатты хроматографияда, әр түрлі органикалық еріткіштер жүйесі көмегімен сараптама жасалынды. Сараптаудың негізінде экстракт құрамында сетроидтар, терпендер, фенол қышқылдары және флавоноид гликозидтерінің бары анықталды. Экстрактіге органикалық еріткіштерімен сұйық-сұйықтық экстракциясын жүргізу нәтижесінде гексан және этилацетат фракциялары алынды. Гексан және этилацетат фракцияларын силикагель бағанасында органикалық еріткіштер жүйесі арқылу жуу нәтижесінде стигмастеролдың 3-O-β-D-галактопиранозиді, изованил қышқылы, кверцетиннің 3-O-β-D-глюкопиранозиді және изорамнетиннің 3-O-α-L-рамнопиранозиді бөлінді. Бөлінген жеке заттардың биологиялық белсенділігін тексеру жоспарлануда.

Түйін сөздер: *Chenopodiaceae* тұқымдасы, *Petrosimonia brachiata*, ультрадыбыстық экстракция, жоғары критикалық флюидті экстракция, хроматография.

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СПОСОБ ОПТИМИЗАЦИИ ПОЛУЧЕНИЯ БИОЛОГИЧЕСКИ АКТИВНЫХ ВЕЩЕСТВ ИЗ РАСТЕНИЯ *PETROSIMONIA BRACHIATA*

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Аннотация. Казахстан богат лекарственными растениями, поэтому его необходимо изучать. В Казахстане произрастают десять видов *Petrosimonia* семейства маревых. Эти растения адаптированы к суровым климатическим условиям засоленных и пустынных территорий страны. Растения *Petrosimonia* – новый вид, до конца не изученный. Китайские ученые изучили виды *Petrosimonia sibirica* и обнаружили, что растение содержит алкалоиды, стероиды, терпены, флавоноиды и фенольные кислоты и обладает антибактериальной активностью

(Wang et al, 2015; Ying et al, 2016). Изучен качественный и количественный состав биологически активных веществ *P. sibirica, P. Glaucescens, P. triandra* и *P. brachiata*, произрастающих в Казахстане. Для приготовления фитопрепаратов из растений *P. Sibirica*, *P. Glaucescens* и *P. triandra* экстрагировали классическим методом мацерации и выделяли отдельные представители биологически активных соединений (Toktarbek et al, 2021). В данном исследовании было проведено фитохимическое исследование *P. brachiata.* Оптимизирован метод получения биологически активных комплексов с высокой эффективностью за счет использования методов сверхкритической флюидной и ультразвуковой экстракции. Эти методы имеют короткое время экстракции, могут проводиться при комнатной температуре, являются экономически эффективными и представляют собой современный экологически чистый химический подход. С помощью сверхкритической флюидной CO_2-экстракции (180 бар, 2 часа) установка была очищена от липофильных веществ. Далее биологически активный комплекс получали путем заливки в растительное сырье 70% растворителя этанол-вода и проведения ультразвуковой экстракции. Полученный комплекс анализировали методом тонкослойной хроматографии с использованием различных систем органических растворителей. На основании нашего анализа в экстракте были обнаружены стероиды, терпены, фенольные кислоты и флавоноидные гликозиды. Гексановую и этилацетатную фракции получали в результате жидкостной экстракции экстракта органическими растворителями. Стигмастерол 3-O-β-D-галактопиранозид, изованилиновая кислота, кверцетин 3-O-β-D-глюкопиранозид и изорамнетин 3-O-α-L-рамнопиранозид выделены промывкой гексановой и этилацетатной фракций на силикагеле. Колонка с системой органических растворителей. Выделенные соединения будут проверены на биологическую активность.

