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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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STUDY OF THE CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF SUBCRITICAL CO_2 EXTRACT FROM *EUPHORBIA HUMIFUSA* WILLD

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Abstract.

Introduction: One of the priority areas of development in pharmacy is the study of local plants possessing antimicrobial activity. In this regard, determining the chemical composition of the CO_2 extract of *Euphorbia humifusa* Willd. with the aim of identifying new promising compounds with antimicrobial and antifungal activity is a pressing issue.

Materials and methods: Using subcritical carbon dioxide extraction, 23 g of CO_2 extract of *Euphorbia humifusa* Willd. was obtained. To determine the potential use of *Euphorbia humifusa* Willd., we analysed the composition of the extract using GC-MS and the antimicrobial activity obtained by CO_2 extraction.

Results: For the first time, a comprehensive study of the CO_2 extract from the aerial parts of *Euphorbia humifusa* Willd., collected during the flowering phase in the Bostandyk district of Almaty Region, was conducted. Using gas chromatography-mass spectrometry (GC/MS) with Wiley 7th edition and NIST'02 libraries, 47 components were identified.

Conclusions: The obtained CO_2 extract of *Euphorbia humifusa* Willd. can be used in pharmaceutical technology as a substance.

Key words. *Euphorbia humifusa* Willd, GC-MS analysis, CO_2 extraction, antimicrobial activity.

Introduction.

In Kazakhstan, ensuring the population's access to affordable medical and pharmaceutical services is one of the most important areas of state policy, in accordance with the objectives of the National Medical Policy [1,2], which represents a complex integrative structure [3]. Implementing this policy requires a comprehensive approach that includes not only effective personnel management [4]—such as planning for seasonal workforce needs [5], developing communication skills [6], or forming pharmaceutical clusters [7]—but also the strategic development of the pharmaceutical industry itself as a high-profit and rapidly growing economic sector, serving as a driver for the country's socio-economic development [8,9].

A key challenge in achieving these goals is the high dependence of Kazakhstan's pharmaceutical market on imported products. In this regard, a priority task is the development of the national pharmaceutical industry [10] by increasing the volume of domestic production. One of the most promising and strategically important directions in this context is the development and manufacture of medicines based on domestic plant materials.

Thus, the scientific assessment of the potential of local flora and the screening of Kazakhstan's biodiversity for the presence of biologically active compounds become practical tools for addressing national tasks. This line of work directly contributes to import substitution, the formation of economic independence and national security [11,12], as well as opens new opportunities for the development of evidence-based phytotherapy and the creation of effective medicines [13,14].

Currently, many promising plant species native to Kazakhstan are already in focus of scientific interest, such as *Nicotiana Tabacum L.* [15], *Echinops Ritro* [16], *Portulaca Oleracea L.* [17], *Brassica Napus L.* [18], *Plantago Major L.* [19], representatives of the *Apiaceae* family [20], *Ferula Asafoetida L.* [21,22] and others. Within this strategic direction and with the aim of further expanding the raw material base of the domestic pharmaceutical industry, this study focuses on the species *Euphorbia humifusa* Willd. This species was selected as the main object of scientific analysis due to its rich content of biologically active compounds and diverse pharmacological potential, making it a promising source for the development of new medicines.

The studied plant, *Euphorbia humifusa* Willd., belongs to the genus Euphorbia of the family *Euphorbiaceae*. The *Euphorbiaceae* family is very diverse in terms of range, distribution and morphology and consists of a variety of species that grow on the mainland as shrubs, weeds, trees or climbing vines. *Euphorbia* is one of the largest genera of flowering plants with more than 2,000 species [23]. One of the species of the genus *Euphorbia* is the annual herbaceous plant *Euphorbia humifusa* Willd. (Figure 1).

Research of herbarium specimens confirmed the wide distribution of *Euphorbia humifusa* Willd. in the flora of Kazakhstan. Analysis of the herbarium specimens showed the widespread distribution of *Euphorbia humifusa* Willd. in the territories of Almaty Region, Zhambyl Region, Mangistau Region, and Central Kazakhstan Region (Figure 2).

Scientific studies have revealed that the key components of *Euphorbia humifusa* Willd. are flavonoids, triterpenes, coumarins, sterols, tannins and phenolic acid derivatives [24].

A literature review demonstrated that various extracts and isolated compounds from *Euphorbia humifusa* Willd. possess a broad spectrum of pharmacological activities, such as antimicrobial activity [25], anti-vitiligo effects [26], anti-tumor effects [27], antidiabetic effect [28], anti-influenza activity [29] and hepatoprotective effects [30].

The aim of this study is to analyse the chemical components and evaluate the antimicrobial properties of CO_2 extract from *Euphorbia humifusa* Willd. with a view to using, it in the future to create medicinal products that demonstrate antimicrobial and antifungal efficacy.

