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PRELIMINARY IDENTIFICATION OF ORGANIC COMPOUNDS IN THE ETHANOL EXTRACT OF THE ROOTS OF THE HERB *FERULA FOETIDA*

Ferula foetida is a traditional medicinal herb, and its roots contain various bioactive components. In this study, dried roots of *Ferula foetida* were used as raw material. The total extract (4.4 g from 80 g of raw material) was obtained by ultrasound-assisted extraction with 96% ethanol, followed by concentration using rotary evaporation. After dispersing the total extract in an aqueous phase, liquid-liquid extraction with petroleum ether (PE) and dichloromethane (DCM) was performed to yield the PE (1.46 g) and DCM (0.19 g) fractions. These fractions were analyzed by gas chromatography-mass spectrometry method (GC-MS) and the results evaluated 32 compounds in PE fraction and 79 components in DCM fraction. This study preliminarily established a sequential extraction method for organic compounds from the roots of this herb, laying a foundation for the subsequent separation, purification, and structural identification of chemical constituents in fractions of different polarities. Preliminary analysis indicates that the PE and DCM fractions are mainly enriched in low-polarity components, which can be further identified by techniques such as HPLC, LC-MS, and NMR.

Keywords: *Ferula foetida*, roots, organic compound extraction, liquid-liquid extraction, preliminary identification.

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Ferula foetida шөп тамырларының этанол сығындысындағы органикалық қосылыстарды алдын ала анықтау

Жемісті Ферула – дәстүрлі дәрілік өсімдік және оның тамырларында әртүрлі биологиялық белсенді компоненттер бар. Бұл зерттеуде жемісті феруланың кептірілген тамыры шикізат ретінде пайдаланылды. Сығындының жалпы көлемі (80 г шикізаттың 4,4 г) 96% этанолмен ультрадыбыстық экстракция арқылы, содан кейін айналмалы булану арқылы концентрациялау арқылы алынды. Барлық сығындыны Сулы фазада таратқаннан кейін PE (1,46 г) және DCM (0,19 г) фракцияларын алу үшін мұнай эфирімен (PE) және дихлорметанмен (DCM) сұйық-сұйық экстракция жүргізілді. Бұл фракциялар газ хроматографиясы-масс-спектрометрия (GC-MS) әдісімен талданды және нәтижесінде PE фракциясындағы 32 қосылыс және DCM фракциясындағы 79 компонент бағаланды. Бұл зерттеу химиялық компоненттерді кейіннен бөлуге, тазартуға және құрылымдық сәйкестендіруге негіз қалап, осы өсімдіктің тамырынан органикалық қосылыстарды дәйекті түрде алу әдісін алдын ала белгіледі. Бұл зерттеу осы шөптің тамырынан органикалық қосылыстарды алудың дәйекті әдісін алдын ала анықтап, кейіннен әртүрлі полярлық фракциялардағы химиялық құрамдастарды бөлуге, тазартуға және құрылымдық сәйкестендіруге негіз қалады. Алдын ала талдау көрсеткендей, PE және DCM фракциялары негізінен төмен полярлықты компоненттермен

ты компоненттермен байытылған, оларды HPLC, LC-MS және NMR сияқты әдістермен одан әрі анықтауға болады.

Түйін сөздер: *Ferula foetida*, тамырлар, органикалық қосылыстарды алу, сұйық-сұйық экстракция, алдын ала сәйкестендіру.

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Предварительная идентификация органических соединений в этанольном экстракте корней травы *Ferula foetida*

Ферула плодовая – традиционное лекарственное растение, а ее корни содержат различные биологически активные компоненты. В данном исследовании в качестве сырья использовались высушенные корни ферулы плодовой. Общий объем экстракта (4,4 г из 80 г сырья) был получен путем экстракции с помощью ультразвука 96%-ным этанолом с последующим концентрированием с помощью ротационного выпаривания. После диспергирования всего экстракта в водной фазе проводили жидкостно-жидкостную экстракцию петролейным эфиром (РЕ) и дихлорметаном (ДСМ) для получения фракций РЕ (1,46 г) и ДСМ (0,19 г). Эти фракции были проанализированы методом газовой хроматографии-масс-спектрометрии (GC-MS), и в результате были получены оценки 32 соединений во фракции РЕ и 79 компонентов во фракции ДСМ. Это исследование предварительно установило метод последовательной экстракции органических соединений из корней этого растения, заложив основу для последующего разделения, очистки и структурной идентификации химических компонентов во фракциях различной полярности. Это исследование предварительно установило метод последовательной экстракции органических соединений из корней этого растения, заложив основу для последующего разделения, очистки и структурной идентификации химических компонентов во фракциях различной полярности. Предварительный анализ показывает, что фракции РЕ и ДСМ в основном обогащены компонентами низкой полярности, которые могут быть дополнительно идентифицированы с помощью таких методов, как ВЭЖХ, ЖК-МС и ЯМР.

