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STUDY OF THE CHEMICAL COMPONENTS OF CO_2 EXTRACTS FROM THE UNDERGROUND PART OF *FERULA ASAFOETIDA* L.

Serzhan Mombekov, Nurgali Rakhymbayev, Kairat Zhakipbekov, Bayan Sagindykova, Symbat Akhatova, Maral Amirov, Iryna Zhuravel, Akmaral Nurmahanova, Murat Ashirov, Arailym Daulbayeva, Sergii Kolisnyk

The aim *of this study was to determine the chemical composition of the CO_2 extract obtained from the underground part of Ferula asafoetida L., a plant widely used in traditional Kazakhstani medicine, with a particular focus on the amino acid and fatty acid profiles.*

Materials and methods. *The plant material was collected in accordance with current Good Agricultural and Collection Practice (GACP) guidelines and underwent thorough pre-processing. A subcritical CO_2 extraction method was applied to preserve thermolabile and volatile components while minimizing solvent residues. Chemical analysis was performed using GC-MS, and the amino acid and fatty acid profiles were determined by gas-liquid chromatography (GLC) based on standardized methods.*

Results*. The resulting CO_2 extract had a high proportion of unsaturated fatty acids (90.2 %), primarily oleic acid (46.1 %) and linoleic acid (43.0 %), along with a complete set of 20 amino acids, including 25.92 % essential amino acids. The major bioactive compounds identified were 9,12-Octadecadienoic acid, ethyl ester (18.61 %) and ethyl oleate (13.18 %), which have potential antioxidant and anti-inflammatory properties. These findings suggest the extract's suitability for pharmaceutical and nutraceutical applications.*

Conclusions*. Although the extract shows promising potential for pharmaceutical applications, further verification through comprehensive pharmacological studies is necessary*

Keywords*: medicinal plants, plant raw materials, Ferula asafoetida L., CO_2 extract, chemical compounds, fatty acids, amino acids, GC-MS*

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1. Introduction

Medicinal plants have long been used for treating a wide range of infections and disorders, serving as an essential source of inspiration for novel therapeutic compounds. The medicinal value of these plants is largely due to the presence of diverse secondary metabolites such as alkaloids, glycosides, tannins, volatile oils, and terpenoids. The World Health Organization estimates that up to 80 % of the world's population relies on traditional medicine, often derived from plant sources [1–3]. The production of pharmaceuticals from plant raw materials plays a key role in revitalizing the domestic pharmaceutical industry [4–6].

The genus *Ferula* (family Apiaceae) is particularly notable as it is the third largest within the family, with species predominantly growing in sandy, mountainous, and arid regions. [7]. These species are distributed across the Mediterranean, North Africa, Western, Central, Middle and East Asia, Northern India, the Far East and the Altai Territory [8]. According to World Flora Online, the genus comprises about 554 accepted species, and over 40 species are known to grow in Kazakhstan, specifically in the regions of Kyzylorda, Zhambyl, Almaty and South Kazakhstan regions [9].

Ferula asafoetida L. is used in traditional medicine to treat various gastrointestinal diseases, such as poor digestion, stomach pain, intestinal parasites, flatulence, respiratory diseases (including flu and asthma), neurological disorders (epilepsy, hysteria, paralysis and depression) and reproductive issues (severe or premature birth, leukaemia, infertility, copious menstruation) [10, 11].

The plant contains resin (40–64 %), endogenous gum (25 %) and volatile essential oil (10–17 %), which include sulfur-containing compounds, coumarins, and terpenes [12, 13].

Despite its extensive traditional use, most studies have focused on the aerial parts of *Ferula asafoetida* L., while the chemical composition of its underground part (taproot) remains less characterized. This study specifically aims to determine the component composition – focusing on the amino acid and fatty acid profiles – of the CO_2 extract obtained from the underground part of *Ferula asafoetida* L. The investigation is intended not only to elucidate the extract's chemical profile but also to assess its potential for pharmaceutical applications, thereby laying the groundwork for future pharmacological and standardization studies.

2. Planning (method logy) of research

In Table 1 a representation of the research planning process is shown.

Table 1

Planning of the research

Step 1	Collection and preparation of plant material
Step 2	Extraction of the CO_2 extract
Step 3	GC-MS analysis of the CO_2 extract
Step 4	Quantitative determination of fatty acid and amino acid profiles

3. Materials and methods

3.1. Plant material and pre-processing

Collection.