To achieve the stated objective of the study, we have defined the following tasks:

1. Obtain a CO_2 extract from the plant *Euphorbia humifusa* Willd.

Figure 1. *Picture of Euphorbia humifusa Willd.*

A - *Collected plant;* ***B -*** *Plant flowers;* ***C -*** *Plant stem.*

Figure 2. *Distribution area of Euphorbia humifusa Willd. within the Republic of Kazakhstan.*

2. Perform a comprehensive chemical analysis of the CO_2 extract of *Euphorbia humifusa* Willd. to identify the main bioactive compounds using gas chromatography-mass spectrometry (GC/MS) and to quantify the key components of the extract.

3. Evaluate the antimicrobial activity of the studied extract against Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*), and Fungal microorganism (*Candida albicans*).

Materials and Methods.

Plant raw material:

Euphorbia humifusa Willd. was collected in autumn (August-September) 2024 in Almaty, Kazakhstan, in accordance with the requirements of Good Agricultural and Collection Practice (GACP). The plant material was identified at the Republican State Enterprise 'Institute of Botany and Phytointroduction' of the Committee for Forestry and Wildlife of the Ministry of Ecology, Geology and Natural Resources of the Republic of Kazakhstan (Reference registration number No. 01-05 / 490).

CO_2 extraction procedure:

For our research, we chose subcritical CO_2 extraction to obtain extract.

Traditional extraction methods have demonstrated limited efficiency, and the resulting extracts contained impurities and residues of organic solvents, which significantly reduced their potential for practical application [31]. As an alternative, subcritical CO_2 extraction technology was chosen, which complies with the principles of green chemistry and modern environmental requirements [32]. This method, being thermosensitive, ensures the gentle extraction of thermolabile and easily oxidisable biologically active components [33].

The extraction was carried out on a carbon dioxide extraction unit (CO_2-5L flow extraction system) at the production facility of "Zhanapharm" Company (a pharmaceutical enterprise, Almaty, Kazakhstan). The process was conducted in a subcritical mode in accordance with the requirements of the National Standard 27658-1910 – LLP-02-2011. Liquefied carbon dioxide (GOST 8050-85) was used as the extractant.

Chemical detection of biologically active compounds:

After obtaining the CO_2 extract, its component composition was analysed using gas chromatography with mass spectrometric detection.

The chemical analysis of the component composition was performed at the Al-Farabi Kazakh National University in the Centre for Analytical, Colloid Chemistry and Rare Element Technology. The study was conducted using gas chromatography with mass spectrometric detection (GC-MS) using an Agilent 7890A/5975C chromatographic system [34].

Chromatographic analysis conditions: sample volume 0.5 µl, sample injection temperature 260°C, no split flow. Separation was performed using a DB-17ms capillary column 30 m long, with an internal diameter of 0.25 mm and a film thickness of 0.25 µm at a constant carrier gas (helium) flow rate of 1 ml/min. The chromatography temperature was programmed from 60°C with a heating rate of 20°C/min to 120°C, then with a heating rate of 10°C/min to 270°C (holding time 10 min) and with a heating rate of 5°C/min to 300°C (holding time 10 min). The analysis time was 44 minutes. Detection was performed in SCAN m/z 34-750 mode. Agilent MSD ChemStation software (version 1701EA) was used to control the gas chromatography system and to record and process the results and data obtained. Data processing included determining retention times, peak areas, and processing spectral information obtained using a mass spectrometric detector. The Wiley 7th edition and NIST'02 libraries (total number of spectra in the libraries – over 550,000) were used to decipher the obtained mass spectra.

Antimicrobial activity determination:

The antimicrobial activity of the extract was tested by the microbiological laboratory of the Scientific Centre for Anti-Infective Drugs JSC, Almaty, Kazakhstan, accredited by the accreditation system of the Republic of Kazakhstan for compliance with the requirements of GOST ISO/IEC 17025-2019 'General requirements for the competence of testing and calibration laboratories (IK)' (No. KZ. T.02.1395 dated 2 October 2018).

The following culture media were selected: Mueller-Hinton broth M 391 (Himedia, India), Mueller-Hinton agar M 173 (Himedia, India), sodium chloride (Mikhailovsky Chemical Reagents Plant, Russia), 96% ethanol (Talgar Alcohol, Kazakhstan), and purified water.

As test strains, microorganisms obtained from the Republican Collection of Microorganisms (RCM, Astana, Kazakhstan) and the American Type Culture Collection (ATCC, USA) were used: the Gram-positive bacterium *Staphylococcus aureus* ATCC 6538-P (ATCC, Kazakhstan); the Gram-negative bacterium *Escherichia coli* ATCC 8739 (ATCC, USA) and the fungus *Candida albicans* ATCC 10231 (ATCC, USA).

