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Chemical Constituents from *Ferula songarica* and *Ferula foetida*: a Dereplication Approach

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Abstract

The genus *Ferula* (Apiaceae) is widely distributed across Central and Southwest Asia and is recognized for its diverse secondary metabolites and traditional medicinal uses. However, the phytochemical composition of *Ferula songarica* and *Ferula foetida* from Kazakhstan remains poorly explored. In this study, UHPLC–ESI–MS/MS-based metabolite profiling coupled with in silico annotation was applied to characterize methanolic extracts of *F. songarica* and *F. foetida*. A total of 20 metabolites were tentatively identified in *F. songarica*, dominated by daucane-type sesquiterpenes, along with flavonol glycosides, prenylated benzoic acids, and a fatty acid. In *F. foetida*, nine metabolites were annotated, primarily caffeoylquinic acid derivatives and fatty acids. Compound identification was achieved with high mass accuracy (<5 ppm) using a curated Apiaceae and *Ferula*-specific database integrated into SIRIUS. Several metabolites were confirmed at Level 1 confidence based on prior isolation in this laboratory, while the remainder were assigned as Level 3 annotations. Mechanistic MS/MS analysis revealed characteristic fragmentation pathways for each compound class. Notably, this study represents the first comprehensive UHPLC–ESI–MS/MS profiling of *F. songarica* and *F. foetida* from Kazakhstan. These findings expand the phytochemical knowledge of *Ferula* and highlight their potential as sources of bioactive natural products.

1. Introduction

The genus *Ferula* L. (Apiaceae) comprises approximately 240 species [1], distributed mainly across Asia, extending westward to the Mediterranean region and northern Africa, with a center of diversity in Central and Southwest Asia [2]. Approximately 50 species occur in Kazakhstan, of which 10 are considered rare [3–4].

Species of *Ferula* have long been utilized in traditional medicine and exhibit diverse applications. Several local taxa, including *F. ferulaeoides*, *F. foetida*, *F. songarica*, *F. varia*, *F. dissecta*, and *F. akitschkensis*, serve as forage plants in steppe and semi-desert ecosystems. The antibiofilm and antimicrobial potential of *F. varia* for dental applications is currently under investigation in Kazakhstan [5]. In traditional medicine, *Ferula* species have been used in Iran for the treatment of diarrhoea, malaria, and microbial diseases [6], and in Saudi Arabia for skin infections [7].

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Pharmacological studies have demonstrated a broad spectrum of biological activities for *Ferula* species, including anti-inflammatory, cytotoxic, antibacterial, antileishmanial, antiviral, antigenotoxic, antitumor, anticoagulant, antioxidant, and α -glucosidase inhibitory effects [8–11].

Ferula songarica is traditionally used to treat body pain, colds, and gastrointestinal disorders. Previous studies from this laboratory reported flavonoids, phenolic acids, and essential oils; however, its chemical composition remains insufficiently characterized [12–13].

Ferula foetida, widely used as a culinary spice and medicinal plant, is consumed in South Asia and employed in Uyghur medicine for gastrointestinal, respiratory, and infectious conditions [2, 11, 14]. It is chemically diverse, containing sesquiterpene coumarins, sulphur-containing compounds, diterpenes, phenolics, acetylenes, fatty acids, sterols, carbohydrates, and flavonoids [14–16]. Its wide distribution in Kazakhstan highlights its potential as a source of bioactive natural products.

This study investigates the phytochemical profiles of methanolic extracts from *Ferula songarica* and *Ferula foetida* collected in Kazakhstan using UHPLC–ESI–MS/MS analysis. Comprehensive characterization of the secondary metabolites present in these species expands the current phytochemical knowledge and underscores their significance as potential sources of biologically active compounds.

2. Experimental

2.1 General experimental procedures

The analytical grade solvents such as hexane, ethyl acetate, dichloromethane, and methanol were used for plant extraction (RCI Labscan Limited, Bangkok). HPLC grade propan-2-ol and acetonitrile (Fisher Scientific Korea Ltd., Seoul) were used for sample clean-up using solid phase extraction, fractionation, and purification, while LC-MS grade acetonitrile (Fisher Chemical™) and formic acid (more than 99% purity, Optima™ LC/MS grade, Fisher Chemical™) were used during LC-MS analysis. High-quality ultra-pure water was obtained from an Arium® Pro ultra-pure water system (Sartorius, Malaysia).