Ключевые слова: *Ferula foetida*, корни, экстракция органического соединения, жидкостно-жидкостная экстракция, предварительная идентификация.

Introduction

Ferula foetida is an annual or perennial herb of the Apiaceae family, native to Central Asia (Kyzylkum Desert, Karakum Desert, Turkmenistan), eastern Iran, western Afghanistan, and western Pakistan. It is one of the most widely distributed species producing asafoetida. Asafoetida (also spelled asafetida) is a dried resin (gum) obtained from the roots or rhizomes of *Ferula* species (Oxford English Dictionary, 1989). In traditional Asian medicine systems, asafoetida resin has been used as a digestant, gastrointestinal tonic, anthelmintic, antispasmodic, carminative, lung purifier, stomachic, and sedative (Zare et al., 2011). More information regarding its current uses and pharmacological effects is needed for future clinical studies.

Ferula foetida is among the most widely distributed species for asafoetida production. It is the main source of asafoetida in eastern Iran, western Afghanistan, western Pakistan, and Central Asia (Karakum Desert, Kyzylkum Desert). Its essential oil contains sulfur compounds (Farhadi et al., 2020). It possesses medicinal value due to bioactive phytochemicals including terpenoids, sulfide derivatives, essential oils, phenolics, and minerals. Recent studies have shown that asafoetida exhibits various promising activities, particularly relaxant, memory-enhancing, neuroprotective, digestive enzyme, antispasmodic, antioxidant, antihypertensive, hepatoprotective, anticancer, antibacterial, anticytotoxic, anthelmintic, anti-obesity, and antagonistic effects (Amalraj & Gopi, 2017).

The aqueous extract of *Ferula foetida* resin at a concentration of 100 mg/mL has shown significant anthelmintic activity against *Pheretima posthuma* (Kareparamban et al., 2012). Ferulic acid (FA), a compound first isolated from *Ferula foetida* and naturally occurring in plant cell walls, exhibits antioxidant, anti-inflammatory, antibacterial, anticancer, neuroprotective, hepatoprotective, cardioprotective, antidiabetic, and photoprotective effects (Lahari et al., 2025).

Current research on *Ferula foetida* has mostly focused on its aerial parts or resin, while systematic chemical identification of organic compounds in its roots remains insufficient. Its extracts and derivatives are used for treating diseases, softening skin, and repelling parasites. Chemical investigation of bioactive compounds from the roots and improvement of extraction techniques hold great potential for significant economic benefits in the market.

This study aims to preliminarily extract, fractionate, and identify the organic components from the roots of this plant, thereby clarifying its major chemical types and providing a scientific basis for subsequent activity-guided isolation and quality evaluation.

Literature review

Our previous study included the comparative results of qualitative and quantitative analyses of the main groups of biologically active complexes in the aerial and underground parts of *Ferula foetida* (Tolpebergen et al., 2024). The study demonstrated that the underground part contained higher amounts of flavonoids (0.24%), polysaccharides (2.80%), tannins (8.50%), and saponins (2.34%), whereas the aerial part was characterized by higher contents of alkaloids (0.56%), coumarins (1.96%), and organic acids (0.16%). In addition, plant authenticity parameters showed moisture contents of 6.00% and 7.40%, and ash values of 10.30% and 13.17% for the aerial and underground parts, respectively. The mineral composition of both plant organs was also investigated, confirming significant differences in their chemical profiles and potential pharmacological value.

The Table 1 and Table 2 reflect the results of quantitative determination of some organic groups and mineral composition of root part of *Ferula foetida*.

