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PHYTOCHEMICAL STUDY AND ELEMENTAL COMPOSITION OF *BRYONIA MELANOCARPA* NABIEV FROM THE FLORA OF KAZAKHSTAN

In the context of increasing interest in the study of medicinal plants of Kazakhstan's flora, special attention is given to species that remain poorly explored in terms of their chemical composition and pharmacognostic value. One such plant is Bryonia melanocarpa, which is considered a potential source of biologically active compounds. The aim of this study was to investigate the phytochemical and elemental composition of the aerial part of B. melanocarpa growing in the natural conditions of Kazakhstan to assess its raw material and therapeutic potential.

The study included an evaluation of the raw material's physicochemical parameters (moisture content – 9.39%, ash content – 7.50%, extract yield – 1.1087 g), spectral analysis of macro- and microelements, as well as chromatographic identification of organic compounds in the ultrasonic extract. The presence of physiologically important elements (K, Ca, Mg, Fe) and biologically active substances such as β-sitosterol, vitamin E, stigmastan-3,5-diene, and alkanes with a high degree of identification was established. The results confirm the pharmacognostic value of B. melanocarpa and substantiate its potential for further research as a promising source of natural compounds for phytotherapy.

Key words: *Bryonia melanocarpa*, phytochemical analysis, elemental composition, chromatography, biologically active compounds, medicinal plant, phytotherapy.

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Қазақстан флорасындағы *Bryonia melanocarpa* nabiev өсімдігіне жүргізілген фитохимиялық зерттеу және элементтік құрамы

Қазақстан флорасындағы дәрілік өсімдіктерді зерттеуге деген қызығушылықтың артуына байланысты, химиялық құрамы мен фармакогностикалық құндылығы жеткілікті зерттелмеген түрлерге ерекше назар аударылуда. Солардың бірі – биологиялық белсенді қасиеттерге ие болуы мүмкін *Bryonia melanocarpa* өсімдігі. Зерттеудің мақсаты – Қазақстанның табиғи жағдайында өсетін *B. melanocarpa* өсімдігінің жер үсті бөлігінің фитохимиялық және элементтік құрамын анықтап, оның шикізаттық және емдік әлеуетін бағалау. Жұмыс барысында өсімдік шикізатының физика-химиялық көрсеткіштері (ылғалдылығы – 9,39 %, күл мөлшері – 7,50 %, экстракт шығымы – 1,1087 г), макро- және микроэлементтердің спектрлік талдауы, сондай-ақ ультрадыбыстық экстракттағы органикалық қосылыстардың хроматографиялық идентификациясы жүргізілді. Зерттеу нәтижесінде физиологиялық маңызды элементтер (K, Ca, Mg, Fe) мен биологиялық белсенді заттар – β-ситостерол, Е дәрумені, stigmastan-3,5-diene және жоғары сәйкестікпен анықталған алкандардың бар екені анықталды. Алынған нәтижелер *B. melanocarpa* өсімдігінің фармакогностикалық құндылығын дәлелдейді және оны фитотерапияда қолдануға арналған табиғи қосылыстардың перспективті көзі ретінде әрі қарай зерттеудің маңыздылығын көрсетеді.

Түйін сөздер: *Bryonia melanocarpa*, фитохимиялық талдау, элементтік құрам, хроматография, биологиялық белсенді заттар, дәрілік өсімдік, фитотерапия.

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Фитохимическое исследование и элементный состав *Bryonia Melanocarpa* nabiev из флоры Казахстана

В условиях растущего интереса к изучению лекарственных растений флоры Казахстана особое внимание уделяется видам, недостаточно исследованным с точки зрения химического состава и фармакогностической ценности. Одним из таких растений является Bryonia melanocarpa, потенциально обладающее биологически активными свойствами. Целью настоящего исследования являлось установление фитохимического и элементного состава надземной части B. melanocarpa, произрастающей в природных условиях Казахстана, для оценки её сырьевого и терапевтического потенциала. В ходе работы определены физико-химические показатели растительного сырья (влажность – 9,39 %, зольность – 7,50 %, выход экстракта – 1,1087 г), проведён спектральный анализ макро- и микроэлементов, а также выполнена хроматографическая идентификация органических соединений в ультразвуковом экстракте. Установлено наличие физиологически значимых элементов (K, Ca, Mg, Fe) и биологически активных веществ, включая β-ситостерол, витамин E, stigmastan-3,5-diene и алканы с высокой степенью идентификации. Полученные результаты подтверждают фармакогностическую ценность B. melanocarpa и указывают на целесообразность её дальнейшего изучения как перспективного источника природных соединений для фитотерапии.