The assessment of the antimicrobial activity of the extract was conducted in accordance with the internal methodological guidelines MI-LM-02 "Determination of the Bactericidal Action of Antimicrobial Agents by the Method of Double Serial Dilutions" (Methodological Instructions of Microbiology Laboratories) [35]. The study employed the method of double serial dilutions in liquid nutrient medium and the disk-diffusion method.

The diameters of the inhibition zones were measured using a caliper with an accuracy of 0.5 mm. The data are presented as mean value ± standard deviation (SD).

All methodologies complied with the standards of the Clinical and Laboratory Standards Institute (CLSI, USA) [36].

Results.

Preparation of plant raw materials *Euphorbia humifusa* Willd. for extraction:

The plant material was dried at room temperature (25±5°C) in a ventilated room at the «Institute of Botany and Phytointroduction». After drying, the raw material was ground to a size of 1–3 mm using an IKA M20 laboratory mill.

Technological parameters of CO_2 extraction and storage of the finished *Euphorbia humifusa* Willd. Extract:

The extraction of the aerial parts of *Euphorbia humifusa* Willd. with carbon dioxide was carried out under the following conditions: the extraction temperature was +18+23°C, the operating pressure was maintained at 57-65 atmospheres, and the process duration was 8 hours. The extract yield reached 23 grams.

The CO_2 extract of *Euphorbia humifusa* Willd. must be stored in a dark, cool place in an airtight glass container at a temperature not exceeding +25°C, protected from direct sunlight. The shelf life under these conditions is two years.

Biologically active compounds of the *Euphorbia humifusa* Willd. CO_2 extract:

An analysis of the chemical composition of the *Euphorbia humifusa* Willd. CO_2 extract using gas chromatography-mass spectrometry (GC-MS) revealed 47 chemical compounds. The chromatogram and the list of identified compounds are presented in Figure 3 and Table 1.

GC-MS analysis of the CO_2 extract of *Euphorbia humifusa* Willd. revealed the presence of fatty acids and their derivatives, terpenes and isoprenoids, steroids and triterpene compounds, tocopherols and vitamins, hydrocarbons (alkanes and alkenes), lactones, and other organic components. The following compounds were identified during the study: among fatty acids and their derivatives, Hexadecanoic acid (9.57 %), Octadecanoic acid (3.99 %), 9,12-Octadecadienoic acid (5.55 %) and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (linolenic acid) (17.78 %); in the group of hydrocarbons, Tetratriacontane (6.45 %) was identified; Among steroids and triterpenoids, γ-Sitosterol (8.25 %), β-Amyrin (2.46 %), Lupeol (11.08 %) and Lanosterol (2.28 %) were identified; in the group of terpenes and isoprenoids, Phytol (4.26 %) was identified.

Results of antimicrobial activity of CO2 extract of *Euphorbia humifusa* Willd.

The aim of this study was to evaluate the antimicrobial activity of CO_2 extract of *Euphorbia humifusa* Willd. The testing was carried out using double serial dilutions in broth and agar diffusion against Gram-positive (*Staphylococcus aureus* ATCC 6538-P) and Gram-negative (*Escherichia coli* ATCC 8739) microorganisms and yeast-like fungi (*Candida albicans* ATCC 10231). The results of the study are presented in Table 2 and Figure 4.

