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COMPONENT COMPOSITION AND ANTIMICROBIAL ACTIVITY OF SUBCRITICAL CO_2 EXTRACT OF *FERULA ASAFOETIDA L.*, GROWING IN THE TERRITORY OF KAZAKHSTAN

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Ferula asafoetida L. has been studied for centuries by many scientists as food products and in traditional medicine, which are relevant for Kazakhstan as well. Recent studies have shown that Ferula asafoetida L. has neuroprotective, memory-enhancing, digestive enzymatic, antioxidant, antispasmodic, hypotensive, hepatoprotective, antimicrobial, antitumor, cytotoxic, and anthelmintic effects.

The aim *of this study is to determine the component composition by GC-MS method and to study the antimicrobial properties of Ferula asafoetida L. extract obtained by CO_2 extraction, which grows in Kazakhstan.*

Materials and methods. *To determine the possibility of using Ferula asafoetida L., we carried out the component composition of the extract obtained by CO_2 extraction in subcritical conditions of the underground part of Ferula asafoetida L. by a certain GC-MS method and also investigated the antimicrobial effect of this extract.*

Results. *The plant raw materials were collected in accordance with GACP requirements. Conducted subcritical CO_2 extraction of plant raw materials showed a 2.5 % extraction yield. The study of the component composition by GC-MS revealed 46.3 % of sulfate compounds. The determination of antimicrobial activities showed high efficacy against gram-positive (Staphylococcus aureus subsp. Aureus, Bacillus subtilis subsp. Spizizenii), gram-negative bacteria (Escherichia coli, Klebsiella pneumoniae subsp. pneumoniae, Salmonella enterica subsp. enterica) and fungi (Candida albicans, Aspergillus niger).*

Conclusions. *The possibility of using the obtained CO_2 extract of Ferula asafoetida L. in the field of pharmaceutical products as a substance and a drug that has a huge antimicrobial effect*

Keywords: *Ferula asafoetida, GC-MS analysis, CO_2 extraction, antimicrobial effect, component composition, plant raw materials, Ferula L., double serial dilutions method, disco-diffusion method*

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1. Introduction

Ferula asafoetida L. has been used for centuries as a spice and in folk medicine to treat various diseases [1].

Ferula asafoetida L. is a rich source of antimicrobial compounds [2]. These species produce alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glucosides, terpenes and phenolic substances, which are secondary metabolites [3]. These chemicals with antimicrobial properties have practical applications in the field of food safety, preventing bacterial and fungal infections [4].

Amalraj, A., & Gobi, S., in their literature reviews, describe the existence of more than 170 species of Ferula, and they discuss its widespread distribution in Iraq, Central Asia, Afghanistan, Africa, Iran, Turkey and Europe [5].

In the Republic of Kazakhstan*, Ferula asafoetida* L. grows in South Kazakhstan, on the banks of the Syr-Darya, in the Chu-Ili mountains, in the Kyzylkum, Turkestan floristic region and in the western part of Kazakhstan [1] (Fig. 1).

It grows on clay plains and in suburban deserts. On loess and fine-grained slopes, river terraces, along streams, in grass-mixed grass and sagebrush-feather grass steppes, in meadows with shrubs, high-grass clearings in the tugai and is a dominant or subdominant in the mixed-grass-ferula, sagebrush-hultemiev-ferula, ferula-eromurus associations [6]. Blooms in March-April; bears fruit in April [7].

The total area of *Ferula asafoetida* L. in the Turkestan region is 2260 hectares. The average yield of plant raw materials ranging from 5940 kg/ha to 14520 kg/ha based on the raw weight on controlled land plots and from 4501 kg/ha to 9650 kg/ha in dry weight. According to official data for 2009, up to 6958 tons of fresh ferula or 5140 tons of dried ferula accumulated in the South Kazakhstan region in Saryagash, Otyrar district, and Arys. Almost all the collected underground parts were exported [8].

In Kazakhstan, *Ferula asafoetida* L. is called *Sasyk kurai, Sasyr, Keurek,* and in other countries, they are named*:* Afghanistan – *Kama, Anguza*, China – *A–wei*, India – *Hing, Hengu, Ingu, Hingu, Inguva, Perungayam, Kayam, Raamathan*, Turkey – *Setan bokosu, Seytan tersi,* Tibet – *Shing–kun*, Greece – *Aza,* Iran – *Rechina fena, Zaz,* Sri Lanka – *Perunkayan,* England, Finland, France, Germany, Italy, Poland, Russia and Spain – *Asafetida* [5].