Table 1

(A) Quantitative determination of the content of some groups of biologically active substances (BAS) in the root part of *Ferula foetida*; (B) The amount of detected micro- and macroelements in the ash of the underground part of *Ferula foetida*

(A)		(B)	
Phytochemical	Root part of <i>Ferula foetida</i> plant, %	Elements	Concentration in root part, mg/g
Moisture content	7,40	Cd	0,0001
Ash	13,17	Ni	0,0002
Extractives	22,69	Pb	0,0030
Organic acids	0,090	Cu	0,0076
Flavonoids	0,237	Mn	0,0206
Polysaccharides	2,80	Zn	0,0011
Alkaloids	0,34	Fe	0,0875
Coumarins	0,76	Ca	4,668
Tannins	8,50	Mg	1,496
Saponins	2,34	Na	1,295
		K	11,676

Methodology

The roots of *Ferula foetida* were collected from the vicinity of Shymkent, Republic of Kazakhstan, during the vegetation period and primary processing procedures of plant raw material as drying and grinding were completed for further phytochemical analyses.

Preparation of Plant Extracts Using Ultrasound-Assisted Extraction

Dried roots of *Ferula foetida* were pulverized using a grinder and passed through a 40-mesh sieve for later use. Accurately weighed 80.00 g of root powder was placed into a 1000 mL conical flask with a stopper. Then, 500 mL of 96% ethanol was added, and ultrasound-assisted extraction was performed at room temperature for 60 min. The mixture was filtered, and the filtrate was collected. The residue was extracted twice more under the same conditions, each time with 500 mL of ethanol. The three filtrates were combined.

The combined filtrate was concentrated under reduced pressure in a water bath at 45°C until no

ethanol odor remained, yielding 4.4 g of a dark-brown syrupy total extract. The extraction yield was calculated to be 5.5% (relative to the dry weight of the plant material).

Preparation of the PE and DCM Fractions by Liquid-Liquid Extraction

The total extract (4.4 g) was dissolved in 100 mL of distilled water and transferred to a separatory funnel. The aqueous solution was extracted three times with petroleum ether (PE, 100 mL each time). The PE phases were combined and concentrated under reduced pressure at 45°C to obtain 1.46 g of the PE extract. The same procedure was done with DCM as solvent.

GC-MS analysis

The PE and DCM fractions of *Ferula foetida* roots was analyzed and characterized by GC-MS. The samples were analyzed by gas chromatography with mass spectrometric detection (7890A/5975C). Sample volume was 2.0 μ L, injection temperature was 280 °C, splitless injection mode. Separation was carried out using a DB-17ms capillary column (30 m length, 0.25 mm inner diameter, 0.25 μ m film thickness) at a constant carrier gas (helium) flow rate of 1 mL/min. The column temperature was programmed from 40 °C, with a heating rate of 5 °C/min, to 300 °C (held for 10 min). The analysis time was 67 minutes. Detection was performed in SCAN mode (m/z 34–750). Agilent MSD ChemStation software (version 1701EA) was used to control the gas chromatography system, record and process the obtained results and data. Data processing included determination of retention times, peak areas, and processing of spectral information obtained from the mass spectrometric detector. For interpretation of the obtained mass spectra, the Wiley 11th edition and NIST'02 libraries were used (the total number of spectra in the libraries is more than 550,000).

Results and discussion

The fractionation of the root extract of *F. foetida* revealed differences in the distribution of compounds according to their polarity (Table 2). The total extract yield was 4.4 g, which is 5.5% of total raw plant mass. The PE fraction was the predominant fraction, accounting for 1.46 g, indicating the abundance of non-polar lipophilic compounds in

the roots. In contrast, the DCM fraction's yield was equal to 0.19 g or 4.31% of total crude extract. The remaining aqueous phase represented approximately 62.5% of the total extract. This value can be explained by the presence of significant amount of polar constituents such as phenolic compounds and polysaccharides.

Table 2

Yield of fractions from the root extract of Ferula foetida

Fraction	Mass (g)	Percentage of total extract (%)	Yield (%)
Total extract	4.4	100	5.50
PE fraction	1.46	33.2	1.83
DCM fraction	0.19	4.31	0.23
Remaining aqueous phase	~2.75	~62.5	~3.43

GC-MS analysis has identified 32 bioactive compounds in PE fraction (Table 3) and 79 constituents in DCM fraction (Table 4) that provide a clearer insight into the therapeutic value of *Ferula foetida*.