Ключевые слова: семейство *Chenopodiaceae, Petrosimonia brachiata,* ультразвуковая экстракция, сверхкритическая флюидная экстракция, хроматография

Introduction

Medicinal products of plant origin have been used since ancient times, and even ancient times, civilisations were famous for using plants for healing. The oldest records date back to approximately 5,000 years when plants were used medicinally. The Greeks developed medicinal plants and herbs. Theophrastus (ca. 300 BC) wrote «Historia Plantarum», or the «History of Plants», one of the most important books on plant pharmacology and ancient natural history, in which he discussed the anatomy of plants and their pharmacological uses (Atanasov et al, 2021).

Much of the knowledge of Greco-Roman medicine was lost during the Middle Ages, but Arab and Islamic scholars from Andalusia and the Middle East were able to preserve and update the practises of that era. This progress in medicine occurred between the 9th and 12th centuries, after which the Renaissance began, and science began to be studied and developed more and more in the Western world. For thousands of years, plant extracts have been used to treat diseases. The past 200 years have witnessed

the discovery, isolation, and determination of the structures of thousands of natural compounds.

The new drug discovery approach has not hindered the study of natural compounds. A study of all newly approved drugs from 1981 to 2014 found that a total of 1211 new drugs were approved worldwide. Among them, 320 were natural compounds or their derivatives. These drugs comprise 32.7% of all authorised drugs in the world in the last 30 years (Li et al, 2018).

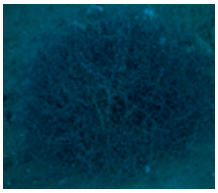
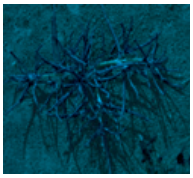
Compounds obtained from natural sources are not only medicinal products but also important tools for the discovery of new aspects of physiology. Currently, there is an interest in systematic research on low-molecular-weight inhibitors of the main steps of biochemical processes. Because many assays involve phenotyping, changes in living cells are likely to provide natural products that are useful as probes in such studies.

Our research objective: Plants belonging to the *Chenopodiaceae* family that grow in saline environments, are viable, and have high physiological capacity. They are widely used in folk medicine for the treatment of digestive, respiratory, genitourinary, and blood vessel disorders (Boneva et al, 2022). There are 11 species of Petrosimonia plants belonging to the *Chenopodiaceae* family worldwide, and 10 species grow in the desert and the desert regions of Kazakhstan. These species are: *P. monandra, P. triandra, P. litwinowii, P. squarrosa, P. hirsutissima, P. crassifolia, P. glaucescens, P. brachyphylla, P. glauca, P. brachiata,* and *P. sibirica.* The chemical compositions of all types of *Petrosimonia* plants have not been fully studied. The qualitative and quantitative compositions of biologically active substances in *P. sibirica, P. Glaucescens, P. triandra*, and *P. brachiata* growing in Kazakhstan were studied. According to the methods of the first edition of the State Pharmacopeia of the Republic of Kazakhstan, qualitative and quantitative analysis of biological active substances was performed, and the authenticity of plant raw materials was determined. The results of the study are shown in table 1 and table 2 (Seitimova et al, 2022).

Table 1. Quantification and authenticity of the main biological active groups of *P. Sibirica, P. Glaucescens, P. triandra,* and *P. Brachiata*

Plant names	*P. triandra*	*P. glaucescens*	*P. brachiata*	*P. sibirica*
Quality indicators of plant raw materials, (%)±SEM				
Humidity	8.09±0.03	5.90±0.04	10.22±0.04	7.81±0.03
Ash content	17.52±0.07	24.50±0.06	20.45±0.15	24.71±0.06
Extractives (80% ethanol-water)	42.70±0.04	46.10±0.02	46.90±0.06	52.90±0.04
Quantification of the main groups of biologically active substances, (%)±SEM				
Saponins	4.53±0.03	8.6±0.03	1.92±0.03	0.6±0.02
Flavonoids	2.55±0.04	4.1±0.03	4.53±0.04	1.92±0.04
Taninns	1.75±0.03	1.1±0.02	0.1±0.01	0.1±0.01
Alkaloids	1.56±0.04	0.27±0.03	0.53±0.04	0.4±0.03
Polysaccharides	1.78±0.01	1.4±0.01	5.14±0.02	4.2±0.04
Free organic acids	0.52±0.03	0.6±0.04	5.51±0.04	3.5±0.04
Coumarins	0.18±0.02	0.9±0.03	0.13±0.03	0.3±0.02