According to the literature, *Ferula asafoetida* L. has antiulcer [9], anti-inflammatory, antinociceptive [10, 11], anticonvulsant [12], antihaemolytic, antioxidant [13–17], antifungal, allelopathic [18–21], antiviral [22], antileishmanial [23], antimicrobial [16, 24], antitumor [25], antidiabetic, antihypertensive, antispasmodic activities [26, 27].

We have reviewed the literature on the antimicrobial activity of various extracts of *Ferula asafoetida* L. obtained using various extractants (Table 1).

Table 1

Data from the literature review of the antimicrobial activity of Ferula asafoetida L. extracts obtained using various extractants

–	Escherichia coli		Staphylococcus aureus		Bacillus subtilis		K. pneumonia		Candida albicans		Aspergillus niger	
–	MIC	Inhibition zone (diameter mm)	MIC	Inhibition zone (diameter mm)	MIC	Inhibition zone (diameter mm)	MIC	Inhibition zone (diameter mm)	MIC	Inhibition zone (diameter mm)	MIC	Inhibition zone (diameter mm)
Mujeeb Ur Rahman et al. [28]: Ferula assafoetidaoil.	110.00 μg/ml	10.3–42.0	180.00 μg/ml	9.0–29.0	165.00 μg/ml	12.0–33.0	–	–	–	–	–	–
S.D. Patil et al. [24]: chloroform, ethyl acetate, ethanol, methanol and aqueous extracts	1000–2000 μg/ml	08.42–19.31	500–2000 μg/ml	08.36–20.11	500–2000 μg/ml	08.22–22.15	1000–2000 μg/ml	08.18–17.14	1000–2000 μg/ml	10.65–20.23	1000–2000 μg/ml	09.34–17.15
Richa Bhatnager et al. [29]: petroleum ether, hexane, hot and cold water, ethanol.	–	7.0–12.0	–	7.0–11.0	–	–	–	7.0–10.0	–	–	–	
Razieh Niazmand, Bibi Marzieh Razavizadeh [30]: hydroethanolic extracts of leaf and gum	62.5±3–400±12 μg/ml	–	62.5±2–300±9 μg/ml	–	–	–	–	–	–	–	50±2–125±5 μg/ml	–
Kavoosi G, Rowshan V. [31]: oleo-gum-resin	65–111 μg/ml	–	17–32 μg/ml	–	15–27 μg/ml	–	–	–	18–28 μg/ml	–	22–36 μg/ml	–
Kavoosi, G. et al. [16]: essential oil	>200 μg/ml	–	125±17 μg/ml	–	80±12 μg/ml		–	–	90±11 μg/ml	–	85±5 μg/ml	–
N. Samadi et al. [32]: essential oil	24000 μg/ml	8.0–9.0	12000 μg/ml	11.5–16.0	12000 μg/ml	12.0–15.0	24000 μg/ml	10.0–20.0	–	–	–	–
Charu Singh and Ramendra Singh Parmar [33]: various extracts of asafoetida resin	–	8.0–16.0	–	4,5–12.0	–	no inhibition zone	–	–	–		–	–
Vikas Shrivastava et al. [34]: various extracts	–	9.2–17.1	–	4.9–13.0	–	no inhibition zone	–	–	–	–	–	no inhibition zone
Divya, K. et al. [35]: volatile oils	5–100 μg/g	14.0–18.0	–	14.0	5–100 μg/g	14.0–28.0	–	–	–	–	–	–
Devanesan et al. [36]: The silver nanoparticles synthesized using asafoetida gum (AsAgNPs)	7.80 μg/ml	12.0	31.20 μg/ml	7.0	–	–	31.20 μg/ml	7.0	15.60 μg/ml	9.0	–	–
Murali Mohan Ch & P. Venkata Smitha [37]: Resin.	–	7.0±1.2	–	5.0±1.5	–	no inhibition zone	–	–	–	no inhibition zone	12.0±0.8	–
Kamble, V. A., & Patil, S. D. [38]: essential oil	–	–	–	–	–	–	–	–	1.25 μg/ml	20.0	no inhibition zone	32.0

The aim of this study is to determine the component composition by GC-MS method and to study the antimicrobial properties of *Ferula asafoetida* L. extract obtained by CO_2 extraction, which grows in Kazakhstan.