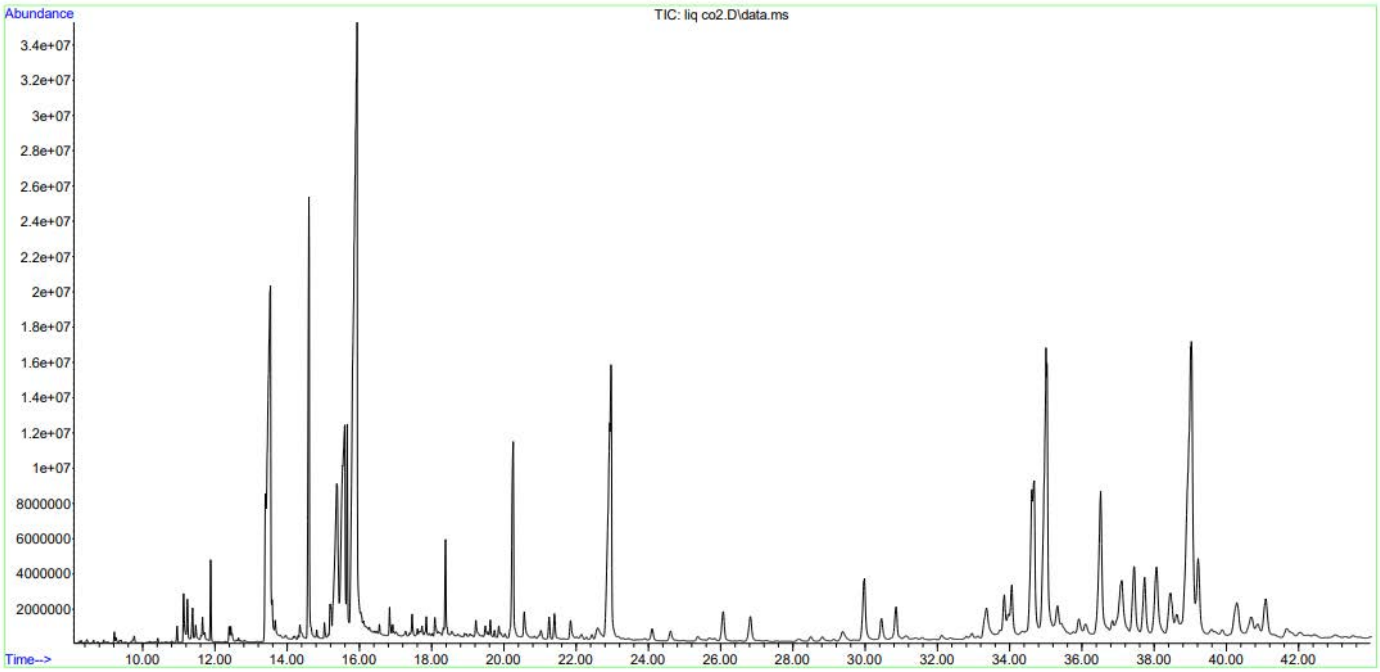
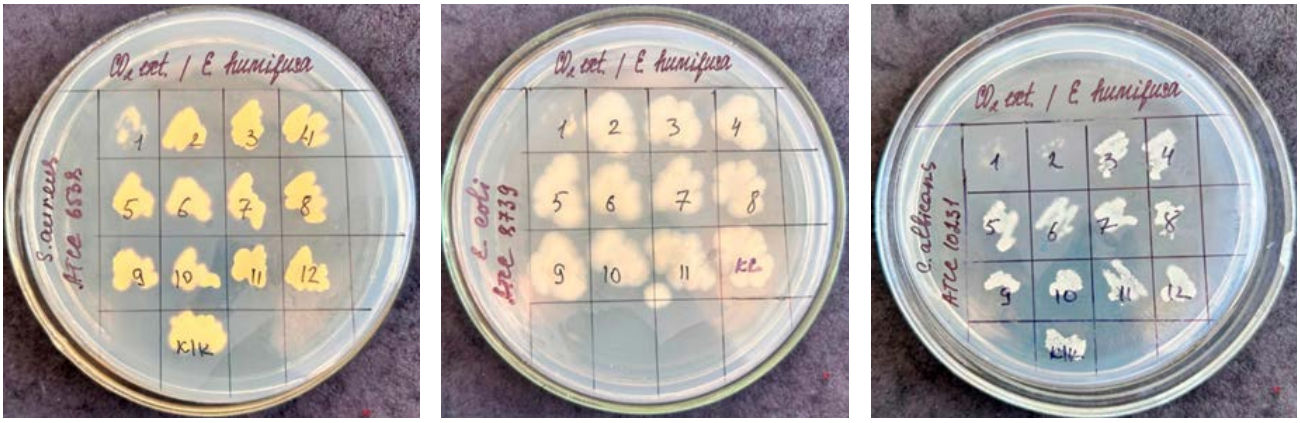


Figure 3. *Chromatogram of CO_2 extract of Euphorbia humifusa Willd.*



Staphylococcus aureus ATCC 6538-P *Escherichia coli* ATCC 8739 *Candida albicans* ATCC 10231

Figure 4. *Results of antimicrobial activity of CO_2 extract of Euphorbia humifusa Willd.*

Staphylococcus aureus ATCC 6538-P *Escherichia coli ATCC 8739* *Candida albicans ATCC 10231*

Figure 5. *Results of antimicrobial activity of CO_2 extract of Euphorbia humifusa Willd. against Staphylococcus aureus ATCC 6538-P, Escherichia coli ATCC 8739 and Candida albicans ATCC 10231.*

Table 1. *Results of GC-MS analysis of CO_2 extract of Euphorbia humifusa Willd.*