Ключевые слова: *Bryonia melanocarpa*, фитохимический анализ, элементный состав, хроматография, биологически активные вещества, лекарственное растение, фитотерапия.

Introduction

Since ancient times, humanity has turned to plants for medicinal purposes to meet its healthcare needs (Assefa et al., 2010). Today, approximately 80% of the world's population still relies on traditional herbal medicine, which is based on local knowledge, cultural traditions, and the use of medicinal plants growing in the surrounding environment (Belhouala et al., 2021).Some plant families possess high medicinal activity, are widely distributed, and exhibit significant species diversity – one such family is the Cucurbitaceae. This family includes nearly 100 genera, primarily found in tropical and subtropical regions (Kocyan et al., 2007). Among these genera, the genus Bryonia holds particular importance as a group of medicinal plants (Tutin,1964). *Bryonia* is one of the smallest genera within the Cucurbitaceae family (Suresh Kumar S. K. et al.,2008). It comprises 10 recognized species (*B. cretica* L., *B. multiflora* Boiss. & Heldr., *B. monoica* Aitch. & Hemsl., *B. aspera* Steven ex Ledeb., *B. alba* L., *B. lappifolia* Vassilcz., *B. melanocarpa* Nabiev, *B. syriaca* Boiss., *B. verrucosa* Aiton, and *B. flexuosa* Yıld.) and 3 recognized subspecies (*B. dioica* Jacq., *B. marmorata* (E. Petit) Jauzein, and *B. acuta* Desf.), which include both dioecious and monoecious forms (http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:30074180-2).

Moreover, all species of *Bryonia* are of Irano-Turanian origin and have successfully adapted to many regions of the Mediterranean (Volz & Renner, 2009). *Bryonia* was well known in ancient times for its medicinal properties. In fact, various diseases have traditionally been treated using *Bryonia* species, including infectious diseases, tissue inflammation, coughs, flu, lung diseases, cancer, hemorrhoids, diabetes, peritonitis, jaundice, typhoid, bone pain, as well as nervous and cardiac disorders (Benarba et al., 2024).

This wide range of therapeutic applications and medicinal properties of the genus can be attributed to the presence of noteworthy phytochemical constituents such as polyphenols, flavonoids, triterpenoid glycosides, sterols, saponins, and alkaloids (Ilhan et al.,2019).

Of particular interest is Bryonia melanocarpa, a perennial plant that is a rare, narrow endemic species of the southeastern Kyzylkum Desert. It is listed in the Red Data Book of Uzbekistan under category 3. The stems are thin, long, climbing, and cling to supports with the help of tendrils. The surface of the stems may be rough or nearly smooth. Bifid (forked) tendrils, a characteristic feature of Indian and some Asian species of the genus, are also observed in *B.*

melanocarpa. The leaves are alternate, on long petioles, with a palmately divided blade, most commonly having five lobes or angles. Leaf surfaces range from slightly pubescent to nearly glabrous, with toothed or slightly wavy margins.

The flowers are unisexual, small, yellowish-green, and grouped in axillary inflorescences. In dioecious plants, male and female flowers develop on separate individuals. In *B. melanocarpa*, the flowers are located in the leaf axils on short pedicels. The calyx is broadly bell-shaped with five teeth. The corolla is five-lobed and fused at the base. There are three stamens, located at the base of the calyx tube. Of the three anthers, two are bilocular and one is unilocular; the anther sacs are curved or S-shaped.

The fruit is a spherical, black, indehiscent berry. The seeds are oblong or ovoid, flattened, with a smooth or slightly rough surface (Rus et al.,2015).