The chromatographic profiles were acquired using a Thermo Scientific analytical ultra-high performance liquid chromatography (UHPLC) system. The LC-MS/MS profiles were analysed using Thermo Scientific Vanquish UHPLC coupled to a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ Mass Spectrometer.

2.2 Plant material

The aerial (stem and leaf) and root parts of *Ferula songarica* were harvested during the flowering phase in June 2023 near the village of Novaya Bukhtarma, about 100 km southeast of Ust-Kamenogorsk, East Kazakhstan region, Republic of Kazakhstan (GPS coordinates: N 49°37'14.5", E 83°28'32.5"). The plant was identified by Dr. Serik Kubentayev from Astana Botanical Garden (Astana, Kazakhstan). The herbarium materials have been deposited in the Herbarium of the Astana Botanical Garden (NUR), with voucher numbers NUR002675–NUR002679.

Roots of *Ferula foetida* (Bunge) Regel were collected in May 2023 at the budding stage, 13.4 km northwest of the Darbaza settlement, Turkestan Region, Kazakhstan (coordinates: N 42°03'57.9", E 068°31'52.6"). Herbarium specimen was identified by Dr. N.G. Gemedzhieva of the Laboratory of Plant Resources of the Institute of Botany and Phytointroduction, (Almaty, Kazakhstan). Voucher specimen was deposited in the same laboratory.

The plant materials were air-dried under a canopy without access to direct sunlight. The dried raw materials were ground to 2 mm.

2.3 Sample Preparation

The powdered aerial and root parts of *Ferula songarica* and the powdered roots of *Ferula foetida* were macerated separately in methanol at a plant material-to-solvent ratio of 3 g per 100 mL. Extractions were carried out over a period of three days at room temperature, employing alternating 12-hour intervals of magnetic stirring and static maceration. The resulting extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator to afford the methanolic root (0.75 g) and aerial (0.13 g) extracts of *Ferula songarica* and the methanolic root extract (0.17 g) of *Ferula foetida*.

The methanol extract (20 mg) was dissolved in 1 mL of 95% methanol and filtered through a 0.45 μ m polytetrafluoroethylene (PTFE) syringe filter. The solution was then subjected to solid-phase extraction using a C18 cartridge (Strata®, Phenomenex, USA) eluted with methanol. The resulting filtrate was filtered through a 0.45 μ m PTFE filter and subsequently dried under a stream of nitrogen gas. A stock solution was prepared by dissolving 5.0 mg of the dried extract in 95% methanol. For UHPLC–ESI–MS/MS profiling, the stock solution was diluted to a final concentration of 250 ppm in 95% methanol.

2.4 UHPLC Profiling

Profiling of the crude extract was achieved on a C18 reversed-phase column (Synergi 4 μ m Hydro-RP 80 Å, 150 $\times$ 4.6 mm, Phenomenex, USA). The injection volume was set at 10 μ L, with a flow rate of 0.8 mL/minute. The mobile phase consisted of ultrapure water (solvent A) and acetonitrile (solvent B). The analysis was carried out using gradient elution, where solvent B was increased gradually from 10% to 100% in 35 min. The chromatographic profile was monitored at a wavelength of 254 nm.

2.5 UHPLC-ESI-MS/MS Profiling

The pretreated methanol extracts of the aerial and root were analyzed using an analytical Accu-core™ Vanquish™ C18 column (100 $\times$ 2.1 mm, 1.5 μ m, Thermo Scientific, Lithuania) with a flow rate of 0.2 mL/min and an injection volume of 1 μ L. The positive ion mode was applied during the analysis. The analysis was based on the gradient elution of ultrapure water (solvent A) and acetonitrile (solvent B), both containing 0.1% formic acid. The analysis was carried out using gradient elution, where solvent B was increased gradually from 10% to 100% in 35 min. The mass spectrometer was set to 60,000 and 15,000 orbitrap resolutions in MS¹ and MS² scan modes, respectively. The spray voltage in positive mode was 3,500 V, with an ion transfer tube temperature of 300 °C, sheath gas of 35 arb, auxiliary gas of 7 arb, vaporizer temperature of 275 °C, and RF lens of 60%. The scan ranged from m/z 150 to 1,500. The Orbitrap detector was utilized for both MS¹ and MS² scan modes. Instrument controls were performed using Thermo Scientific Xcalibur, Orbitrap Fusion 4.2 Tune, and Chromeleon7, while data processing was executed using FreeStyle™ software version 1.6.