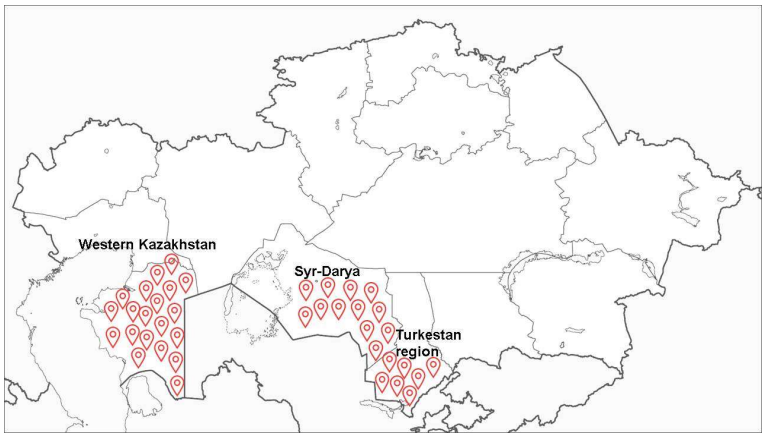

Fig. 1. Distribution map of *Ferula asafoetida* L. in Kazakhstan

2. Planning (methodology) of research

In Table 2, a representation of the research planning process is shown.

Table 2

Planning of the research

Step 1	Critical evaluation of literature data on the chemical composition, distribution, therapeutic properties, and antimicrobial activity of *Ferula asafoetida* L., as well as the name in different countries
Step 2	Obtaining CO_2 extract from the collected underground part of *Ferula asafoetida* L.
Step 3	GC-MS analysis of CO_2 extract of *Ferula asafoetida* L.
Step 4	Determination of antimicrobial activity by double serial dilutions and disco-diffusion method of CO_2 extract of *Ferula asafoetida* L.

3. Materials and methods

3. 1. Plant material

Underground parts of *Ferula asafoetida* L. were collected in the Arys Turkestan region of Kazakhstan in April 2019 at the savannah and identified (No. 01-08/2) by the Institute of Botany and Phyto-introduction under the Committee of Science of the Ministry of Education and Science of the Republic of Kazakhstan.

The photo was taken while collecting medicinal plant raw materials in the Arys settlement of South Kazakhstan Turkestan region (April 2019, Fig. 2).

Fig. 2. *Ferula asafoetida* L. growing in the Kazakhstan region Turkestan

The plant raw materials were collected from the Turkestan region of Kazakhstan in compliance with the requirements of the Good Agricultural And Collection Practice (GACP). The plant material was dried at the room temperature of 25 °C±5 °C, equipped with ventilation. After drying, the raw material was ground to 1–3 mm using an IKA M20 laboratory mill.

3. 2. Extraction procedure

Next, we selected subcritical CO_2 extraction to obtain the optimal form of extraction.

The low efficiency of traditional extraction methods, and many disadvantages of the extract, such as contamination of the extract and residues of organic solvents in it, prevented the further use of the resulting natural active ingredient [39]. To eliminate these shortcomings, the technology of subcritical CO_2 extraction (SCCE) is used, which is safe for the environment and satisfies the current desire of the whole world for «green» technology [40]. This method is sensitive to high temperatures and allows the most effective way to obtain an easily oxidizable and decomposable ingredient [41].

The process of extracting *Ferula asafoetida* L. using carbon dioxide from the roots was carried out according to the following parameters: temperature +17–21 °C and working pressure 40–51 atmospheres, extraction time 11 hours. The percentage of extraction yield was 2.5 %. The resulting CO_2 extract is fat-soluble.

The extraction was obtained using a carbon dioxide extraction plant (installation of carbon dioxide flow-through extraction-5L) in the Zhanapharm (Pharmaceutical company, Almaty city of Kazakhstan), under subcritical conditions according to the institution national standard 27658-1910 – LLP-02-2011, and liquefied carbon dioxide was used as an extractant (national standard 8050-85).

3. 3. Chemical detection of active compounds

After obtaining the extract, we determined the component composition of this extract by the GC-MS method.

The chemical detection of active compounds analysis was carried out at the Department of Analytical, colloidal Chemistry And Technology Of Rare Elements Center, Al-Farabi Kazakh National University by gas chromatography with mass spectrometric detection using an Agilent 7890B/5977A chromatography [42].