The morphological characteristics of Bryonia melanocarpa – such as the black color of its berries, the structure of its stamens, and the shape of its seeds – distinguish this species from other members of the genus and make it easily identifiable in natural conditions (Benarba & Belhouala, 2024). It grows on stabilized sandy dunes and ridges, typically in shrublands dominated by white saxaul (Haloxylon persicum) and Calligonum species. The plant occurs rarely and sporadically, forming small populations of only a few dozen individuals. It is poisonous and not consumed by livestock.

The main threat to this species is the degradation of desert ecosystems caused by overgrazing and the cutting of saxaul and Calligonum for fuel (Tojibaev et al., 2021).

Thus, an in-depth study of the morphological, anatomical, chemical, and pharmacological characteristics of Bryonia melanocarpa represents a relevant and promising area of scientific research aimed at expanding the raw material base of medicinal plants and developing new phytotherapeutic agents. Despite its limited distribution range, this plant possesses high biological potential due to its rich chemical composition and content of physiologically active compounds. However, current data on the morphological structure, elemental composition, and pharmacological significance of *B. melanocarpa* remain extremely limited, underscoring the relevance and scientific importance of further research on this species.

Materials and methods

Plant Material and Species Identification

The aerial parts of *Bryonia melanocarpa* Nabiev were used as the study material. Plant material was collected during the active vegetation period in 2025 in the Kyzylkum Desert, Turkestan Region, Kazakhstan (42.197774° N, 67.792683° E) (Figure 1).

Figure 1
Flowering plant of Bryonia melanocarpa in the Kyzylkum Desert



Species identification was performed at the Department of Biodiversity and Bioresources, Al-Farabi Kazakh National University. Identification was based on diagnostic morphological characteristics using relevant taxonomic keys and comparison with herbarium specimens.

After collection, the plant material was dried in the shade at room temperature, mechanically crushed, and stored in paper bags under dry conditions until further analysis. The prepared material was subsequently used for the determination of physicochemical parameters, mineral composition, and phytochemical composition.

Determination of Moisture Content

Moisture content was determined gravimetrically as the mass loss of the plant material after drying to constant weight. This parameter represents the loss of hygroscopic moisture and other volatile substances under the specified drying conditions.

Approximately 1.0 g of pre-dried and powdered plant material was accurately weighed into a previously dried and weighed weighing bottle (büx). The sample was dried in a drying oven at 100–105 °C and repeatedly weighed until a constant mass was obtained.

The moisture content was calculated according to the following equation:

$$X = \frac{(M - M_1)}{M} * 100$$

where M is the mass of the initial plant material (g), and M_1 is the mass of the plant material after drying to constant weight (g) (Antonova et al., 2017).

Determination of Total Ash Content

Approximately 3.0 g of the powdered plant material was accurately weighed and evenly distributed in a previously ignited and weighed porcelain crucible. The sample was initially heated gradually on a hot plate to promote slow charring and removal of volatile components while minimizing the risk of sample loss.

Following preliminary charring, the crucible was transferred to a muffle furnace for further ashing. The sample was heated at approximately 500 °C until a constant mass was obtained. When incomplete combustion of the organic material was observed, the residue was cooled, moistened with distilled water or a saturated ammonium nitrate solution, evaporated on a water bath, and re-ignited. This procedure was repeated when necessary until complete ashing was achieved.

After ashing, the crucible was cooled in a desiccator and weighed. The total ash content was calculated on a dry-mass basis according to the following equation:

$$X = \frac{m * 100 * 100}{M_1 * (100 - W)}$$

where m is the mass of the plant sample (g), M_1 is the mass of the ash residue (g), and W is the percentage mass loss during drying (%) (Grigoryevna, 2017).

Determination of Mineral Element Composition

For elemental analysis, 5.0 g of accurately weighed powdered plant material was placed in a previously ignited and weighed porcelain, quartz, or platinum crucible. The sample was distributed evenly over the bottom of the crucible and gradually heated on a hot plate to ensure controlled charring and evaporation of volatile components.