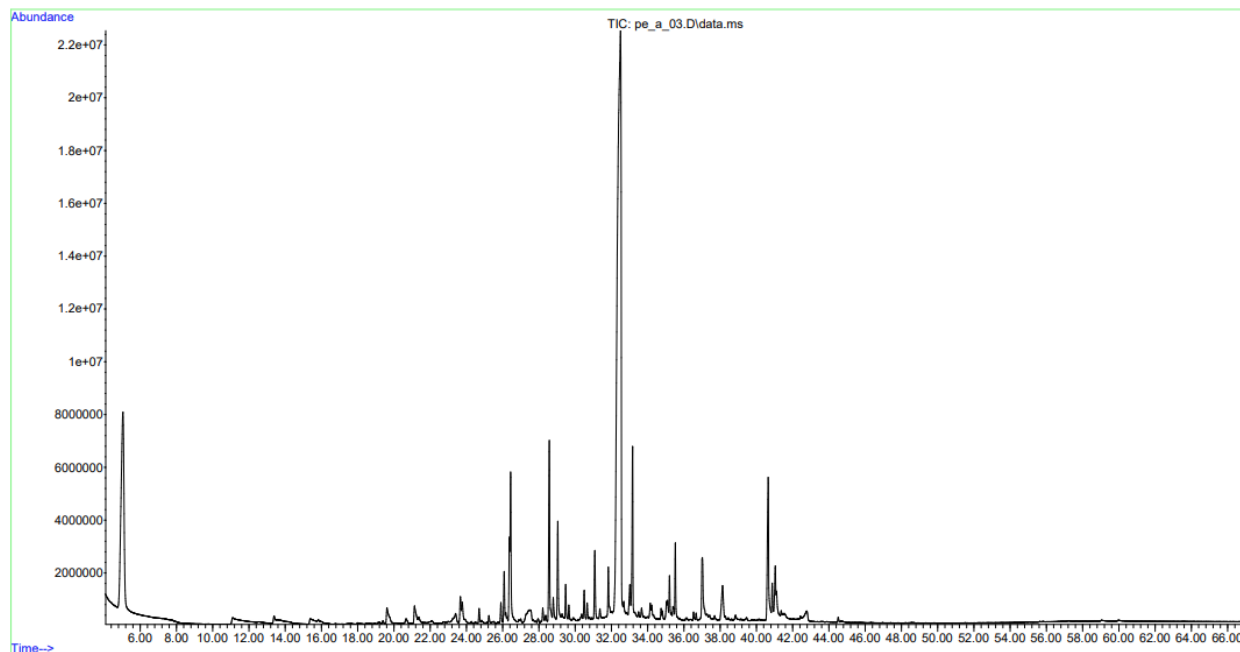
β -Caryophyllene exhibits various pharmacological activities, including anti-inflammatory, analgesic, antioxidant, cardioprotective, immunomodulatory, nephroprotective, neuroprotective, and intestinal protective effects (Guo et al., 2025). β -Caryophyllene can directly inhibit cancer cell proliferation, induce cell cycle arrest, and promote apoptosis in multiple cancers, including lung (Ahmed et al., Gbadebo et al., 2025; 2022; Wittig et al., 2024), colorectal (Dahham et al., 2021), ovarian (Arul et al., 2020), bladder (Yu et al., 2021), liver (Ahmad et al., 2024; Xiu et al., 2022), pancreatic (Di Giacomo et al., 2022), and breast cancer (Di Sotto et al., 2022).

Caryophyllene oxide possesses significant anti-lymphoma and cytotoxic activities, reduces cancerous lung cell growth, and shows a synergistic anti-nociceptive effect when combined with acetaminophen (Espinosa-Juárez et al., 2024; Ramírez-Santos et al., 2024; Shabana et al., 2023).

9,12-Octadecadienoic acid (Z,Z)- (also known as linoleic acid) exhibits anticancer, anti-obesity, and anti-inflammatory effects (Du et al., 2024).