2.6 UHPLC-ESI-MS/MS-based chemical profiling

2.6.1 Database export and building

A metabolite database containing SMILES (Simplified Molecular Input Line Entry System) codes was accessed via the Universiti Teknologi MARA library to retrieve reported compounds from the Apiaceae (Umbelliferae) family. Additionally, a manually curated database specific to the genus *Ferula* was constructed based on comprehensive literature sources. The combined databases, comprising a total of 3722 compounds, were imported and stored in the SIRIUS software (version 5.6.3) for structure prediction and dereplication analysis.

2.6.2 Compound annotation

Compound annotation involved several sequential steps, including conversion of raw MS data using MSConvert, data processing with MZmine, and annotation using SIRIUS software. The procedure was adapted from Abd Karim et al. with slight modifications [17]. Specifically, the noise levels for MS¹ and MS² were set to 5.0×10^4 and 0, respectively, for all samples, including the aerial and root parts of *Ferula songarica* and the roots of *Ferula foetida*.

3. Result and Discussion

The aerial and root parts of *Ferula songarica* were subjected to tentative annotation using UHPLC-ESI-MS/MS. The annotation of those extracts was acquired using a modified run time of 35 min. A mass accuracy of less than 5 ppm was observed. For the aerial and root parts of *Ferula songarica*, a total of twenty metabolites with a molecular weight of 273 to 641 g/mol were annotated, including terpenoids representing the predominant class (thirteen compounds), four flavonoid glycosides, two prenylated benzoic acids, and a single fatty acid (Table 1). Most compounds were matched to the developed family- and genus-level databases, whereas six compounds were identified using online databases.

Quercetin-3-*O*- β -D-glucopyranoside **1**, isorhamnetin-3-galactoside **3**, 9,12,13-trihydroxy-10-octadecenoic acid **5**, kuhistanicaol G **6**, 6-(*p*-hydroxybenzoyl)lancerotriol **7**, kuhistanol D **10**, akiferidin **11**, akiferidin **13**, microferin **14**, ferutin **15**, microferin **16**, 3-farnesyl-*p*-hydroxybenzoic acid **17**, akichenin **18**, and *p*-coumaroyl jaeskeanadiol **19** matched entries in the Apiaceae and *Ferula* databases. The percentage of similarity was over 70%.

The assignment of confidence levels for compound identification (Levels 1-3) followed classification framework proposed by Schrimpe-Rutledge et al. which defines five tiers of identification confidence (Levels 1-5) [18]. Level 1 represents the highest confidence and requires confirmation using a reference standard, supported by two orthogonal analytical techniques, such as tandem mass spectrometry (MS/MS), liquid chromatography (LC), or nuclear magnetic resonance (NMR). Level 2 corresponds to putative identification based on MS/MS fragmentation patterns matched against spectral libraries without a reference standard. Level 3 entails tentative structural assignment based solely on MS¹ data. Level 4 is limited to molecular formula determination from accurate mass measurements, whereas Level 5 relies on mass accuracy without structural annotation.

Table 1. Compound annotation in the methanol extract of aerial part and roots of *Ferula songarica* by UHPLC-ESI-MS/MS