Table 2. Comparative conditions for extracting plant raw materials

Plant Names	*Petrosimonia triandra*	*Petrosimonia glaucescens*	*Petrosimonia brahiata*	*Petrosimonia sibirica*
Plant pictures				
Extraction type	First, lipophilic substances were removed using super critical fluid extraction, followed by extraction using the maceration method.	Maceration method	First, lipophilic substances were removed using super critical fluid extraction, followed by extraction using ultrasonic method.	Maceration method
Solvent	80% ethanol-water	80% ethanol-water	80% ethanol-water	80% ethanol-water
Extraction time	For super critical fluid extraction 2 hours and for maceration 72 hours	72 hours	For super critical fluid extraction 2 hours and for ultrasonic extraction 30 minutes	72 hours
Extraction temperature	Room temperature	Room temperature	40 ^{O}C	Room temperature
Biological activity of the obtained extracts	Antibacterial activity	No activity	Anti-inflammatory activity	No activity

It is important to use modern advanced technologies to obtain ecologically clean products and reduce factors that have an indirect effect on the extraction and distribution of biologically active substances from plant raw materials, especially secondary metabolites. The potential of CO_2 supercritical fluids and ultrasonic extraction to solve these problems is enormous (Nguyen et al, 2023; Shi et al, 2023; Herrero, 2024).

Materials and methods

Materials

The plant raw material was as follow:

The research object was the above-ground parts of *P.brachiata* plant belonging to the *Chenopodiaceae* family. This plant was collected in September 2023 from the saline land of the Enbekshikazakh district of the Almaty region. *Petrosimonia's* specie plant was identified with the help of leading specialists at the Institute of Botany and phytoproduction, Almaty. Collected plant was dried at room temperature and protected from light. The dried plant raw material was ground to a diameter of 4 mm using sieves.

Solvents used:

1) Common solvents:

Ethanol and water were used as extractants for extracting plant raw materials.

2) Solvents for NMR spectroscopy:

Dimethylsulfoxide DMSO-d6 (Cambridge Isotope Laboratories, Inc.), Methanol CD3OD-d4 (Cambridge Isotope Laboratories, Inc.), and Acetone C3D6O-d6 (Cambridge Isotope Laboratories, Inc.).

Chromatography materials

Normal phase thin-layer chromatography (Aluminum Silica gel 60 F254) Merck KgaA; Reversed phase thin-layer chromatography (Glass Silica gel 60 RP–18 F254S) Merck Millipore; Silica gel 60, 0.04 – 0.063 mm (230 – 400 μm) Merck; Silica gel 60, 0.063 – 0.200 mm (70 – 230 μm) Merck, and Column.

Methods

Research methods

For extraction procedure: Supercritical fluid CO_2 extraction and ultrasonic extraction; Isolation and purification procedure: chromatography; Structural elucidation procedure: ^{1}H-NMR - AVANCE NEO-400, at 400 MHz and ^{13}C-NMR, BB, DEPT - AVANCE NEO-400 at 100, 125 and 150 MHz; two-dimensional: NMR ^{1}H-^{13}C HSQC, HMBC, ^{1}H-^{1}H COSY-45 ^{0}C, NOESY, IR -spectroscopy (Bruker Vector 22, Japan), EI-MS (JEOL 600H-1, Inlet: Direct Probe), FAB-MS (JEOL 600H-2, Inlet: Direct Probe), and melting points were determined by Buchi M-560 apparatus.