Table 3*Phytocompounds identified from PE fractions*

№	Retention time, min	Name of compounds	Molecular formula	Probability of identification, %	Content, %
1	5,05	Toluene	C ₇ H ₈	98	12,54
2	13,39	Methyl sec-butyl disulphide	C ₅ H ₁₂ S ₂	87	0,21
3	19,62	Disulfide, bis(1-methylpropyl)	C ₈ H ₁₈ S ₂	94	1,79
4	20,68	Butane(dithioic) acid, methyl ester	C ₅ H ₁₀ S ₂	68	0,22
5	21,14	Tioxolone	C ₇ H ₆ O ₂ S	65	0,81
6	21,37	(E)-1-(But-1-en-1-yl)-2-(sec-butyl)disulfane	C ₈ H ₁₆ S ₂	66	0,28
7	23,41	3-Vinyl-1,2-dithiacyclohex-5-ene	C ₆ H ₈ S ₂	89	0,59
8	23,67	Thymol	C ₁₀ H ₁₄ O	67	0,71
9	24,70	Caryophyllene	C ₁₅ H ₂₄	92	0,35
10	26,09	cis-Raphasatin	C ₆ H ₁₀ S ₂	66	1,45
11	26,44	Di-n-butyl dithiophosphinic acid	C ₈ H ₁₉ PS ₂	67	5,41
12	28,57	1,3-Dioxolane, 2-butyl-4-methyl-	C ₈ H ₁₆ O ₂	70	4,21
13	29,66	sec-Butyl t-butyl sulfide	C ₈ H ₁₈ S	77	0,49
14	30,36	Phenol, 2,6-dimethoxy-4-(2-propenyl)-	C ₁₁ H ₁₄ O ₃	82	0,35
15	30,50	Caryophyllene oxide	C ₁₅ H ₂₄ O	91	0,84
16	31,09	3H-1,2-Dithiole	C ₃ H ₄ S ₂	64	1,93
17	31,83	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	C ₁₂ H ₁₆ O ₃	93	2,37
18	32,50	6-(Methylthio)hexa-1,5-dien-3-ol	C ₇ H ₁₂ OS	70	52,73
19	32,68	2,5-Octadecadiynoic acid, methyl ester	C ₁₉ H ₃₀ O ₂	65	0,81
20	33,02	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	90	1,19
21	33,50	aR-Turmerone	C ₁₅ H ₂₀ O	68	0,35
22	33,67	Spiro[4.5]dec-6-en-8-one, 1,7-dimethyl-4-(1-methylethyl)-	C ₁₅ H ₂₄ O	71	0,68
23	34,15	Pentadecanoic acid	C ₁₅ H ₃₀ O ₂	76	0,52
24	35,21	Pentadecanoic acid, ethyl ester	C ₁₇ H ₃₄ O ₂	74	1,19
25	37,02	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	80	2,35
26	37,28	Ethyl 9-hexadecenoate	C ₁₈ H ₃₄ O ₂	70	0,08
27	38,13	1-(Methylthio)-3-(2-propenylthio)-1-propene	C ₇ H ₁₂ S ₂	66	1,46
28	38,85	Ethyl 14-methyl-hexadecanoate	C ₁₉ H ₃₈ O ₂	68	0,22
29	40,88	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	90	0,82
30	41,04	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	88	2,16
31	41,37	1,4,7-Androstatrien-3,17-dione	C ₁₉ H ₂₂ O ₂	65	0,11
32	42,78	Thiocyanic acid, ethyl ester	C ₃ H ₅ NS	70	0,76

Figure 1*Chromatogram of the PE fraction of Ferula foetida root part*

n-Hexadecanoic acid demonstrated strong anti-inflammatory effects via COX-1 and COX-2 inhibition (Purushothaman et al., 2025). Tetradecanoic acid (also known as myristic acid) can promote the proliferation and differentiation of neural stem cells in vitro and alleviate hippocampal aging associated with GABA signaling (Shang et al., 2022). 3-Vinyl-1,2-dithiacyclohex-5-ene, one of the main

active components of garlic extract, has been confirmed to possess broad-spectrum antibacterial activity (Chen et al., 2018). Benzene, 1,2,3-trimethoxy-5-(2-propenyl)- (also known as elemicin), present in an essential oil at a content of 72.34%, exhibits significant antioxidant, anti-inflammatory, antidiabetic, and antibacterial activities (Gogoi et al., 2022).

Table 4*Phytocompounds identified from DCM fractions*

№	Retention time, min	Name of compounds	Molecular formula	Probability of identification, %	Content, %
1	9,75	Ethanone, 1-(2-methylcyclopropyl)-	$C_6H_{10}O$	73	0,49
2	9,91	Formamide, N,N-dimethyl-	C_3H_7NO	71	0,22
3	10,77	2-Furanmethanol	$C_5H_6O_2$	74	0,20
4	12,15	Cyclohexanone	$C_6H_{10}O$	82	0,12
6	13,85	Eucalyptol	$C_{10}H_{18}O$	78	0,23
7	14,74	Hexane, 1,1-diethoxy-	$C_{10}H_{22}O_2$	77	0,19
8	15,32	2(5H)-Furanone, 5,5-dimethyl-	$C_6H_8O_2$	93	0,21
9	17,38	Disulfide, ethyl 1-methylpropyl	$C_6H_{14}S_2$	68	0,33
11	18,07	Glycerin	$C_3H_8O_3$	82	1,24
12	19,43	(+)-2-Bornanone	$C_{10}H_{16}O$	94	0,19
13	20,63	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	88	0,23