No.	Retention time, min	Compounds	Probability of identification, %	Percentage content, %
1	9.22	Dodecanoic acid	88	0.08
2	9.27	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-	83	0.05
3	9.77	Vanillin	80	0.11
4	10.42	Caryophyllene oxide	73	0.03
5	10.96	Fumaric acid, ethyl 2-methylallyl ester	74	0.12
6	11.14	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	87	0.46
7	11.24	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	90	0.40
8	11.38	Tetradecanoic acid	90	0.31
9	11.89	2-Pentadecanone, 6,10,14-trimethyl-	93	0.52
10	12.40	Pentadecanoic acid	82	0.11
11	13.53	Hexadecanoic acid	94	9.57
12	13.67	Oleic Acid	80	0.36
13	14.36	Heptadecanoic acid	79	0.39
14	14.60	**Phytol**	91	4.26
15	15.38	**Octadecanoic acid**	82	3.99
16	15.59	9,12-Octadecadienoic acid (Z,Z)-	92	5.55
17	15.67	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	96	1.57
18	15.93	**9,12,15-Octadecatrienoic acid, (Z,Z,Z)**	93	17.78
19	16.84	Pentacosane	89	0.37
20	17.46	4,8,12,16-Tetramethylheptadecan-4-olide	84	0.32
21	18.39	Heptacosane	91	1.08
22	19.49	17-Pentatriacontene	75	0.19
23	20.25	Nonacosane	83	2.77
24	21.26	Squalene	92	0.23
25	21.40	Triacontane	90	0.30
26	22.97	Tetratriacontane	90	6.45
27	24.11	1-Pentatriacontanol	77	0.20
28	25.37	Dotriacontane	85	0.09
29	26.07	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	78	0.59
30	28.50	γ-Tocopherol	72	0.09
31	28.82	1,30-Triacontanediol	67	0.11
32	29.98	α-Tocopherolquinone	75	1.40
33	30.46	Vitamin E	91	0.46
34	33.36	Campesterol	86	1.16
35	33.86	Stigmasterol	82	0.89
36	34.68	4α,14-Dimethyl-5α-ergosta-8,24(28)-dien-3β-ol	87	2.79
37	35.01	**γ-Sitosterol**	90	8.25
38	35.33	Phytonadione	76	0.99
39	37.11	**β-Amyrin**	87	2.46
40	37.45	9,19-Cyclolanost-24-en-3-ol, (3β)-	90	1.88
41	37.74	6a,14a-Methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy-	78	1.55
42	38.07	Lanosterol	85	2.28
43	38.45	9,19-Cyclolanostan-3-ol, 24-methylene-, (3β)-	80	1.44
44	39.03	**Lupeol**	91	11.08
45	39.22	D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3β)-	81	2.13
46	40.29	Stigmasta-4,24(28)-dien-3-one, (24E)-	84	1.48
47	41.10	Lanosterol	80	1.28

Table 2. *Results of testing the antimicrobial activity of the Euphorbia humifusa Willd. CO_2 extract.*

Test strains	MIC values, mg/mL	Result (growth inhibition)
Staphylococcus aureus ATCC 6538-P	**1 000**	+
Escherichia coli ATCC 8739	**1 000**	+
Candida albicans ATCC 10231	**1 000**	+

**MIC values are expressed in mg/mL. The initial extract concentration was 1000 mg/mL.*

Table 3. *Antimicrobial activity zones of CO_2 extract of Euphorbia humifusa Willd.*

Test strains	Zone of inhibition, mm
	Average value
Staphylococcus aureus ATCC 6538-P	15.33 ± 1.15
Escherichia coli ATCC 8739	14.0 ± 0.5
Candida albicans ATCC 10231	11.67 ± 0.58

The data obtained show that the CO_2 extract of *Euphorbia humifusa* Willd. has the same antimicrobial and antifungal activity, with a MIC value of 1000 mg/mL against all test strains: *Staphylococcus aureus* ATCC 6538-P, *Escherichia coli* ATCC 8739 and *Candida albicans* ATCC 10231.

The results of testing the antimicrobial activity of the test sample using the agar diffusion method are presented in Figure 5 and Table 3.

The results of the study of the antimicrobial activity of the CO_2 extract of *Euphorbia humifusa* Willd., presented in Table 3 and Figure 5, demonstrate the following indicators:

1. Against *Staphylococcus aureus* ATCC 6538-P an inhibition zone of 15.33 ± 1.15 mm was recorded.
2. Against *Escherichia coli* an inhibition zone of 14.0 ± 0.5 mm was observed.
3. Antifungal activity against *Candida albicans* ATCC 10231 is demonstrated by an inhibition zone of 11.67 ± 0.58 mm.

Discussion.

Our results confirm that the composition of biologically active substances in *Euphorbia humifusa* Willd. extract varies significantly depending on ecological growth conditions, the phenological phase at the time of raw material collection and technological extraction parameters. This variability directly affects the severity of the antimicrobial effect, highlighting the need to standardise both the raw materials and the extraction processes to ensure consistent pharmacological action. Our data on antimicrobial activity are consistent with reports from several authors [25,37-39].

Previous studies have shown that triterpenoids play a key role in the formation of antimicrobial properties and are among the main secondary metabolites of the species [40]. As part of our ongoing search for natural compounds with antimicrobial activity in the CO_2 extract of *E. humifusa's* aerial parts, we isolated nine triterpenoid compounds. This correlates well with the data of Jianbo Lin et al. [23], who identified two active triterpenoid components in the methanol fraction of petroleum ether – cycloart-23E-en-3β-25-diol and 24-methylene cycloartanone — with pronounced antimicrobial activity, as well as with the results of Xia R. F. et al. [41], who demonstrated the antimicrobial effect of isolated triterpenoids against several of clinically significant strains (*Staphylococcus aureus, S. epidermidis, Bacillus cereus, Listeria monocytogenes, Escherichia coli, Enterobacter sakazakii*). Reports by Li Z. J. et al. [42] on the antifungal activity of ellagic acid (EA) from *E. humifusa* against *Candida* and *Trichophyton rubrum strains*, both in vitro and in vivo, expand the range of potential targets for extracts of this species. Additionally, fractionated extracts (petroleum ether, chloroform, ethyl acetate, butanol, and residual methanol) demonstrated activity against *Staphylococcus aureus, Escherichia coli* and *Candida albicans* [43], confirming the contribution of several chemical classes to the cumulative effect.