The underground parts of *Ferula asafoetida* L. were collected in May 2023 from the Akaltyn settlement in the Turkestan Region, Kazakhstan (Coordinates: 42.025667° N, 68.014881° E). The collection was conducted under optimal weather conditions and in full compliance with the principles of Good Agricultural and Collection Practice (GACP) guidelines, emphasising proper identification of plant species, selection of appropriate harvesting time, control of drying conditions, and prevention of contamination to ensure the quality and reproducibility of medicinal plant raw materials [14]. The plant material was authenticated by Dr. Akmaral Nurmahanova, a botanist at Al-Farabi Kazakh National University, based on morphological characteristics such as root structure, leaf shape, and colour, as well as comparison with herbarium specimens using standard taxonomic and morphological. The underground parts of *Ferula asafoetida* L. were collected under optimal conditions (Fig. 1.). A voucher specimen was deposited at the Herbarium of Al-Farabi Kazakh National University under the voucher number No. 5-05/23.

Fig. 1. *Ferula asafoetida* and its underground parts

Photo taken during the collection of raw materials in Akaltyn, Turkestan Region, Kazakhstan (Coordinates: 42.025667° N, 68.014881° E), May 2023. (Photo by Nurgali Rakhymbayev).

Pre-processing.

After collection, the material was air-dried in a shaded, well-ventilated area at approximately 25 °C to prevent the degradation of thermolabile components. Once dried, the plant material was milled using an IKA M20 laboratory mill to obtain a uniform particle size of 1–3 mm, thereby enhancing the extraction efficiency. The moisture content of the milled material was determined, and the processed material was stored in airtight containers under controlled conditions (18±2 °C; 60±5 % relative humidity).

The technological scheme for the preparation of raw materials in the underground part of *Ferula asafoetida* L. is shown in Fig. 2.

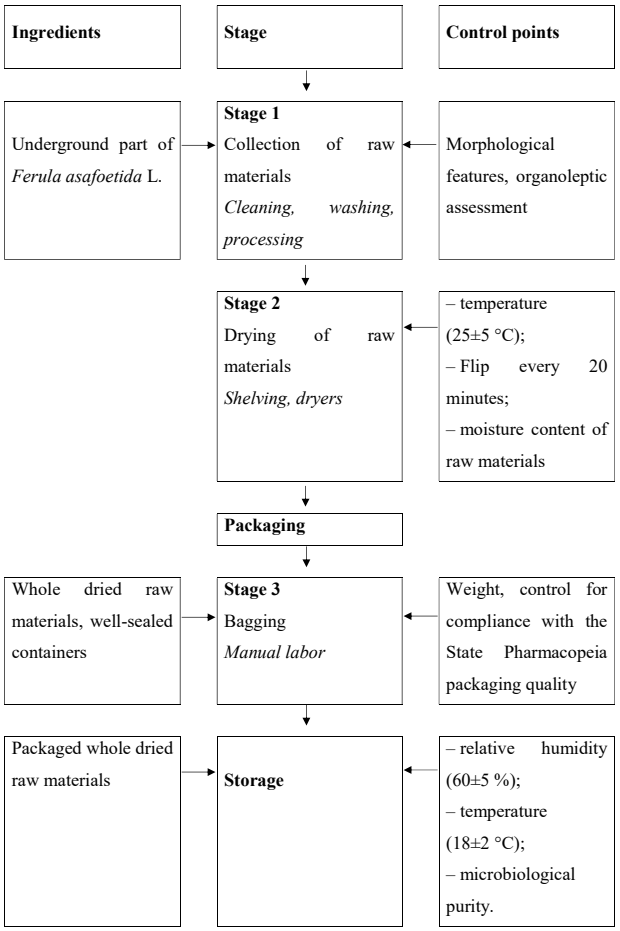

Fig. 2. Technological scheme of raw material procurement *Ferula asafoetida* L.

3. 2. Extraction procedure

Subcritical CO_2 extraction was employed to obtain the extract from the roots of *Ferula asafoetida* L. In this procedure, 1 kg of dried and milled plant material (particle size 1–3 mm) was loaded into a 5-L extraction vessel. The extraction was performed using liquefied CO_2 as the extractant under the following conditions: temperature: +17–21 °C; working pressure: 40–51 atm; extraction Time: 11 hours.