The partially charred material was subsequently transferred to a muffle furnace for complete ashing. In cases where the ash contained incompletely combusted organic residues, the sample was cooled, moistened with distilled water or a saturated ammonium nitrate solution, evaporated on a water bath, and re-ignited. Ashing was continued at approximately 500 °C until a constant mass was obtained.

The resulting ash was subsequently calcined at 600 °C until a homogeneous grey residue was obtained. The crucible was then cooled in a desiccator before further analysis.

A control experiment was performed simultaneously using a solution containing the same acid concentration as that used for sample preparation.

Elemental composition was determined using the ASSIN atomic emission spectrometric system (Carl Zeiss, Germany). Approximately 300 mg of the ash residue was introduced into a direct-current arc, and the resulting emission spectra were recorded in the 2100–3600 Å wavelength range using a DFS-13 spectrograph with a reciprocal linear dispersion of 1 Å/mm. The analytical sensitivity ranged from 10^{-2} to 10^{-5}.

The accuracy of the analytical measurements was controlled using the certified copper sludge reference material SHM-M in accordance with TSO 2962-84 and TSO 2964-84 (Vdovenko-Martynova & Zhilina, 2019).

Preparation of Plant Extracts by Ultrasonic-Assisted Extraction

Ultrasonic-assisted extraction (UAE) was used to obtain extracts from the powdered aerial parts of *B. melanocarpa*. Ultrasonic treatment facilitates mass transfer by disrupting the diffusion boundary layer and enhancing penetration of the extraction solvent into plant tissues, thereby promoting the release of biologically active compounds.

Briefly, 5.0 g of powdered plant material was transferred into a 200-mL flask, and 40 mL of 70% (v/v) ethanol was added at a plant material-to-solvent ratio of 1:8 (w/v). The suspension was subjected to ultrasonic treatment three times for 1 h per cycle using a KQ5200B ultrasonic bath operating at a frequency of 40 kHz.

Following extraction, the combined extract was filtered through filter paper and concentrated to dryness using an EYELA N-1300 rotary evaporator at 50 °C (Popova & Potoroko, 2018).

GC–MS Analysis of Organic Compounds

The chemical composition of the ethanol extract was determined by gas chromatography coupled with mass spectrometry (GC–MS) using an Agilent 7890A gas chromatograph coupled to a 5975C mass selective detector (Agilent Technologies, USA).

A 0.5-µL aliquot of the extract was injected at an injector temperature of 280 °C in splitless mode. Chromatographic separation was performed using a DB-35MS capillary column (30 m × 0.25 mm i.d.; film thickness, 0.25 µm). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min.

The oven temperature was initially set at 60 °C and then increased to 280 °C at a rate of 5 °C/min, with a final hold time of 15 min. The initial temperature was not held (0 min).

Mass spectra were acquired in SCAN mode over an *m/z* range of 34–750. Instrument control, data acquisition, and chromatographic data processing were performed using Agilent MSD ChemStation software (version 1701EA).

The obtained chromatograms were evaluated based on retention times and peak areas. Individual compounds were tentatively identified by comparison of their mass spectra with reference spectra in the Wiley 11th Edition and NIST'02 mass spectral libraries. The libraries contained more than 550,000 reference spectra. Compound identification was based on the degree of spectral similarity between the experimental and library spectra (Maksotovna, 2023).

Results and discussion

A comprehensive analysis of the qualitative and chemical composition of the plant material *B. melanocarpa* was conducted during the study. At the first stage, the main physicochemical parameters of the plant material were determined, and its suitability for storage and processing was evaluated. The obtained qualitative characteristics are presented below (Table 1).

Table 1
Results of Authenticity Analysis of Plant Material

Name of Parameter	Quantitative Results
Moisture Content	9.39 %
Ash Content	7.50 %
Extract	1.1087 г

Physicochemical Characteristics of the Plant Material

The main physicochemical characteristics of the aerial parts of *B. melanocarpa* are presented in Table 1. The moisture content of the dried plant material was 9.39%, while the ash content was 7.50%. These parameters characterize the physicochemical state of the investigated plant material and provide baseline information for its further analytical processing.