Continuation of the table

№	Retention time, min	Name of compounds	Molecular formula	Probability of identification, %	Content, %
14	21,39	Nonanoic acid	C ₉ H ₁₈ O ₂	65	0,16
15	21,73	Benzyl nitrile	C ₈ H ₇ N	90	0,14
17	22,26	Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester	C ₁₂ H ₂₀ O ₂	85	0,07
18	22,70	Benzaldehyde, 2,5-dimethyl-	C ₉ H ₁₀ O	91	0,29
20	24,51	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	C ₁₅ H ₂₄	82	0,26
21	25,01	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	88	0,34
22	25,98	N-Acryloylmorpholine	C ₇ H ₁₁ NO ₂	73	0,08
23	26,17	Eugenol	C ₁₀ H ₁₂ O ₂	82	0,07
24	27,82	Methyleugenol	C ₁₁ H ₁₄ O ₂	76	0,14
26	28,54	2,4-Di-tert-butylphenol	C ₁₄ H ₂₂ O	93	2,06
27	30,28	Ethanone, 1-(2-hydroxyphenyl)-	C ₈ H ₈ O ₂	78	0,12
28	32,10	Sucrose	C ₁₂ H ₂₂ O ₁₁	75	2,60
30	32,95	2,5-Pyrrolidinedione, 3-(1-aminoethylidene)-4-methyl-	C ₇ H ₁₀ N ₂ O ₂	74	0,20
31	33,03	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	74	0,33
32	33,14	Diethyl Phthalate	C ₁₂ H ₁₄ O ₄	78	0,19
33	34,55	Ethyl α-d-glucopyranoside	C ₈ H ₁₆ O ₆	84	0,81
34	35,03	Pentadecanoic acid	C ₁₅ H ₃₀ O ₂	72	0,39
36	35,83	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	75	0,12
39	36,60	3-Deoxy-d-mannonic lactone	C ₆ H ₁₀ O ₅	68	0,49
40	37,03	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	92	3,28
41	37,45	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	95	17,49
42	38,38	Heptadecanoic acid	C ₁₇ H ₃₄ O ₂	76	0,24
43	38,86	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C ₁₇ H ₂₄ O ₃	92	0,48
47	40,08	Dibutyl phthalate	C ₁₆ H ₂₂ O ₄	91	0,48
48	40,61	(E)-9-Octadecenoic acid ethyl ester	C ₂₀ H ₃₈ O ₂	92	2,60
49	40,93	Linoleic acid ethyl ester	C ₂₀ H ₃₆ O ₂	93	8,28
50	41,17	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	75	15,36
51	41,50	9,12-Octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	94	14,22
52	41,76	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	C ₁₈ H ₃₀ O ₂	82	0,43
53	42,71	Fumaric acid, decyl 2-dimethylaminoethyl ester	C ₁₈ H ₃₃ NO ₄	88	0,18
55	43,91	Eicosanoic acid, ethyl ester	C ₂₂ H ₄₄ O ₂	72	0,09
57	44,07	Eicosanoic acid	C ₂₀ H ₄₀ O ₂	71	0,28
61	46,44	9-Octadecenamide, (Z)-	C ₁₈ H ₃₅ NO	73	0,12
64	48,21	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₁₉ H ₃₈ O ₄	92	1,31
65	48,34	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	C ₂₃ H ₃₂ O ₂	85	1,07
66	48,75	Bis(2-ethylhexyl) phthalate	C ₂₄ H ₃₈ O ₄	94	0,92
69	50,93	Squalene	C ₃₀ H ₅₀	95	15,92
71	51,25	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	C ₂₁ H ₄₀ O ₄	83	2,18

Continuation of the table

No	Retention time, min	Name of compounds	Molecular formula	Probability of identification, %	Content, %
72	51,57	9,12-Octadecadienoic acid (Z,Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₂₁ H ₃₈ O ₄	87	1,17
74	52,41	13-Docosenamide, (Z)-	C ₂₂ H ₄₃ NO	72	0,30
77	55,37	β-Tocopherol	C ₂₈ H ₄₈ O ₂	88	0,17
78	56,66	Vitamin E	C ₂₉ H ₅₀ O ₂	80	0,04
79	60,41	Stigmasterol	C ₂₉ H ₄₈ O	82	0,89