Preparation of extracts

The plant raw material was treated to eliminate lipophilic substances using supercritical fluid CO_2 extraction at 180 bar, followed by extraction with 80% ethanol-water at a ratio of 1:8 for 2 hours at room temperature twice by an ultrasonic extractor. The prepared extracts were combined and concentrated under vacuum at a temperature of 45-50 ^{0}C. The suspension was prepared by adding 500 ml of distilled water to 177 g of dry extract. Sequential liquid-liquid extraction was performed by adding hexane and ethyl acetate to the suspension according to polarity, and all extracts were desolvated in a rotary evaporator at temperatures not exceeding 50 ^{o}C.

The hexane extract was qualitatively analyzed by TLC (solvent system n-Hexane: ethyl acetate 9:1 → 1:9), a solution of $Ce(SO_4)_2$ in 15% H_2SO_4 was used as a reagent. The presence of lipophilic substances such as steroids and terpenes was determined in this study.

General experimental procedure for isolation of compounds

Silica gel column chromatography was used to separate 10 g of hexane extract into fractions and isolate individual compounds. The column was first washed with 100% hexane solvent, and then chromatography was performed by increasing the concentration of the polar solvent (ethyl acetate, methanol) according to the hexane:ethyl acetate 10:1 →1:10, 100% ethyl acetate, and ethyl acetate:methanol 10:1 →7:3. The fractions were combined with similar Rf values and spot colors in TLC to obtain 10 (H1-H10) fractions. Each fraction was concentrated under mild conditions using a rotary evaporator. Fraction H10 was isolated as a pure compound, corresponding to **1** (35 mg).

The ethyl acetate fraction was qualitatively analyzed with the help of TLC (solvent system n-Hexane:ethyl acetate:acetone 8:1:1), as well as a solution of the determining reagent $Ce(SO_4)_2$ in 15% H_2SO_4 was sprinkled, and as a result of analyzing the color of the spots formed under UV light (254 and 366 nm), the extract contained phenolic acids, flavonoid aglycones, flavonoid glycosides, and alkaloidal compounds were detected (Li, 2024).

5 g of dried and powdered ethyl acetate extract was chromatographed in SG-60 column chromatography with increasing polarity: 100% hexane → hexane-ethyl acetate (in different ratios) → 100% ethyl acetate → ethyl acetate-methanol (in different ratios) → 100% methanol, resulting in 11 fractions (E1-E10) were obtained. Substance **2** (29 mg) was separated from E2 fraction. Pure compound **3** (42 mg) and subfraction E8S1 were separated from fraction E8. The subfraction E8S1was purified by chromatography on a Sephadex LH-20 column with 100% methanol, and compound **4** (7 mg) was isolated.

The structures of isolated compounds were determined by melting point, IR, FAV-MS, and NMR data analyses.

Results and Discussions

Compounds **1-4** were isolated for the first time from the *Petrosimonia brachiata* species.

Compound **1** is a white crystalline powder, molecular formula $C_{35}H_{58}O_6$, molecular weight m/z 574, melting point 264-266 °C, a single spot in thin-layer chromatography (solvent system dichloromethane:methanol 9:1) after treatment with 15% H_2SO_4 showed a deep green.

The ^{1}H-NMR spectrum (DMSO, 500MHz) of compound **1** showed six methyl groups: δ_H 0.65 (3H, c, H-18), 0.79 (3H, д, J = 8.1, H-29), 0.80 (3H, д, J = 6.9, H-27), 0.84 (3H, d, J = 6.3, H-26), 0.95 (3H, d, J = 6.3, H-21), 0.99 (3H, s, H-19); proton occupying one olefinic position δ_H 5.15 (1H, br.d, J = 4.8, H-6); two protons 4.83 (1H, dd, J = 8.4, 15, H-23) and 4.97 (1H, dd, J = 8.4;15.0, H-22); and one anomeric proton showed chemical shift values of 4.19 (1H, d, J = 7.8, H-1′).