These parameters were chosen based on established protocols and were maintained in accordance with the national standard 27658-1910 (LLP-02-2011) and national standard 8050-85. The process yielded an extract with a yield of 2.5 % (w/w) relative to the raw material. The resulting CO_2 extract is fat-soluble. The extraction was carried out using a CO_2 flow-through extraction system (5-L capacity) at Zhanapharm (Pharmaceutical Company, Almaty, Kazakhstan) [15].

3. 3. Chemical detection of active compounds by GC–MS

The component composition of the CO_2 extract was determined using gas chromatography-mass spectrometry (GC–MS) [16]. Analysis was performed on an Agilent 7890B/5977A system equipped with a WAXetr capillary column (30 m in length, 0.25 mm internal diameter, 0.25 µm film thickness). A sample volume of 1.0 µL was injected at an entry temperature of 240 °C with a split ratio of 1:10. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The column temperature was programmed to increase from 40 °C (0 min) to 260 °C at a heating rate of 10 °C/min, followed by a 20-minute hold at 260 °C. Detection was carried out in SCAN mode over the mass range m/z 34–850. Data acquisition and processing were performed using Agilent MSD ChemStation software (version 1701EA). Compound identification was accomplished by comparing the acquired mass spectra with entries in the Wiley 7th edition and NIST'02 libraries, which collectively contain over 550,000 reference spectra [17, 18].

3. 4. Determining the number of amino acids was performed by gas-liquid chromatography (GLC)

To quantify amino acids, 1 g of the sample was hydrolyzed in 6N hydrochloric acid at 105 **°C** for 24 hours in sealed vials under an argon stream to prevent oxidation. The hydrolysate was evaporated to dryness using a rotary evaporator (40–50 °C, atmospheric pressure) and dissolved in sulfosalicylic acid. After centrifugation (1500 rpm, 5 min), the supernatant was purified through an ion-exchange resin column (Dauks 50, H-8, 200–400 mesh), followed by sequential washing with deionized water and 0.5N acetic acid until achieving a neutral pH.

Amino acids were eluted using 6N NH_4OH and further purified by evaporation. The residue was derivatized with acetic anhydride, triethylamine, and acetone, followed by extraction with ethyl acetate for gas-liquid chromatography (GLC) analysis. GLC was performed using a Carlo Erba 4200 gas chromatograph under the following conditions:

– flame ionization detector (FID): 300 °C;

– evaporator temperature: 250 °C;

– column temperature program: 110 °C→185 °C (60 °C/min)→250 °C (32 °C/min, held until complete elution);

– stationary phase: polar mixture (Carbowax® 20M, Silar® 5CP, Lexan® on Chromosorb® W AW, 120–140 mesh).

Amino acid quantification was performed using an external standard mixture (Altex) containing known concentrations of amino acids. Peak areas of the analyzed samples were compared to those of the standard solution, and concentrations were calculated based on a pre-established calibration curve [19].

3. 5. Determination of fatty acids was performed by gas-liquid chromatography (GLC)

The fatty acid composition of the CO_2 extract was determined using gas-liquid chromatography after derivatization to fatty acid methyl esters (FAMEs). For this, the extract was subjected to lipid extraction using a chloroform: methanol (2:1, v/v) mixture at a sample-to-solvent ratio of 1:20, ensuring efficient separation of lipid components. The mixture was thoroughly shaken for 5 minutes and then filtered through a paper filter to obtain a clear lipid extract. The filtrate was transferred to a round-bottom flask and evaporated to dryness under atmospheric pressure using a rotary evaporator at a bath temperature of 30–40 °C.

Following evaporation, the dried lipid residue was subjected to methylation by adding 10 mL of methanol and 2–3 drops of acetyl chloride. The reaction was conducted at 60–70 °C for 30 minutes, allowing the conversion of fatty acids into their respective methyl esters. Upon completion, the methanol was evaporated under reduced pressure, leaving behind the methylated lipid fraction. The resulting residue was then dissolved in 5 mL of hexane to obtain a clear solution suitable for chromatographic analysis.