The analyzed chromatographic profile shows a complex mixture of compounds with a clear predominance of lipophilic constituents. The dominant chemical classes are fatty acids and their derivatives (esters, glycerides), which represent the majority of fraction, including palmitic acid (17.49%), stearic acid (15.36%), and linoleic acid derivatives (8.28–14.22%). Another significant group is unsaturated hydrocarbons and triterpenoids, with squalene being one of the key components (15.92%). Squalene is a triterpenoid compound composed of six isoprene units and serves as a precursor for the biosynthesis of triterpenes, steroid hormones, and cholesterol (Wu et al., 2022). Owing to its multiple double bonds, squalene exhibits various biological activities, including antioxidant, antitumor, antibacterial, hypolipidemic, detoxifying, and skin-protective effects (Cheng et al., 2024).

In addition, the sample contains phenolic compounds and aromatic derivatives (eugenol, benzaldehyde derivatives), as well as esters and phthalates, which contribute to minor but diverse fractions. Carbohydrates and sugar derivatives (e.g., sucrose) are also present in moderate amounts. Smaller amounts of terpenoids and oxygenated monoterpenes (eucalyptol, camphor derivatives) and sterols (stigmasterol, vitamin E derivatives) were detected. Vitamin E functions as a key antioxidant, protecting membrane lipids as well as low-density lipoprotein (LDL) in circulation, and showing activity in tissues such as the lungs, liver, and adrenal glands (Permadi et al., 2025).

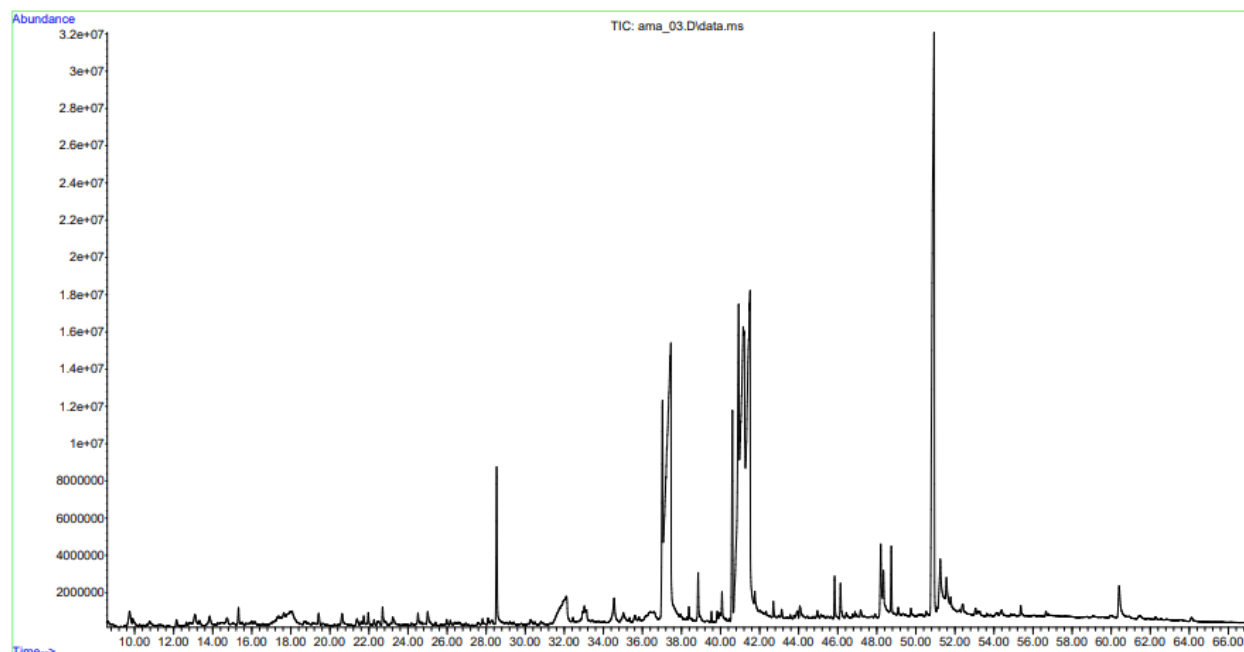
Stigmasterol is a plant sterol found in various herbs and crops such as soybean and tobacco, and it has attracted considerable interest due to its pharmacological properties, including anti-inflammatory,

antidiabetic, antioxidant, and cholesterol-lowering effects (Zhang et al., 2022). Several studies suggest that stigmasterol may serve as a promising therapeutic compound.