By analyzing the ^{13}C-NMR spectrum, this compound was found to contain 35 carbon signals. A chemical shift of δ_C 100.76 indicates the presence of a monosaccharide molecule with an anomeric carbon C-1′, whereas chemical shift values of δ_C 69.87, 73.22, 75.54, and 76.15 indicate four methanes and methylene at δ_C 61.43 chemical shifts C-2′, C-3′, C-4′, C-5′, and C. According to the C-6′ carbons, the product was found to be β-D-galactopyranose.

The δ_C 78.72 shift region corresponds to the C-3 carbon atom bonded to the alcohol hydroxyl group. Chemical shifts δ_C 121.71 (C-6), δ_C 137.92 (C-23), δ_C 128.79 (C-22), and δ_C 139.97 (C-5) represent olefinic carbons in the sterol molecule. The value of J = 7.8 Hz for the anomeric proton H-1′ shows that it is in an axial position to the proton H-2′, which indicates that this galactopyranoside fragment is bound to the sterol fragment in the *β* position.

Based on ^{1}H- and ^{13}C-NMR chemical shift values and physical data, it was proved that compound **1** is stigmasterol 3-O-β-D-galactopyranoside.

Compound **2** was isolated from the ethyl acetate extract. They were subjected to a qualitative reaction with TLC (Hexane:Ethyl acetate 5:1) using 15% H_2SO_4 and a brown spot formed. These compounds exhibited purple under UV light.

Compound **2** is a white crystalline compound with a melting point of 208–210 °C. The mass spectrum of the isolated compound EI-MS m/z 168.1 $[M]^+$; molecular formula corresponds to $C_8H_8O_4$. The valence vibration band in the IR spectrum defines the C=O group at 1682 cm^{-1} and the OH group at 3484 cm^{-1}. Absorption at 1598 and 1523 cm^{-1} indicates C=C groups in the aromatic ring, and the band at 1206 cm^{-1} indicates a C-O bond.

In the ^{1}H-NMR (CD_3OH, 500MHz) spectrum of the compound **2**, the chemical shift signal at δ_H 3.83 indicates the presence of an OCH_3 group in the molecular structure of the compound. The high-field signals δ_H chemical shifts at 7.61, 7.44, and 6.86 identify the H-2, H-6, and H-5 protons of the aromatic ring, respectively.

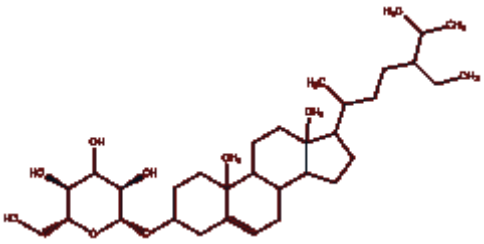
The ^{13}C-NMR spectrum of the compound showed 8 signals with 3 methine, 4 quaternary and 1 methyl carbons. The δ_C 55.5 signal is the chemical shift characteristic of the carbon atom in the methoxy group. And the δ_C 167.2 signal region indicates the C=O group of the acidic fragment of the molecular structure. The high-field signals at δ_C 149.1 and 151.2 indicate the shift characteristics of the C-3 and C-4 carbons, respectively, in the aromatic ring. ^{1}H-NMR, ^{13}C-NMR spectral data, etc. based on its physical properties, compound **2** was identified as isovanillic acid.