Chromatographic analysis conditions: sample volume 1.0 µl, sample entry temperature 240 °C, with a flow division of 1:10. The separation was performed using a WAXetr chromatographic capillary column with a length of 30 m, an internal diameter of 0.25 mm and a film thickness of 0.25 microns at a constant carrier gas velocity

(helium) of 1 ml/min. The chromatography temperature is programmed from 40 °C (0 min exposure) to 260 °C with a heating rate of 10 °C/min (20 min exposure). Detection is carried out in the SCAN mode m/z 34-850. The Agilent MSD ChemStation software (version 1701EA) was used to control the gas chromatography system and record and process the results and data obtained. Data processing included the determination of retention time and peak areas, as well as the processing of spectral information obtained using a mass spectrometric detector. To decipher the obtained mass spectra, the Wiley 7th edition and NIST'02 libraries were used (the total number of spectra in the libraries is more than 550 thousand).

3. 4 Antimicrobial activity determination

Antimicrobial activity was carried out in the microbiology laboratory of the Scientific Center for Anti-infectious Drugs, Almaty of Kazakhstan, which is accredited in accordance with the standards of ST RK ISO 9001-2009 «Quality management System. Requirements» and GOST ISO/IEC 17025-2007 «General requirements for the competence of testing and calibration laboratories» (certificate of accreditation No. KZ I.02.1252 dated 26.12.2011), in 2018 – again in the German Federal Bureau for Standards of Good Laboratory Practice (GLP).

Muller-Hinton agar (Himedia, India), Muller-Hinton broth (Himedia, India), Saburo agar (Himedia, India), Saburo broth (Himedia, India), sodium chloride, (Mikhailovsky Chemical Reagents Plant, Russia), ethanol 96 % (Talgar Alcohol, Kazakhstan), purified water were chosen to select the nutrient medium.

The procedure for determining antimicrobial activity was carried out according to the internal methodological instructions of MI-LM-02, «Determination of the bactericidal action of antimicrobial agents by the method of double serial dilutions» (Methodological instructions of the laboratories of microbiology) [43] and the research methods used are regulated by the Institute of Clinical and Laboratory Standards, USA (CLSI – Clinical and Laboratory Standards Institute, USA) [44]. The study was carried out by two methods – the method of double serial dilutions in a liquid nutrient medium and the disco-diffusion method.

Testing of antimicrobial activity of CO_2 extract of *Ferula asafoetida* L. was carried out concerning 7 strains of microorganisms obtained from the American Type Culture Collection (ATCC, USA): gram-positive bacteria: *Staphylococcus aureus subsp. aureus* ATCC® 6538P™; *Bacillus subtilis subsp. spizizenii* ATCC® 6633™; gram-negative bacteria: *Escherichia coli* ATCC® 11229™; *Klebsiella pneumoniae subsp. pneumoniae* ATCC® 700603™; *Salmonella enterica subsp. enterica* ATCC® 14025™; fungi: *Candida albicans* ATCC® 10231™; *Aspergillus niger* ATCC® 16404™.

The activity of the sample was taken as 1 *mg/ml* or 1000 μg/ml (100 %) for the entire CO_2 extract of *Ferula asafoetida* L.

4. Results

Ferula asafoetida L. The study of the chemical composition of the CO_2 extract revealed 34 chemical compounds. The list and chromatogram of these chemical bonds are shown in Fig. 3 and Table 3.

As shown in Table 2, di-n-butyldithiophosphinic acid (11,91 %), disulfide, bis(1-methylpropyl) (9.63 %), 1,2-Dithiolane (4.26 %), Tioxolone (3.46 %), 6-(Methylthio)hexa-1,5-dien-3-ol (2.51 %), 1,4-Dithiane-2,5-dione, 3,6-dimethyl-(2.49 %), Thiophene, 2,3,4-trimethyl-(0.13 %), di-n-butyldithiophosphinic acid (11.91 %), were found from the CO_2 extract. Some of the presented chemical compounds are referred to as sulfur compounds. The total percentage of sulfur compounds in the extract is 46.3 %.