No.	Tentative identification	RT (min)	Molecular formula	[M+H] ⁺	[M–H] [–]	[M+H] ⁺ Fragment ions	[M–H] [–] Fragment ions	Ref.
1	Quercetin-3- <i>O</i> - β -D-glucopyranoside ^{a,b,g}	5.68 ^a 5.71 ^b	C ₂₁ H ₂₀ O ₁₂	465.1050	463.0875	303.0513, 145.0494, 127.0388, 91.0385, 85.0280, 61.0281	343.0445, 301.0341, 300.0265, 271.0234, 255.0294, 178.9983	Kahraman et al., 2019 [19]
2	Quercetin-3- <i>O</i> - α -L-arabinopyranoside ^{b,g}	6.31	C ₂₀ H ₁₈ O ₁₁	ND	433.0768	ND	301.0342, 300.0266, 271.0244, 255.0283, 178.9982, 151.0030	Harborne et al., 1999 [20]
3	Isorhamnetin-3-galactoside ^{a,b,g}	6.61 ^a 6.62 ^b	C ₂₂ H ₂₂ O ₁₂	479.1210	477.1033	317.0677, 145.0486, 127.0390, 97.0285, 91.0390, 85.0281	315.0498, 314.0426, 299.0193, 286.0474, 271.0244, 243.0294	Doganca et al., 1991 [21]
4	Quercetin 3-(6''-ferulylglucoside) ^{a,b,g}	8.53 ^a 8.55 ^b	C ₃₁ H ₂₈ O ₁₅	641.1531	639.1346	339.1087, 21.0976, 303.0513, 77.0547	463.0870, 315.0486, 301.0341, 300.0266	Niemann, 2006 [22]
5	9,12,13-Trihydroxy-10-octadecenoic acid ^{b,h}	11.33	C ₁₈ H ₃₄ O ₅	ND	329.2319	ND	229.1442, 211.1333, 209.1163, 171.1022, 127.1122, 125.0964	Nagai et al., 2002 [23]
6	Kuhistanicaol G ^{d,f}	13.49	C ₂₂ H ₃₀ O ₅	ND	373.2004	ND	137.0964, 137.0241, 136.0159, 95.0496, 93.0339, 92.0260	Chen et al., 2000 [24]
7	6-(<i>p</i> -Hydroxybenzoyl)lancerotriol ^{d,f}	15.79	C ₂₂ H ₃₀ O ₅	ND	373.2012	ND	235.1693, 217.1585, 207.1746, 137.0236, 136.0159, 92.0260	Alkhatib et al., 2008 [26]
8	4-Hydroxy-3-(4-hydroxy-3,7,11-trimethyldodeca-2,6,10-trienyl)benzoic acid ^{d,f}	16.02	C ₂₂ H ₃₀ O ₄	ND	357.2057	ND	295.2056, 255.1732, 229.1590, 147.0890, 107.0496, 106.0416	No match for this compound was found in either the Apiaceae or <i>Ferula</i>
9	3-(3,7-dimethyl-4-oxo-oct-6-enyl)-4-hydroxy-5-(4-hydroxy-3-methyl-but-2-enyl)benzoic acid ^{b,i}	17.03	C ₂₂ H ₃₀ O ₅	ND	373.2008	ND	341.1754, 273.1122, 203.1068, 203.0712, 149.0235, 121.0288	No match for this compound was found in either the Apiaceae or <i>Ferula</i>
10	Kuhistanol D ^{d,f}	17.67	C ₂₂ H ₃₀ O ₄	ND	357.2059	ND	187.1120, 175.1118, 150.0317, 147.0815, 106.0418, 93.0339	Chen et al., 2000 [25]

Continuation of Table 1

No.	Tentative identification	RT (min)	Molecular formula	[M+H] ⁺	[M–H] [–]	[M+H] ⁺ Fragment ions	[M–H] [–] Fragment ions	Ref.
11	Akiferidin ^{b,d,f}	19.37 ^d 19.38 ^b	C ₂₂ H ₃₀ O ₅	ND	373.2009 ^b	ND	(219.1751, 153.0191, 152.0115, 149.0971, 109.0292, 08.0215) ^d ; (219.1754, 153.0192, 152.0116, 149.0963, 109.0290, 108.0213)	Kushmuradov et al., 1978 [27]
12	[(3 <i>R</i> ,3 <i>aS</i> ,4 <i>S</i> ,8 <i>aR</i>)-3-hydroxy-8 <i>a</i> -methyl-6-methylidene-3-propan-2-yl-2,3 <i>a</i> ,4,5-tetrahydro-1 <i>H</i> -azulen-4-yl] 4-hydroxybenzoate ^{c, f}	19.37	C ₂₂ H ₂₈ O ₄	357.2079	ND	203.1801, 61.1327, 147.1169, 37.0233, 123.1167, 119.0853	ND	No match for this compound was found in either the Apiaceae or <i>Ferula</i>
13	Akiferidin ^{d,f}	21.05	C ₂₇ H ₃₆ O ₇	ND	471.2379	ND	371.1869, 153.0190, 152.0113, 109.0290, 108.0211, 81.0343	Kushmuradov et al., 1978 [27]
14	Microferin ^{a,c,f}	21.51 ^c 21.52 ^a	C ₂₂ H ₂₈ O ₃	341.2131 ^a 341.2107 ^c	ND	203.1796, 61.1323, 147.1166, 123.1164, 121.0280, 119.0851) ^a ; 203.1797, 161.1326, 147.1168, 123.1166, 121.0282, 119.0853) ^c	ND	Golovina et al., 1978 [28]; Kahraman et al., 2019 [19]; Saidkhodzhaev et al., 1993 [29]
15	Ferutinin ^{b,d,f}	21.51 ^d 21.54 ^b	C ₂₂ H ₃₀ O ₄	ND	357.2061 ^d 357.2060 ^b	ND	(219.1749, 149.0970, 137.0240, 136.0164, 93.0342, 92.0264) ^d ; (219.1746, 149.0967, 137.0238, 136.0159, 93.0339, 92.0262) ^b	Tamemoto et al., 2001 [30]; Alkhatib et al., 2008 [26]; Lhuillier et al., 2005 [31]