Our extract was found to contain Lupeol (11.08%) and β-amyrin (2.46%), which have been previously described as possessing antimicrobial activity [44]. A significant proportion of the components were also fatty acids, including Hexadecanoic acid (9.57 %) and 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (linolenic acid) (17.78 %). Such acids are known to disrupt the integrity of microbial cell membranes, causing a pronounced antimicrobial effect [45]. The biological effect we observed is likely the result of the combined action of triterpenoids and lipophilic acids, including possible synergism.

Methodological aspects also proved critical for interpreting the activity. In the serial dilution test, the extract exhibited a bacteriostatic effect only in its undiluted form inhibitory concentration (MIC) of 1000 mg/mL, whereas no activity was observed at 500 mg/mL. It is important to directly note the key methodological limitation identified in our study: significant antimicrobial activity of the extract was observed only at very high concentrations (MIC = 1000 mg/mL). We interpret the sharp loss of effect upon dilution not as an absence of potentially active compounds, but rather as a direct indication of their extremely low solubility, stability, or bioavailability in the aqueous environment of the test system used. This fundamental limitation of the current experimental model implies that revealing the full therapeutic potential of the extract requires further research. On the other hand, the disk-diffusion method revealed significant antimicrobial activity for the CO_2 extract, which may reflect locally high concentrations near the agar surface and/or other specific diffusion patterns of lipophilic molecules in a solid phase. A comparison of our data with reports on ethanolic extracts [25,46] and fractions of varying polarity [47] suggests that optimizing the composition (enrichment with active triterpenoid fractions) and the physicochemical parameters of the carrier formulation could enhance its in vitro efficacy and reproducibility.

The practical significance of the obtained results lies in substantiating the rationale for the targeted standardization of *E. humifusa* extracts based on triterpenoid markers (e.g., lupeol, β-amyrin, cycloartane derivatives) while controlling the content of fatty acids. To overcome the limitation associated with the requirement for high concentrations in broth tests, further purification and fractionation of the extract, as well as the development of pharmaceutical formulations enhancing the solubility and bioavailability of lipophilic components (including solubilization approaches), appear promising. At the next stage, it would be advisable to determine quantitative activity parameters (MIC/MBC, MFC), investigate potential synergy between the isolated triterpenoids and fatty acids, and evaluate the activity against an expanded panel of clinically significant bacterial and fungal pathogens.

Overall, our results are consistent with existing literature data [23,25,37-46] and confirm the potential of *E. humifusa* as a source of antimicrobial compounds. The variable phytochemical profile remains a key factor determining the severity of the effect and requires attention to raw material standards and extraction technology, which is important for subsequent translation into applied developments.

Conclusion.

For the first time, a subcritical CO_2 extract (yield 23 g) was obtained from the aerial parts of *Euphorbia humifusa* Willd. harvested in the Almaty Region, using gentle extraction parameters (18-23°C, 57-65 atm, 8 h). This approach ensures the extraction of thermolabile lipophilic metabolites and guarantees a reproducible and scalable process.

According to GC-MS data, 47 compounds were identified, with fatty acids and their derivatives (including 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (linolenic acid) 17.78 %; Hexadecanoic acid, 9.57 %), triterpenoids (Lupeol, 11.08 %), phytosterols (γ-sitosterol, 8.25 %), and isoprenoids (Phytol, 4.26 %) being the dominant components, collectively forming the characteristic lipophilic profile of the extract.

Antimicrobial testing confirmed the extract's activity against clinically relevant test strains: inhibition zones measured 15.33 ± 1.15 mm (*Staphylococcus aureus* ATCC 6538-P), 14.0 ± 0.5 mm (*Escherichia coli* ATCC 8739) and 11.67 ± 0.58 mm (*Candida albicans* ATCC 10231) in the disk-diffusion test; in serial dilution tests, an antimicrobial/antifungal effect was observed only at the minimum inhibitory concentration (MIC) of 1000 mg/mL.

The combination of data indicates that the biological activity is due to the combined contribution of triterpenoids and fatty acids, and the obtained metabolite profile can serve as a basis for standardizing the extract using marker compounds (e.g., Lupeol, γ-sitosterol) while controlling the content of key acids.