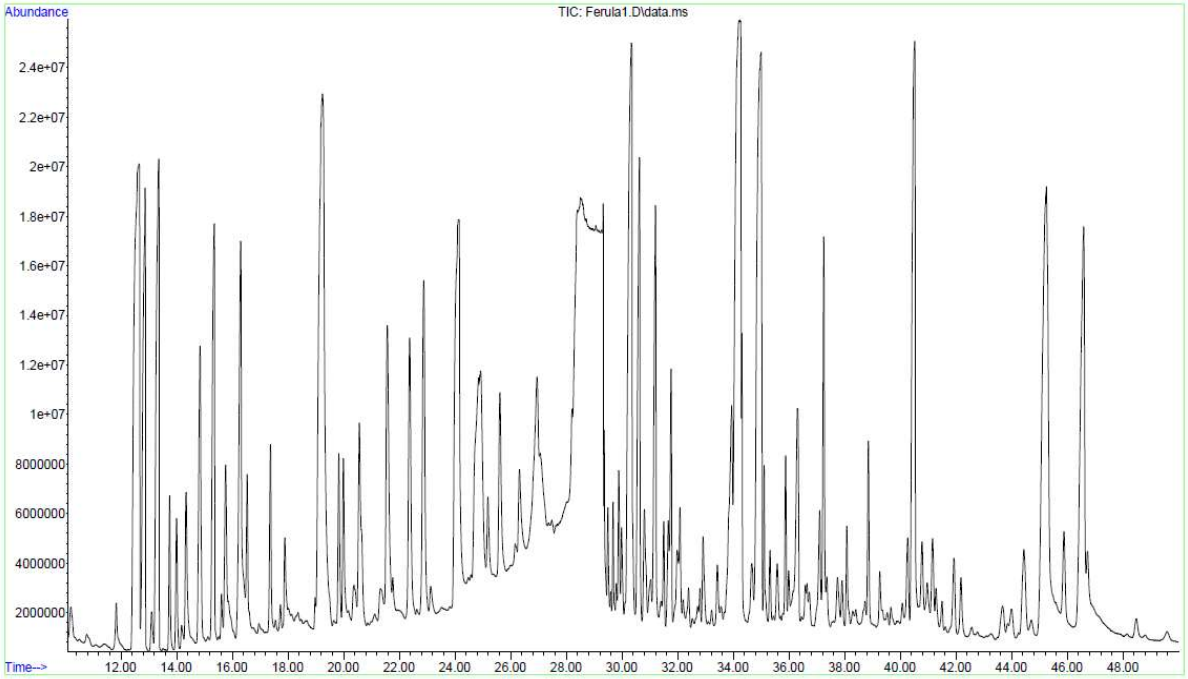

Fig. 3. Chromatogram of *Ferula asafoetida L.* extract obtained by CO_2 extraction

Table 3

Chemical compounds identified during the study of the component composition of *Ferula asafoetida* L. extract obtained by CO_2 extraction

No.	Compound	Holding time, min	Percentage content, %
1	Thiophene, 2,3,4-trimethyl-	10.2	0.13
2	Tetradecane	11.8	0.43
3	Disulfide, bis(1-methylpropyl)	12.6	9.63
4	1,2-Dithiolane	12.9	4.26
5	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, cis-	13.8	0.76
6	1,4-Dithiane-2,5-dione, 3,6-dimethyl-	14.8	2.49
7	Tioxolone	15.3	3.46
8	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	15.8	1.76
9	Pulegone	17.4	1.09
10	Butane(dithioic) acid, methyl ester	17.7	0.16
11	Butanoic acid, 3-methyl-	17.9	1.07
12	di-n-butyldithiophosphinic acid	19.2	11.91
13	1,3-Dioxolane, 2-butyl-4-methyl-	24.1	8.96
14	6-(Methylthio) hexa-1,5-dien-3-ol	26.2	2.51
15	Tetradecanoic acid, ethyl ester	26.9	10.32
16	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	29.5	0.91
17	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	29.7	0.66
18	Spathulenol	29.9	1.21
19	Methyl 6,8-octadecadiynoate	30.0	0.71
20	3,4-Methylenedioxypropiophenone	32.1	0.84
21	Ethyl oleate	34.2	14.13
22	Tributyl phosphorotrithioite	35.6	0.79
23	Eicosanoic acid, ethyl ester	37.1	1.21
24	Tetradecanoic acid	37.2	2.88
25	Pentadecanoic acid	38.1	0.80
26	Cyclopentadecanone, 2-hydroxy-	39.3	0.76
27	Docosanoic acid, ethyl ester	40.3	0.90
28	Palmitoleic acid	41.0	0.67
29	Heptadecanoic acid	41.3	0.44
30	Sclareolide	41.9	0.83
31	Heptadecanoic acid	42.2	0.56
32	Squalene	43.7	0.45
33	Oleic acid	45.2	10.87
34	Bis(2-ethylhexyl) phthalate	45.9	1.44
Total			100

Next, we conducted research on the antimicrobial activity of the CO_2 extract of *Ferula asafoetida* L.

One of the mandatory components of the design of an experiment to study any effect is the setting of controls, including a reference substance that has the same effect. That is why antibiotics, which are widely used in practice, were taken as a reference substance. In addition, there is currently a trend of transition from synthetic drugs to herbal drugs, so we have shown that the studied extract is not inferior in efficiency to synthetic antibiotics and can be one of the promising candidates for further development of an antimicrobial drug of plant origin.