The extraction of 5 g of dry plant material with 70% ethanol yielded 1.1087 g of dry extract, corresponding to an extraction yield of 22.17%. The obtained yield indicates that a measurable proportion of the plant material was extracted under the applied ultrasonic extraction conditions.

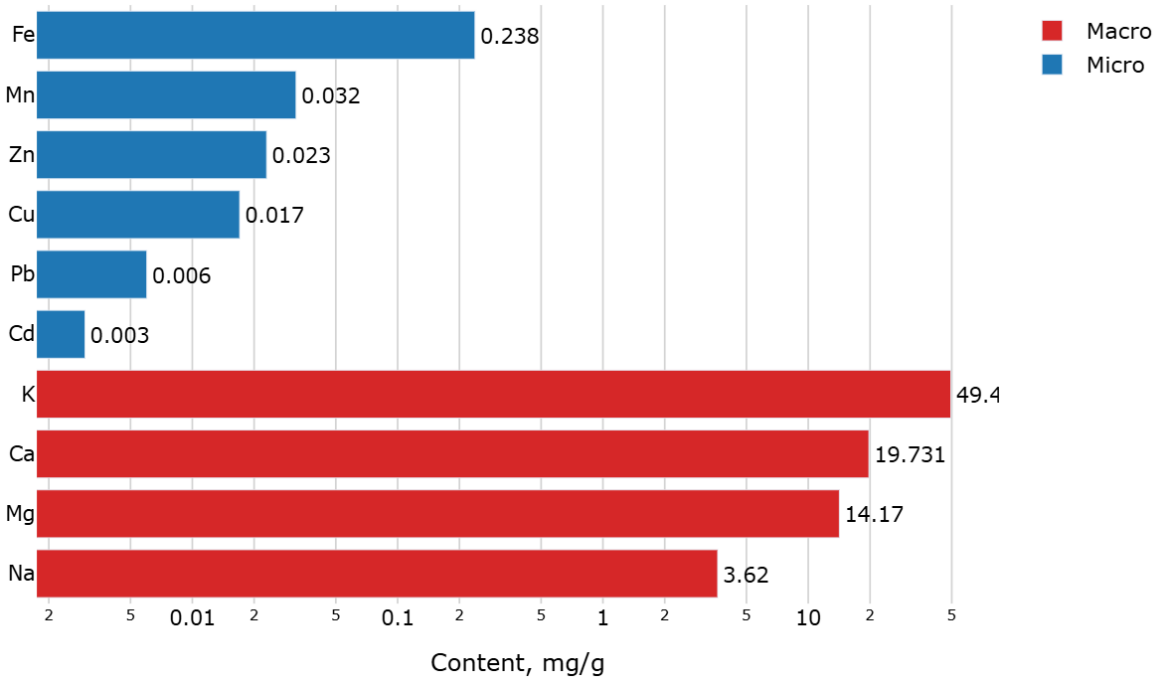
However, these values should be interpreted as characteristics of the investigated sample and extraction procedure rather than as evidence of compliance with pharmacopoeial requirements, since specific quality limits for *B. melanocarpa* were not established or cited in the present study.

Elemental Composition

The elemental composition of *B. melanocarpa* was determined after mineralization/ashing of the plant material. The concentrations of the detected elements are presented in Figure 2.

Figure 2

Content of Macro- and Microelements in the Ultrasonic Extract of B. melanocarpa (mg/g) Macrolements, Microelements



Note: Ni was not detected (0.000 mg/g).

Among the analyzed macroelements, potassium showed the highest concentration (49.476 mg/g), followed by calcium (19.731 mg/g), magnesium (14.170 mg/g), and sodium (3.620 mg/g). Among the investigated trace elements, iron had the highest concentration (0.238 mg/g), followed by manganese (0.032 mg/g), zinc (0.023 mg/g), copper (0.017 mg/g), lead (0.006 mg/g), and cadmium (0.003 mg/g). Nickel was not detected under the applied analytical conditions.

The obtained data characterize the elemental composition of the investigated plant material. The presence of potassium, calcium, magnesium, and iron indicates that these elements constitute measurable mineral components of the aerial parts of *B. melanocarpa*. However, elemental composition alone does not provide evidence of a specific physiological or therapeutic effect of the plant, and therefore the detected concentrations should be considered primarily as compositional characteristics.