The practical significance of the results lies in demonstrating the potential of CO_2 extract of *E. humifusa* as a pharmaceutical substance for the development of plant-derived antimicrobial agents and in defining the key technological parameters for its production.

REFERENCES

1. Zhakipbekov K, Posylkina O, Zhumabayev N, et al. Analysis of the current state of the pharmaceutical market of the Republic of Kazakhstan. ScienceRise: Pharm Sci. 2023;2:57-67.
2. Almurzayeva A, Zhakipbekov K, Ashirov M. Competitiveness of the pharmaceutical industry of Kazakhstan. Eur J Clin Pharmacol. 2022;78:S87.
3. Issatayeva N, Datkhayev U, Datkayeva G, et al. Public-private partnership as a factor in the development of the pharmaceutical industry economy of the Republic of Kazakhstan. ScienceRise: Pharm Sci. 2024;5:96-103.
4. Datkhayev U, Shopabaeva A, Zhakipbekov K, et al. Determination of seasonal demand for pharmaceutical staff. Int J Pharm Sci Rev Res. 2016;36:105-111.
5. Umurzakhova G, Zhakipbekov K, Sultanbekov A, et al. Analyzing the ways to improve personnel management in the pharmaceutical industry of Kazakhstan. J Glob Pharma Technol. 2017;9:223-237.
6. Zhakipbekov K, Umurzakhova G, Sultanbekov A, et al. Communication skills as one of the main competences of pharmacists. Ann Trop Med Public Health. 2018;11:62.
7. Serikbayeva EA, Zhakipbekov KS, Umurzakhova GZh, et al. Methods of assessing the possibility of forming a branch regional clusters in RK. Sys Rev Pharm. 2020;11:425-434.
8. Shertaeva C, Tulemissov S, Botabayeva R, et al. Improvement of medicine provision of patients with a chronic obstructive lung illness on the basis of pharmaceutical and economical investigations. Life Sci J. 2014;11:16-23.
9. Li Z, Li X. Will innovation of pharmaceutical manufacturing improve perceived health? Front Public Health. 2021;9:647357.
10. Datkhayev UM, Sakipova ZB, Ustenova GO, et al. Validation of spectrophotometric method for determination of thiamazole in liquids by dissolution test for the transdermal form. Pharm Chem J. 2019;53:572-576.
11. Mazur I, Dilbarkhanov B, Kuracha X, et al. Periodontal status and bone metabolism in women in reproductive and postmenopausal periods. Horm Mol Biol Clin Investig. 2020;41.
12. Nandi S, Sikder R, Nag A, et al. Updated aspects of alpha-solanine as a potential anticancer agent: mechanistic insights and future directions. Food Sci Nutr. 2024;12:7088-7107.
13. Grudzinskaya L, Gemejiyeva N, Karzhaubekova Z. The Kazakhstan medicinal flora survey in a leading families volume. Bull Karaganda Univ. Biol Med Geogr Ser. 2020;100:39-51.
14. Sam S. Importance and effectiveness of herbal medicines. J Pharmacogn Phytochem. 2019;8:354-357.
15. Ashirov MZ, Datkhayev UM, Myrzakozha DA, et al. Study of cold-pressed tobacco seed oil properties by gas chromatography method. Scientific World J. 2020;2020:8852724.
16. Turgumbayeva A, Zhanat T, Zhakipbekov K, et al. A review on the medicinal plant Echinops ritro species: phytochemistry and biological activity. Farmacia. 2023;71:455-462.
17. Tleubayeva MI, Abdullabekova RM, Datkhayev UM, et al. Investigation of CO_2 extract of Portulaca oleracea for antioxidant activity from raw material cultivated in Kazakhstan. Int J Biomater. 2022;2022:6478977.