In the course of the research, by the method of double serial dilutions in a liquid nutrient medium, the minimum inhibitory concentrations (MIC) of CO_2 extract were determined. The obtained research data is presented in Table 4.

The results indicate the presence of antimicrobial activity of CO_2 extract of *Ferula asafoetida* L. in relation to the tested strains of conditionally pathogenic microorganisms. Antimicrobial property is noted for both representatives of bacteria and fungi.

Table 4

Results of antimicrobial activity testing

Test strain	MIC values, μg/ml
Staphylococcus aureus	7.81±2.28
Bacillus subtilis	31.25±9.0
Escherichia coli	15.63±4.5
Klebsiella pneumoniae	15.63±4.5
Salmonella enterica	15.63±4.5
Candida albicans	62.5±17.4
Aspergillus niger	62.5±17.4

When studying the bactericidal activity, it was determined that the culture of Staphylococcus aureus was the most susceptible to the studied extract, the MIC in re-

lation to it was 7.81 μg/ml. Concerning representatives of bacteria of the *Enterobacteriaceae family*, antimicrobial activity was 15.63 μg/ml for cultures of *Escherichia coli, Salmonella enterica and Klebsiella pneumonia,* which also indicate greater sensitivity of the latter to the extract.

Thus, the test sample of CO_2 extract of *Ferula asafoetida* L. exhibits bactericidal and fungicidal effects against all the studied museum strains of microorganisms. The extract showed the greatest antimicrobial activity against S. aureus strains and representatives of *Enterobacteriaceae* family bacteria; the MIC was 7.81 μg/ml and 15.63 μg/ml, respectively. The fungicidal effect is recorded at a concentration of 62.5 μg/ml, both for the yeast-like fungus and the mycelial fungus *Aspergillus niger.*

The second method for determining the antimicrobial activity of the CO_2 extract of *Ferula asafoetida* L. was carried out by the disk-diffusion method. The results are shown in Table 5, Fig. 4–10.

As shown in Table 4, Fig. 4–10. the CO_2 extract of *Ferula asafoetida* L. *is* more active than the comparison drug amoxicillin in the relationship of *S. aureus* and the spore bacterium *B. subtilis* by 1.2 times, *E. coli* (also when tested by serial dilutions) by 1.5 times and *S. enterica* by 1.4 times. In addition, this extract showed fungicidal activity against *Candida albicans* 1.5 times more than fluconazole.

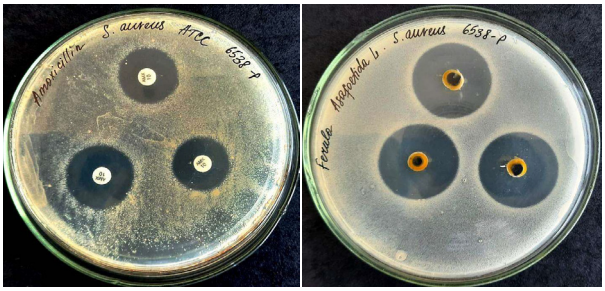

Fig. 4. Results of antimicrobial activity of CO_2 extract of *Ferula asafoetida* L. and amoxicillin against *Staphylococcus aureus* ATCC 6538-P

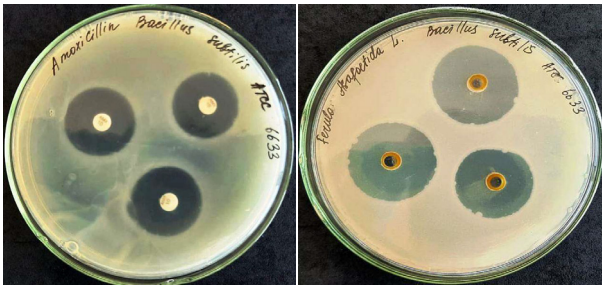

Fig. 5. Results of antimicrobial activity of CO_2 extract of *Ferula asafoetida* L. and amoxicillin against *Bacillus subtilis* ATCC 6633

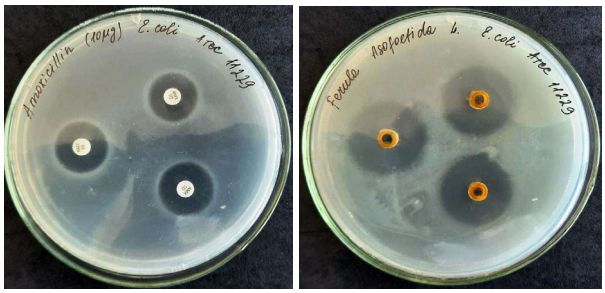

Fig. 6. Results of antimicrobial activity of CO_2 extract of *Ferula asafoetida* L. and amoxicillin against *Escherichia coli* ATCC 11229