Compound **3** was isolated as a yellow powder with a melting point of 231–232 °C. FAB-MS corresponding to the molecular formula $C_{21}H_{20}O_{12}$ showed a molecular ion peak $[M+H]^+$ at m/z 464.14. ^{1}H and ^{13}C NMR spectra in CD_3OH solvent showed that the aromatic protons were resolved as a single ABX system (B ring). The δ_H 7.849 (d, J = 2.3 Hz, H-2′), δ_C 117.90 for δ_H 6.871 (d, J = 8.3 Hz); δ_C 116.3 and δ_H 7.586 for H-5′ (dd,J = 8.3, 2.3 Hz) δ_C 123.05 for H-6′. Another ABX-based (A ring) system δ_H 6.204 (brs), δ_C 100.02 and δ_H 6.407 (brs), δ_C 94.85 assigned to H-6 and H-8 protons, respectively. The ^{13}C values of C-3′ and C-4′ were 150.08, which were assigned 145.94 respectively. The anomeric sugar protons δ_H appeared at 5.16 (d, J = 7.53 Hz); δ_C is 103.95. An anomeric proton coupling constant J = 7.53 Hz confirmed the β-linkage of the sugar. Based on ^{1}H- and ^{13}C-NMR chemical shift values and physical data, it was proved that compound **3** is quercetin 3-O-β-D-glucopyranoside.

Compound **4** was isolated as a yellow amorphous powder with an FAB-MS 462.40 at an m/z $[M]^+$ molecular formula corresponding to $C_{22}H_{22}O_{11}$.

In the ^{1}H NMR (C_3D_6O) spectrum of compound **4**, three protons of the methoxy group exhibited a singlet signal in the region of δ_H = 3.94. Aromatic protons of ring B show doublet signals at 6.97 (1H, d, J=8.45, H-3′) and 8.05 (1H, d, J=2, H-6′), and 7.66 (1H, dd, J=8.5, J= 2.05, H-2′) proton was in the doublet doublet displacement level. In addition, we observed the aromatic H-6 and H-8 protons of ring A at the 6.28 (1H, t) and 6.53 (1H, t) triplet signal shifts, respectively. The anomeric H-1″ proton of the sugar moieties gave a doublet signal in the chemical shift region of 5.02 (1H, d, J= 2.05).

Using ^{13}C NMR and HMBC spectra, the binding sites of the sugars were determined. By comparing the results of the physicochemical analysis with the literature data, compound **4** was identified as isorhamnetin 3-O-α-L-rhamnopyranoside (Bojilov et al, 2023).



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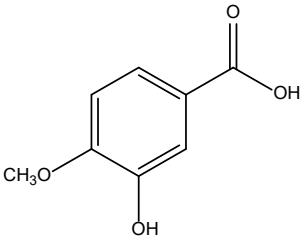

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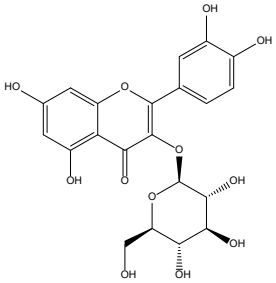

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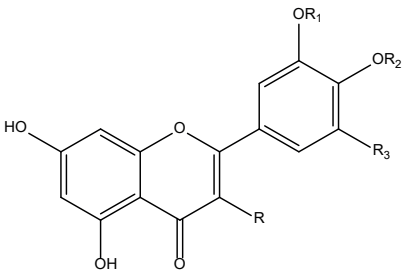

R= O-α-Rhamp, R_1=OCH_3 , R_2=R_3=H

4

Conclusion

First, a phytochemical study was conducted on *P. brachiata.* Plant raw materials were extracted using modern methods, including supercritical fluid extraction and ultrasound extraction. The obtained extract was subjected to liquid-liquid extraction using hexane and ethyl acetate solvents. The qualitative composition of the hexane and ethyl acetate fractions was analysed by chromatography, and the presence of steroids, terpene substances, and flavonoid classes was determined. For further purification of the hexane and ethyl acetate fractions, silica gel column chromatography was performed; stigmasterol 3-O-β-D-galactopyranoside, isovanillic acid, quercetin 3-O-β-D-glucopyranoside, and isorhamnetin 3-O-α-L-rhamnopyranoside compounds were isolated, and modern physicochemical methods, including ^{1}H-NMR, ^{13}C-NMR, 2D NMR, EI-MS, FAB-MS, and IR spectroscopy were used to determine their structures. The isolated compounds will be tested for biological activity.

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