The pharmacological actions of these substances precisely align with the traditional use of asafoetida as an effective remedy for improving digestion, relieving muscle pain, treating digestive system infections, and alleviating inflammation.

This study integrates traditional knowledge with modern scientific techniques and emphasizes the necessity of validating herbal remedies through rigorous scientific inquiry. These findings not only confirm the traditional uses of asafoetida but also open up further prospects for exploring its bioactive compounds. Such research may pave the way for the development of novel therapeutic agents derived from natural products. Although this study provides some insights, further investigations are still needed to explain exactly how the identified phytochemicals exert their effects, this is particularly important for *in vivo* studies, which could reveal the bioavailability and pharmacokinetics of these compounds and further enhance our understanding of their therapeutic efficacy in clinical settings.

Furthermore, potential synergistic effects among the identified compounds may lead to more effective treatment strategies. With the growing global interest in natural products, further exploration of *Ferula foetida* is necessary. Its multi-target pharmacological activities make it suitable for integration into modern medical practice, especially in the treatment of chronic and infectious diseases. Its potent anti-inflammatory, antioxidant, antimicrobial, and neuroprotective effects position this plant as an ideal candidate among herbal remedies.

Figure 2*Chromatogram of the DCM fraction of Ferula foetida root part*

Extraction Efficiency Analysis

Ultrasound-assisted extraction with 96% ethanol gave a total yield of 5.5%, which is close to the reported yields (3–8%) of ethanol extracts from asafoetida roots, indicating that the extraction conditions are reasonable (Khajeh et al., 2005). Compared with traditional heat-reflux extraction, ultrasound-assisted extraction has the advantages of shorter extraction time, lower temperature, and reduced degradation of heat-sensitive components.

Recommendations for Further Studies

For PE and DCM fractions: Silica gel column chromatography (petroleum ether–ethyl acetate gradient elution) analysis is recommended, with focus on volatile and semi-volatile components.

Aqueous phase fraction: Sequential extraction with ethyl acetate and n-butanol is suggested to further isolate and enrich polar compounds such as glycosides and phenolic acids.

Conclusion

In this study, the roots of *Ferula foetida* were preliminarily fractionated using ultrasound-assisted ethanol extraction combined with liquid-liquid extraction, and bioactive compounds of PE and DCM fractions were identified by GC-MS analysis, thereby clarifying the medicinal potential of this plant. The identified

compounds include sesquiterpenes, fatty acids, sulfur-containing compounds, and phenylpropanoids. These components exhibit a wide range of pharmacological activities, such as anti-inflammatory, antioxidant, anticancer, antibacterial, neuroprotective, cardioprotective, and immunomodulatory effects, which precisely explain the traditional use of asafoetida for improving digestion, relieving muscle pain, treating digestive system infections, and alleviating inflammation.

This study integrates traditional knowledge with modern scientific approaches and emphasizes the necessity of rigorous experimental validation in herbal research. These findings not only confirm the traditional uses of asafoetida but also open new prospects for further exploration of its bioactive compounds. However, further *in vivo* studies are still needed to reveal the bioavailability, pharmacokinetics, and mechanisms of action of these compounds in clinical settings. Moreover, potential synergistic effects among the identified compounds may lead to more effective therapeutic strategies.

With the growing global interest in natural products, continued investigation of *Ferula foetida* is of great significance. Its multi-target pharmacological activities make it suitable for integration into modern medical practice, particularly demonstrating its potential as an ideal herbal candidate in the treatment of chronic and infectious diseases.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization, J.J. and A.X.; Methodology, A.X., A.B. and Q.L.; Software, N.N., A.X. and A.M.; Validation, A.M., N.N. and A.X.; Formal

Analysis, A.X., Q.L. and J.J.; Investigation, A.X., A.M. and A.B.; Resources, J.J.; Data Curation, J.J., Q.L. and A.B.; Writing – Original Draft Preparation, A.X., A.M., J.J.; Writing – Review & Editing, A.M. and N.N.; Visualization, A.M., N.N. and J.J.; Supervision, J.J.; Project Administration, J.J.; Funding Acquisition, J.J.

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