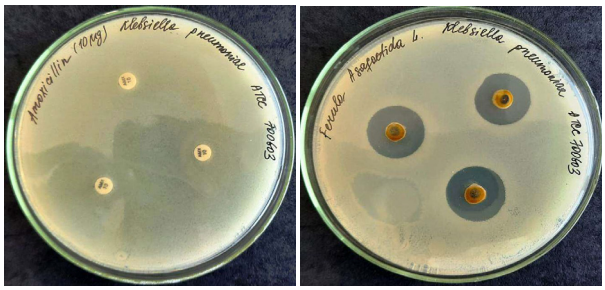

Fig. 7. Results of antimicrobial activity of CO_2 extract of *Ferula asafoetida* L. and amoxicillin against *Klebsiella pneumoniae* ATCC 700603

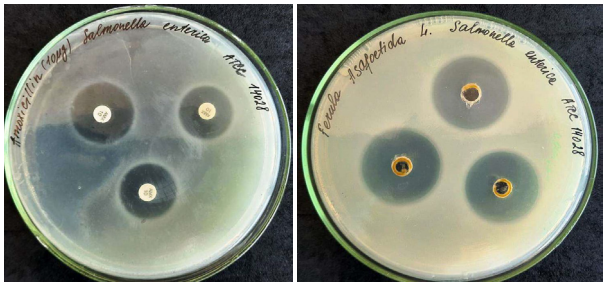

Fig. 8. Results of antimicrobial activity of CO_2 extract of *Ferula asafoetida* L. and amoxicillin against *Salmonella enterica* ATCC 14028

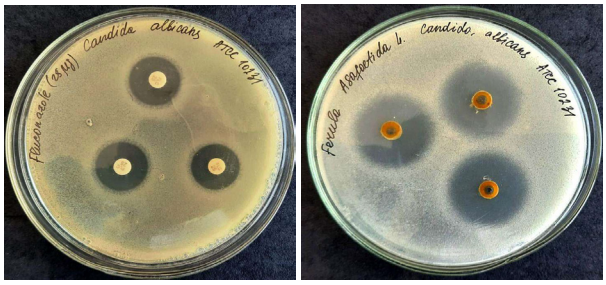

Fig. 9. Results of antimicrobial activity of CO_2 extract of *Ferula asafoetida* L. and fluconazole against *Candida albicans* ATCC 10231

Table 5

Results of determination of the antimicrobial activity of CO_2 extract of *Ferula asafoetida* L. by disk-diffusion method

Extract	*S. aureus*	*B. subtilis*	*E. coli*	*K. pneumoniae*	*S. enterica*	*C. albicans*	*A. niger*
	30.0±0.00	30.0±1.00	30.33±0.58	24.0±1.00	30.67±0.58	32.67±0.58	20.67±1.15
AMX (10 mcg)	25.67±1.15	26.3±1.15	20.33±0.58	6.0±0.00	22.67±1.15	–	–
FLC (25 mcg)	–	–	–	–	–	21.33±1.15	
KTl (10 mcg)	–	–	–	–	–	–	6.0±0.00

Note: AMX (10 μg) –amoxicillin; d Ø – 6,0 mm; FLC (25 μg) – fluconazole; d Ø – 6,0 mm; KTl (10 μg)– ketoconazole; d Ø – 6,0 mm

Fig. 10. Results of antimicrobial activity of CO_2 extract of *Ferula asafoetida* L. and ketoconazole against *Aspergillus niger* ATCC 16404

During our research, we found that the growth inhibition zone of *K. pneumoniae* was 24.0±1.00 mm, while the comparison drug amoxicillin has no activity, and also for the mycelial fungus *Aspergillus niger*, the growth suppression zone of this extract was 20.67±1.15 mm, while the comparison drug ketoconazole does not show activity.

Thus, from the conducted research, it was found that the CO_2 extract of *Ferula asafoetida* L. has antimicrobial activity both when tested by the serial dilution method and by the disk-diffusion method.

5. Discussion

The content of chemical compounds in *Ferula asafoetida* L. extract may vary depending on the climatic conditions in the regions where the plant grows and the time of harvest and processing.