An aliquot of the hexane layer was analyzed using a Carlo Erba 4200 gas-liquid chromatography system (Cornaredo, Italy) equipped with a capillary column (30 m×0.25 mm, 0.25 µm). Helium was used as a carrier gas at a constant flow rate, ensuring optimal separation of fatty acid components. The GC oven temperature was set at 188 °C for 1 hour, with an injector temperature of 188 °C and the detector temperature of 230 °C.

Identification of individual fatty acid methyl esters was performed by comparing their retention times and mass spectra with those available in the spectral library of the National Institute of Standards and Technology (NIST11) [19].

3. 6. Statistical Data Analysis

Experimental results are expressed as mean±standard deviation (SD) based on three independent replicates. Statistical analysis was performed using Microsoft Excel to calculate means and standard deviations.

4 Results

4. 1. Characteristics of the CO_2 extract

The obtained CO_2 extract of *Ferula asafoetida* L. was a dark brown, viscous liquid with a characteristic aromatic odour. The extraction yield was 2.5 % (w/w) relative to the raw material, with a residual moisture content of less than 5 %, determined according to method 2.2.32 SPH RK Volume 1, «Weight loss during drying». The extract demonstrated solubility in nonpolar solvents, confirmed by qualitative solubility testing in hexane, chloroform, and ethyl acetate, indicating its fat-soluble nature.

4. 2. GC-MS analysis of the CO_2 extract

A total of 59 compounds were identified in the CO_2 extract of *Ferula asafoetida* L. using GC–MS analysis. The most abundant and biologically relevant compounds are summarized in Table 2, while the full list of detected components is available upon request. The GC chromatogram of the extract is presented in Fig. 3.

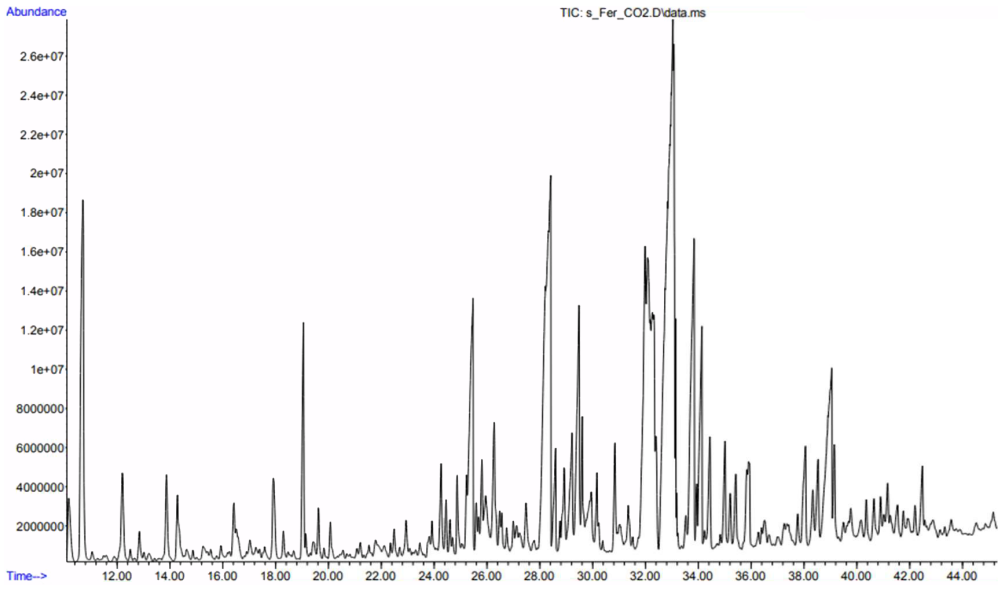

Fig. 3. GC-MS chromatogram of *Ferula asafoetida* L. CO_2 extract

Table 2

GC-MS results of the subcritical CO_2 extract of *Ferula asafetida* L.

No.	Retention time (min)	Component name	Probability of identification (%)	Content (%)
1	10.69	Thiophene, 2,5-diethyl-	81	3.13
2	25.44	Ethyl 13-methyl-tetradecanoate	91	5.01
3	28.37	Hexadecanoic acid, ethyl ester	86	10.01
4	29.46	*cis*-10-Heptadecenoic acid, methyl ester	76	3.98
5	31.99	Ethyl Oleate	89	13.18
6	33.03	9,12-Octadecadienoic acid, ethyl ester	87	18.61
7	33.81	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	94	5.19
8	34.11	Phytol	92	2.45
9	39.02	Nonacosane	92	4.95

The major components detected in the extract include 9,12-Octadecadienoic acid, ethyl ester (18.61 %), ethyl Oleate (13.18 %), hexadecanoic acid, ethyl ester (10.01 %), and thiophene, 2,5-diethyl- (3.13 %). Fatty acid esters made up the majority of the extract, confirming its high lipid fraction.