In addition to the mineral composition, chromatographic analysis was performed to characterize the organic constituents of the B. melanocarpa extract. The identified compounds and their relative contents are presented in Table 2, while the corresponding GC–MS chromatogram is shown in Figure 3.

Table 2

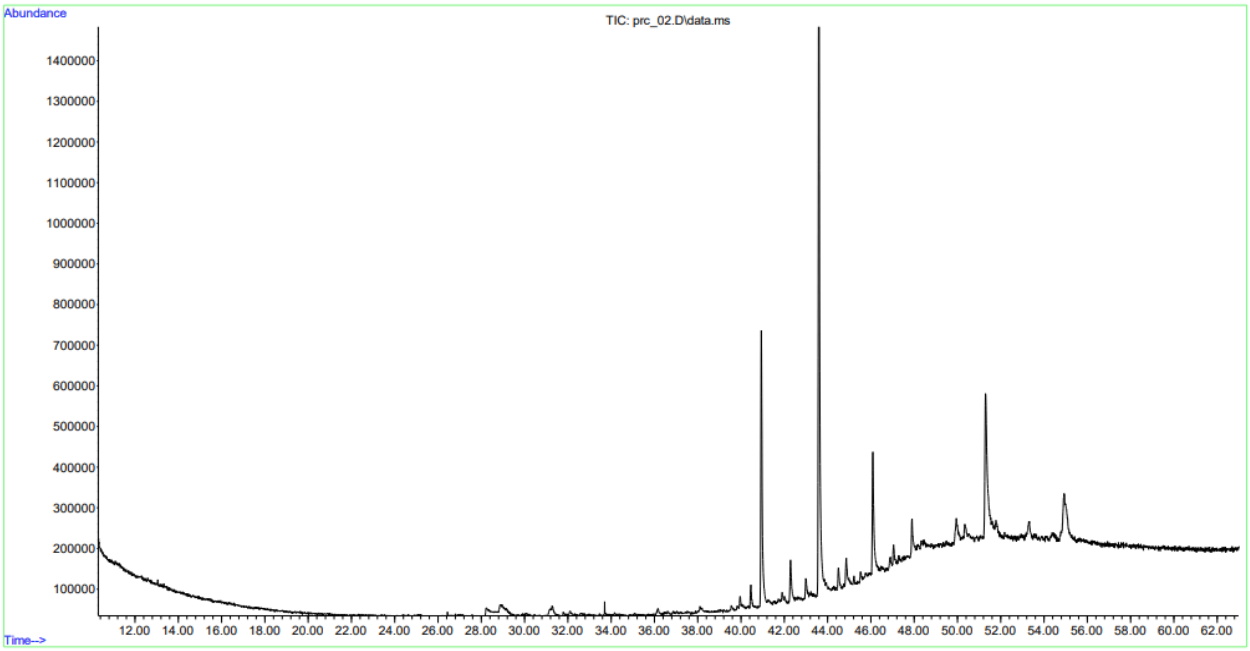
Results of Chromatographic Analysis of Organic Compounds in the Plant Extract

No.	Retention Time, min	Compound	Peak Area	Identification, %	Percentage Content, %
1	31.28	Phthalic acid, butyl hex-3-yl ester	3,367,671	78	2.105
2	39.96	Diisooctyl phthalate	1,770,510	66	1.107
3	40.94	Octacosane	28,289,057	89	17.686
4	43.60	Hexacosane	72,809,008	92	45.519
5	46.09	Tetratetracontane	14,691,274	80	9.185
6	47.05	Stigmastan-3,5-diene	2,388,585	65	1.493

Continuation of the table

No.	Retention Time, min	Compound	Peak Area	Identification, %	Percentage Content, %
7	47.90	Vitamin E	3,853,201	72	2.409
8	51.30	β-Sitosterol	32,784,891	84	20.496

Figure 3
Chromatogram of B. melanocarpa Extract



GC–MS Profile of the Ethanol Extract

GC–MS analysis of the 70% ethanol extract of *B. melanocarpa* revealed eight major chromatographic components with library spectral matches ranging from 65% to 92% (Table 2). The identified compounds belonged predominantly to long-chain alkanes and sterol-related compounds.