18. Turgumbayeva A, Tileuberdi N, Zhakipbekov K, et al. Antimicrobial efficacies of Brassica napus L. essential oils/ nanoparticles composites. J Nanostruct. 2021;11:524-533.
19. Turgumbayeva A, Zhakipbekov K, Shimirova Z, et al. Study of phytochemical compounds of Plantago major leaves grown in Kazakhstan. Pharmacia. 2022;69:1019-1026.
20. Kozhanova K, Alimanova A, Datkhayev U, et al. Unlocking the potential: exploring pharmacological properties from Apiaceae family. Pharmacia. 2025;72:1-14.
21. Mombekov S, Rakhymbayev N, Zhakipbekov K, et al. Study of the chemical components of CO_2 extracts from the underground part of Ferula asafoetida L. ScienceRise: Pharm Sci. 2025;1:115-122.
22. Rakhymbayev N, Datkhayev U, Sagindykova B, et al. Component composition and antimicrobial activity of subcritical CO_2 extract of Ferula asafoetida L. growing in the territory of Kazakhstan. ScienceRise: Pharm Sci. 2023;2:82-91.
23. Kemboi D, Peter X, Langat M, et al. A review of the ethnomedicinal uses, biological activities, and triterpenoids of Euphorbia species. Molecules. 2020;25:4019.
24. Liu R, Kong L. Lipid constituents from Euphorbia humifusa Willd. Nat Prod Res Dev. 2005;17:437-439.
25. Wu X, Ma GL, Chen HW, et al. Antibacterial and antibiofilm efficacy of preferred fractions and compounds from Euphorbia humifusa against Staphylococcus aureus. J Ethnopharmacol. 2023;116177.
26. Yuan Y, Rouzimaimaiti R, Hasan A, et al. A new anti-vitiligo unsaturated fatty glycoside from Euphorbia humifusa Willd. Nat Prod Res. 2023;37:2225-2231.
27. Zhao X, Zhang C, Qian X, et al. Research progress on the anti-tumor effects of Euphorbia humifusa. Discov Oncol. 2024;15:761.
28. Rakotondrabe TF, Fan M, Guo M. Exploring potential antidiabetic and anti-inflammatory flavonoids from Euphorbia humifusa with an integrated strategy. Front Pharmacol. 2022;13:980945.
29. Chang SY, Park JH, Kim YH, et al. A natural component from Euphorbia humifusa Willd displays novel broad-spectrum anti-influenza activity by blocking nuclear export of viral ribonucleoprotein. Biochem Biophys Res Commun. 2016;471:282-289.
30. Du H, Yang K, Yang J, et al. Euphorbia humifusa Willd. ex Schltdl. mitigates liver injury via KEAP1-NFE2L2-mediated ferroptosis regulation: network pharmacology and experimental validation. Vet Sci. 2025;12:350.
31. Fan XD, Hou Y, Huang XX, et al. Ultrasound-enhanced subcritical CO_2 extraction of lutein from Chlorella pyrenoidosa. J Agric Food Chem. 2015;63:4597-4605.
32. Hawthorne SB, Krieger MS, Miller DJ. Analysis of flavor and fragrance compounds using supercritical fluid extraction coupled with gas chromatography. Anal Chem. 1988;60:472-477.
33. Bleve M, Ciurlia L, Erroi E, et al. An innovative method for the purification of anthocyanins from grape skin extracts using liquid and sub-critical carbon dioxide. Sep Purif Technol. 2008;64:192-197.
34. Alimzhanova MB, Abilev MB, Kuandykova MM, et al. Rapid screening method for total petroleum hydrocarbons in water samples by solid-phase microextraction and GC-MS. Eurasian Chem Tech J. 2012;14:177.
35. Clinical and Laboratory Standards Institute (CLSI). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard (M07-A10). Wayne, PA: CLSI; 2015. https://clsi.org/media/1928/m07ed11_sample.pdf
36. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing (M100). Wayne, PA: CLSI; 2018. https://clsi.org/media/1930/m100ed28_sample.pdf
37. Wu L, Zhou PJ, Wang XF. The application of antibacterial components of Euphorbia humifusa Willd on silk fabrics. Adv Mater Res. 2012;441:315-319.
38. Ding M, Wang X, Man J, et al. Antibacterial and hemostatic polyvinyl alcohol/microcrystalline cellulose reinforced sodium alginate breathable dressing containing Euphorbia humifusa extract based on microfluidic spinning technology. Int J Biol Macromol. 2023;239:124167.
39. Xiao J, Gu H, Zhang J, et al. A comprehensive review of the phytochemistry, pharmacology and other applications of Euphorbiae humifusae herba. Molecules. 2025;30:1094.
40. Huang PZ, Yang HY, Zhang LJ, et al. Anti-inflammatory activities of triterpenoids from Euphorbia humifusa Willd. Phytochem Lett. 2023;54:129-132.
41. Xia RF, Zhang L, Zhang Y, et al. Structurally diverse triterpenoids with antibacterial activities from Euphorbia humifusa. Bioorg Chem. 2024;153:107915.
42. Li ZJ, Zhang Y, Yang Y, et al. Antifungal activity of ellagic acid in vitro and in vivo. Phytother Res. 2015;29:1019-1025.
43. Lin J, Zhao H, Hang B, et al. Antimicrobial and antitumor activities of crude extracts and isolated compounds from Euphorbia humifusa. Asian J Chem. 2013;25:3957-3960.
44. Musa NM, Sallau MS, Oyewale AO, et al. Antimicrobial activity of lupeol and β-amyrin (triterpenoids) isolated from the rhizome of Dolichos pachyrhizus Harm. Adv J Chem. 2024;1-14.
45. Desbois AP, Smith VJ. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Appl Microbiol Biotechnol. 2010;85:1629-1642.
46. Choi MY. Antibacterial activity of Euphorbia humifusa extracts on food-borne pathogenic bacteria. Korean J Community Living Sci. 2010;21:13-18.
47. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 33rd ed. Wayne, PA: CLSI; 2023. (CLSI supplement M100).