4. 3. Interpretation of key findings

The CO_2 extract of *Ferula asafoetida* L. was rich in unsaturated fatty acid esters, including 9,12-octadecadienoic acid, ethyl ester and ethyl oleate, which are commonly associated with anti-inflammatory and antioxidant properties. The detection of thiophene, 2,5-diethyl- and phytol suggests the presence of bioactive terpenoids with potential antimicrobial activity.

The fatty acid composition (as seen in Table 2) aligns with previous findings on lipid-rich plant extracts, reinforcing the suitability of subcritical CO_2 extraction for obtaining bioactive compounds. The extract's lipid fraction suggests its potential use in pharmaceutical or nutraceutical applications, particularly in lipid-based formulations.

4. 4. Amino acid and fatty acid composition

Amino acid profile.

A total of 20 amino acids were identified in the extract, with essential amino acids constituting 25.92 % of the total composition and nonessential amino acids comprising 74.08 % (Table 3).

Table 3

Amino acid profile of *Ferula asafetida* L.

No.	Amino Acid	Content (mg/100 g)	%	No.	Amino Acid	Content (mg/100 g)	%
	Essential Amino Acids				Nonessential Amino Acid		
1	Leucine	390±5.20	4.48	10	Ornithine	2±0.20	0.02
2	Isoleucine	372±4.80	4.27	11	Alanine	702±6.10	8.06
3	Valine	260±3.90	2.99	12	Glycine	242±3.50	2.78
4	Threonine	232±2.80	2.66	13	Glutamate	2704±8.70	31.09
5	Methionine	70±1.40	0.80	14	Proline	464±4.20	5.33
6	Phenylalanine	303±3.10	3.48	15	Serine	348±2.50	4.00
7	Lysine	305±3.70	3.50	16	Aspartate	1226±6.30	14.08
8	Tryptophan	82±0.90	0.94	17	Cystine	33±0.80	0.38
9	Histidine	244±2.20	2.80	18	Oxyproline	2±0.20	0.02
–	–	–	–	19	Tyrosine	325±2.90	3.73
–	–	–	–	20	Arginine	400±3.50	4.59
Total			25.92 %	Total			74.08 %

Fatty acid profile.

The fatty acid content of the *Ferula asafoetida* L. CO_2 extract was analyzed and is presented in Table 4. The dominant fatty acids included oleic acid (46.1 %), linoleic acid (43 %), palmitic acid (7.3 %), and stearic acid (1.9 %).

Table 4

Fatty acid profile of *Ferula asafoetida* L.

No.	Fatty Acid	C number: number of double bonds	Class	Content, %
1	Myristic acid	C14:0	Saturated	0.2±0.01
2	Pentadecanoic acid	C15:0	Saturated	0.4±0.02
3	Palmitic acid	C16:0	Saturated	7.3±0.20
4	Palmitoleic acid	C16:1	Monounsaturated	0.8±0.02
5	Stearic acid	C18:0	Saturated	1.9±0.05
6	Oleic acid	C18:1	Monounsaturated	46.1±0.50
7	Linoleic acid	C18:2	Polyunsaturated	43.0±0.60
8	Linolenic acid	C18:3	Polyunsaturated	0.3±0.01
Total				100.00±0.81

5. Discussion

The composition of bioactive compounds in plants is influenced by several factors, including climatic conditions, soil composition, harvesting time, and processing methods [20].

Understanding these variables is essential for optimizing extraction methods and ensuring the reproducibility of bioactive compound profiles.

5. 1. Interpretation of key findings

The GC-MS analysis of the *Ferula asafoetida* L. CO_2 extract identified a diverse range of compounds, including terpenes, fatty acids, and thiophenes. One of the notable compounds, thiophene, 2,5-diethyl- (3.13 %), has been widely studied for its antimicrobial properties [21]. However, its lower concentration in our extract, compared to previous studies, may be attributed to differences in extraction methods or variations in plant growing conditions.