Hexacosane represented the major detected component, accounting for 45.519% of the total chromatographic peak area. β-Sitosterol was the second most abundant component (20.496%), followed by octacosane (17.686%) and tetratetracontane (9.185%). Lower relative contents were observed for vitamin E (2.409%), phthalic acid, butyl hex-3-yl ester (2.105%), diisooctyl phthalate (1.107%), and stigmastan-3,5-diene (1.493%).

The high relative abundance of hexacosane and other long-chain hydrocarbons indicates that these compounds constitute major components of the analyzed GC–MS profile. However, their biological significance for *B. melanocarpa* cannot be established solely from their detection in the extract.

β-Sitosterol has been widely investigated as a phytosterol, and previous studies have reported anti-inflammatory, immunomodulatory, and lipid-related biological activities for this compound (Ostlund, 2002). Similarly, vitamin E is recognized for its antioxidant properties and its role in protecting biological membranes from oxidative damage (Traber & Atkinson, 2007). In the present study, these compounds were detected in the *B. melanocarpa* extract; however, their reported biological activities should not be interpreted as experimentally demonstrated pharmacological effects of the whole plant extract.

The long-chain hydrocarbons hexacosane and octacosane have been reported in the chemical profiles of various plant species. Their detection in *B. melanocarpa* contributes to the characterization of the species' phytochemical profile. Further studies using purified compounds and biological assays are required to determine whether these constituents contribute to any pharmacological activity of the plant.

Two phthalate derivatives, phthalic acid, butyl hex-3-yl ester and diisooctyl phthalate, were also detected. Because phthalate compounds can originate from plastic laboratory materials, tubing, solvents, containers, or other sources of analytical contamination, their occurrence should be interpreted cautiously. Confirmation of these compounds as genuine endogenous constituents of *B. melanocarpa* would require additional analyses using appropriate procedural blanks and independently prepared samples.

The chromatographic profile therefore provides preliminary information on the chemical composition of the 70% ethanol extract of *B. melanocarpa*. Nevertheless, library-based GC–MS identification should be considered tentative unless supported by authentic standards and/or additional spectroscopic confirmation.

Conclusion

The present study provides preliminary data on the physicochemical, elemental, and phytochemical characteristics of the aerial parts of *Bryonia melanocarpa* Nabiev collected in the Kyzylkum Desert, Kazakhstan.

The moisture content of the investigated plant material was 9.39%, while the ash content was 7.50%. Extraction with 70% ethanol yielded 1.1087 g of dry extract from 5 g of plant material, corresponding to an extraction yield of 22.17% under the applied ultrasonic extraction conditions.

Elemental analysis demonstrated the presence of potassium, calcium, magnesium, sodium, iron, manganese, zinc, copper, lead, and cadmium, whereas nickel was not detected under the applied analytical conditions. These results contribute to the characterization of the mineral composition of *B. melanocarpa*.

GC–MS analysis of the ethanol extract revealed eight major chromatographic components, with hexacosane, β-sitosterol, octacosane, and tetratetracontane representing the predominant detected compounds. Vitamin E and stigmastan-3,5-diene were also detected. The identification of phthalate derivatives requires cautious interpretation because their presence may be associated with analytical or laboratory contamination.

The results expand the available chemical information on the poorly investigated species *B. melanocarpa*. Nevertheless, the present study provides compositional rather than pharmacological evidence. Further investigations, including analysis of additional populations, replicated sampling, confirmation of GC–MS identifications using authentic standards, assessment of potential contaminants, and biological activity assays, are required to evaluate the pharmacological potential of the species.

Author contributions

Kulvira Zharylkasynovna Adilova: Conceptualization, Investigation, Methodology, Writing – review and editing; Talgat Saduakasovich Ibragimov: Supervision, Validation, Data curation; Zhasulan Koilybayev: Writing – original draft, Formal analysis; Aliya Yerkenovna Kusniyeva: Formal analysis; Nazerke Baqytqyzy Milissova: Software, Visualization.

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