Razavizadeh B. and Niazmand R. studies have found high content of carvacrol and α-bisabolol in hydroalcoholic extract of ferula leaf, gum – (Z)-b-ocimene and (E)-1-propenyl-sec-butyl-disulfide. [30]. Kavoosi G. et al. [31], and also Amalraj A. et al. identified the lowest radical scavenging and the heterocyclic disulfide – 1,2-Dithiolane (Divya K. et al. 1,2-dithiolane – 5.7 % [35]) highest antibacterial and antifungal effects [5]. Kavoosi G. et al., with co-authors, studied that sulfur compounds exhibit antibacterial properties [16]. Thus, the results of our research show that the types and levels of sulfur compounds of *Ferula asafoetida* L. can be used as a determination of the main source of this plant, as well as to identify the high quality of *Ferula asafoetida* L. for pharmaceutical use [45]. And also, this antifungal activity may be attributed, at least in part, to the presence of phenols, flavonoids and sesquiterpenes in the extracts [36].

In addition, in our studies, we identified a chemical compound 1,3-Dioxolane, 2-butyl-4-methyl- (8.96 %). According to research by Küçük et al., this compound and its derivatives have shown antimicrobial activity [46]. And also the largest amount is fatty acid ether – ethyloleate (14.13 %), unsaturated fatty acid – oleic acid (10.87 %), which reduces the frequency of cardiovascular diseases, has an anti-inflammatory effect, and fatty acid ether – tetradecanoic acid, ethyl ether (10.32 %) have antioxidant, antimicrobial, anticancer, cosmetic, hypercholesterolemic activity [47–49].

As a result of comparing the data obtained on the antimicrobial activity of the CO_2 extract of *Ferula asafoetida* L. with the data indicated in Table 1, we found that the antimicrobial activity of the CO_2 extract of *Ferula asafoetida* L. exceeds that of extracts obtained using chloroform, ethyl acetate, ethanol, methanol, petroleum ether, hexane, hot and cold water as an extractant. But, at the same time, the antimicrobial activity of *Ferula asafoetida* L. essential oils obtained by hydrodistillation showed high efficiency in relation to *Escherichia coli* and *Bacillus subtilis* than compared with CO_2 extract.

Research limitations. A limitation of the study could be considered that during the GC-MS study of the composition of CO_2 extract of the *Ferula asafoetida* L., some chemical compounds were not identified due to the absence of their characteristics in the automatic library of the NIST02 and Wiley 7th edition database.

Prospects for further research. In further studies, it is advisable to analyze the component composition of CO_2 extract, as well as essential oils and resin of *Ferula asafoetida* L., depending on their stages of germination, climatic conditions and place of growth. Also, phytochemical and pharmacological studies of *Ferula asafoetida* L. can show the prospect of creating new pharmaceutical preparations in the form of a galenic drug or soft dosage forms (gels, ointments and creams).

6. Conclusions

In conclusion, based on the literary review of *Ferula asafoetida* L., it has been established that medicinal plant raw materials have wound healing, anti-inflammatory, antinociceptive, anticonvulsant, antioxidant, antifungal, antiviral, antileishmanial, antimicrobial, antitumor, antidiabetic, hypotensive, antispasmodic, antihemolytic effects. This plant is widely distributed in the southern and western regions of Kazakhstan. To obtain medicinal plant raw materials *Ferula asafoetida* L. was harvested in April after the end of the vegetative period in accordance with the requirements of the GACP. From the obtained medicinal plant raw materials, a CO_2 extract was obtained under subcritical conditions, the yield of which was 2.5 %. The component composition of the obtained CO_2 extract of *Ferula asafoetida* L. was investigated by the GC-MS method. 34 chemical bonds were identified from the CO_2 extract, of which 8 (46.3 %) were sulfate compounds. During the study of the antimicrobial effect of CO_2 extract of *Ferula asafoetida* L. by the method of double serial dilutions and the disk-diffusion method, the high antimicrobial property was proved against gram-positive (*Staphylococcus aureus subsp. Aureus, Bacillus subtilis subsp. Spizizenii*), gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae ssp. pneumoniae, Salmonella enterica subsp. enterica*) and fungi (*Candida albicans, Aspergillus niger*). A wide range of antimicrobial properties of this extract is associated with the presence of sulfate compounds in the chemical composition. Reactive sulfate compounds form disulfide bonds with free sulfhydryl groups of enzymes and violate the integrity of the bacterial membrane [50].

Given the above, the CO_2 extract of *Ferula asafoetida* L. can be used in the pharmaceutical industry as a medicinal herbal substance, for the development of drugs with antimicrobial action, in particular, ointments or gels for external use.

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 on reasonable request.

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