The presence of ethyl oleate (13.18 %) and 9,12-octadecadienoic acid, ethyl ester (18.61 %), two predominant fatty acid esters, is particularly relevant as these compounds exhibit antioxidant and anti-inflammatory activity [22]. Their significant presence suggests that the CO_2 extract may have potential applications in pharmaceuticals and cosmetics, particularly in lipid-based formulations.

Additionally, phytol (2.45 %) and nonacosane (4.95 %) were detected, which are known to enhance extract stability and contribute to antioxidant effects [23]. These findings support the potential of *Ferula asafoetida* L. extract as a source of bioactive compounds beneficial for human health.

Although previous studies have reported on the chemical composition of *Ferula asafetida* L., there is limited data specifically on the amino acid and fatty acid profile of its underground parts. The fatty acid composition in our study, including the high content of ethyl oleate and linoleic acid esters, differs from data obtained using conventional solvent-based extraction methods, likely due to the selective nature of CO_2 extraction. Other authors have reported variations in lipid profiles when using different extraction techniques, emphasizing the importance of method selection in obtaining bioactive compounds.

We chose subcritical CO_2 extraction to obtain the optimal extract, given the low efficiency of traditional methods and numerous disadvantages such as contamination and organic solvent residues. The subcritical CO_2 extraction (SCCE) technology, which operates under mild conditions, enables the safe and efficient extraction of thermolabile and easily oxidizable compounds while maintaining their structural integrity [24, 25]. This environmentally friendly approach not only improves extract purity but also enhances the yield of bioactive components. The differences observed in our study compared to previously reported compositions may be attributed to the high selectivity of CO_2 extraction, which favours certain classes of lipophilic compounds.

Future studies should focus on a more detailed comparison of amino acid and fatty acid composition using different extraction methods to fully understand the influence of extraction techniques on the chemical profile of *Ferula asafetida* L. Our findings demonstrate the potential of CO_2 extraction as a superior method for obtaining high-quality bioactive extracts with potential pharmaceutical and cosmetic applications.

5. 2. Amino acid and fatty acid composition

The amino acid analysis (Table 3) revealed that 25.92 % of amino acids were essential, while 74.08 % were non-essential. Essential amino acids such as leucine (4.48 %), isoleucine (4.27 %), and valine (2.99 %) play a crucial role in protein synthesis and metabolic processes [26, 27]. Their presence in significant amounts underscores the nutritional value of the extract and its potential use in dietary supplements.

Regarding the fatty acid profile (Table 4), 9.8 % of the total fatty acids in the CO_2 extract were saturated, while 90.2 % were unsaturated. The high proportion of unsaturated fatty acids, including oleic acid (46.1 %) and linoleic acid (43 %), suggests potential cardiovascular benefits, aligning with studies that associate these compounds with reduced cholesterol levels and anti-inflammatory properties [28–32]. The low content of saturated fatty acids (9.8 %) indicates a favourable lipid composition, minimizing risks associated with excessive saturated fat intake.

5. 3. Relevance of fatty acid composition

Unsaturated fatty acids are known to contribute to membrane fluidity, metabolic regulation, and biosynthesis of biologically active substances. Linoleic acid, an essential polyunsaturated fatty acid, is widely found in plant oils

and cannot be synthesized by the human body. Interestingly, the linoleic acid content in *Ferula asafoetida* L. extract is comparable to that found in soybean oil (42–53 %), further reinforcing its potential as a nutraceutical ingredient.

Saturated fatty acids, on the other hand, tend to be more stable but are associated with increased cholesterol levels if consumed in excess. In our study, the total saturated fat content (9.8 %) was lower than that found in many commercial plant oils, suggesting that *Ferula asafoetida* L. CO_2 extract may be a healthier alternative for lipid-based formulations.

Practical relevance. The practical relevance of this study lies in the extraction and identification of bioactive compounds from *Ferula asafoetida* L. CO_2 extract, providing insight into its potential pharmaceutical and nutraceutical applications. The identification of biologically active substances, particularly amino and fatty acids, supports its use in medicinal and functional formulations.

The scientific novelty of the study is confirmed by the patent granted by the Ministry of Justice of the Republic of Kazakhstan, National Institute of Intellectual Property (Patent No. 35010, 20.08.2021), covering a method of obtaining carbon dioxide extract from roots of *Ferula asafoetida* L.

Research limitations. One limitation of this study is that some chemical compounds in the CO_2 extract were not identified, as they were not available in the NIST02 and Wiley 7th edition spectral libraries. Future studies should aim to expand the compound identification process using advanced high-resolution mass spectrometry techniques.

Prospects for further research. Further research should focus on analyzing the component composition, amino acids and fatty acids of *Ferula asafoetida* L. CO_2 extract across different growth stages and climatic conditions. Additionally, phytochemical and pharmacological studies should be conducted to evaluate bioactivity, toxicity, and possible therapeutic applications, supporting the development of new pharmaceutical formulations.

Further studies should explore seasonal and geographical variations, confirm pharmacological activity, optimize extraction methods, and assess formulation stability. The *Ferula asafetida* L. CO_2 extract shows promise for pharmaceutical and nutraceutical applications, pending further validation.

6. Conclusions

The chemical composition of the CO_2 extract from the underground part of *Ferula asafoetida* L. was analyzed using GC-MS for volatile compounds and GLC for amino acid and fatty acid profiles. The extract was found to be a rich source of unsaturated fatty acids, particularly linoleic acid (43 %) and oleic acid (46.1 %), which are known for their cardioprotective and anti-inflammatory properties. Additionally, the presence of essential amino acids (25.92 %), such as leucine, isoleucine, and valine, highlights its nutritional potential.

Given its high content of bioactive lipids and amino acids, the CO_2 extract of *Ferula asafoetida* L. demonstrates the pharmacological potential for use in nutraceuticals, functional foods, and lipid-based drug formulations. Furthermore, the low level of saturated fatty acids (9.8 %) suggests that this extract could serve as a healthier alternative to conventional lipid sources.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

The data will be made available at a reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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Serzhan Mombekov*, PhD, Associate Professor, School of Pharmacy, S. D. Asfendiyarov Kazakh National Medical University, Tolebi str., 94, Almaty, Kazakhstan, 050000

Nurgali Rakhymbayev, PhD, School of Pharmacy, S. D. Asfendiyarov Kazakh National Medical University, Tolebi str., 94, Almaty, Kazakhstan, 050000

Kairat Zhakipbekov, PhD, Associate Professor, School of Pharmacy, S. D. Asfendiyarov Kazakh National Medical University, Tolebi str., 94, Almaty, Kazakhstan, 050000

Bayan Sagindykova, Doctor of Pharmaceutical Sciences, Professor, Faculty of Pharmacy, South Kazakhstan Medical Academy, Al-Farabi ave., 1, Shymkent, Kazakhstan, 160001

Symbat Akhatova, Master of Natural Sciences, Faculty of Natural Science and Informatization, I. Altynsarin Arkalyk Pedagogical Institute, Auelbekova str., 17, Arkalyk, Kazakhstan, 110300

Maral Amirov, Master of Natural Sciences, Faculty of Natural Science and Informatization, I. Altynsarin Arkalyk Pedagogical Institute Auelbekova str., 17, Arkalyk, Kazakhstan, 110300

Iryna Zhuravel, Doctor of Chemical Sciences, Professor, Department of Pharmaceutical Technology, Standardization and Certification of Medicines, National University of Pharmacy, Hryhoriia Skovorody str., 53, Kharkiv, Ukraine, 61002

Akmaral Nurmahanova, PhD, Associate Professor, Faculty of Biology and Biotechnology, Al Farabi Kazakh National University, Al-Farabi ave., 71, Almaty, Kazakhstan, 050040

Murat Ashirov, Lecturer, School of Pharmacy, S. D. Asfendiyarov Kazakh National Medical University, Tolebi str., 94, Almaty, Kazakhstan, 050000

Arailym Daulbayeva, PhD Student, School of Pharmacy, S. D. Asfendiyarov Kazakh National Medical University, Tolebi str., 94, Almaty, Kazakhstan, 050000

Sergii Kolisnyk, Doctor of Pharmaceutical Sciences, Professor, Department of General Chemistry, National University of Pharmacy, Hryhoriia Skovorody str., 53, Kharkiv, Ukraine, 61002

***Corresponding author:** Nurgali Rakhymbayev, e-mail: rakhimbayev.n@kaznmu.kz; Serzhan Mombekov, e-mail: mombekov.s@kaznmu.kz*