Continuation of Table 1

No.	Tentative identification	RT (min)	Molecular formula	[M+H] ⁺	[M–H] [–]	[M+H] ⁺ Fragment ions	[M–H] [–] Fragment ions	Ref.
16	Microferinin ^{a,c,f}	21.78 ^c 21.80 ^a	C ₂₃ H ₃₀ O ₄	371.2231 ^a 371.2233 ^c	ND	(203.1793, 175.1479, 151.0386, 133.1008, 123.1164, 19.0851) ^a ; (203.1796, 175.1480, 151.0387, 133.1009, 123.1165, 119.0852) ^c	ND	Golovina et al., 1978 [28]; Kahraman et al., 2019 [19]; Saidkhodzhaev et al., 1993 [29]
17	3-Farnesyl- <i>p</i> -hydroxybenzoic acid ^{c,d,f}	22.16 ^c 22.19 ^d	C ₂₂ H ₃₀ O ₃	343.2262	341.2111	219.1019, 205.0863, 193.0859, 179.0703, 165.0546, 151.0387	257.1171, 204.0787, 176.0840, 150.0320, 134.0371, 106.0421	Chen et al., 2000 [26]
18	Akichenin ^{b,d,f}	22.95 ^d 22.98 ^b	C ₂₇ H ₃₆ O ₆	ND	455.2429 ^d 455.2430 ^b	ND	(355.1898, 137.0236, 136.0159, 93.0338, 92.0260) ^d ; (355.1897, 137.0237, 136.0159, 93.0339, 92.0261) ^b	Kadyrov et al., 1976 [32]
19	<i>p</i> -Coumaroyl jaeskeanadiol ^{a,f}	23.93	C ₂₄ H ₃₂ O ₄	385.2393	ND	203.1797, 175.1480, 165.0546, 123.1165, 119.0853, 105.0695	ND	Arnoldi et al., 2004 [33]
20	(5 <i>S</i>)-1-[(3 <i>E</i>)-4,8-dimethylnona-3,7-dienyl]-5-ethenyl-5-methylcyclohexene ^{a,f}	27.32	C ₂₀ H ₃₂	273.2589	ND	231.2106, 217.1953, 203.1795, 149.1324, 135.1167, 133.1010	ND	No match for this compound was found in either the Apiaceae or <i>Ferula</i>

a – ions produced in positive mode (aerial part)

b – ions produced in negative mode (aerial part)

c – ions produced in positive mode (root)

d – ions produced in negative mode (root)

e – cinnamic acid and derivative

f – terpenoid

g – flavonoid

h – fatty acid

i – Prenylated benzoic acid

ND – not detected

In this study, compound annotation was performed using Sirius software, which applies in silico fragmentation prediction and therefore corresponds to Level 3 identification, as structures are proposed without experimental validation. Advancement to Level 1 confidence would require compound isolation followed by comprehensive structural elucidation using high-resolution mass spectrometry (HRMS) and NMR spectroscopy. Such analyses provide unequivocal structural confirmation and represent the highest standard of confidence in compound identification.

All compounds are tentatively identified (Level 3), except for quercetin-3-*O*- β -D-glucopyranoside **1**, quercetin-3-*O*- α -L-arabinopyranoside **2**, isorhamnetin-3-*O*- β -D-galactopyranoside **3**, and 3-farnesyl-*p*-hydroxybenzoic acid **17**. The com-

pounds were previously isolated from *F. songarica* from this laboratory [13], thus, assigned to Level 1 confidence. The base peak chromatogram of the aerial part and roots of *F. songarica* is shown in Fig. 1.

Quercetin-3-*O*- β -glucopyranoside **1** was previously isolated from an ethanol extract of the aerial parts of *F. caspica* M. Bieb. [19]. Quercetin-3-*O*- α -L-arabinopyranoside **2** was isolated from *Foeniculum vulgare* (Apiaceae) [20]. Isorhamnetin-3-*O*-galactoside **3** was obtained from the aerial parts of *Ferulago aucheri* Boiss. (Apiaceae) [21]. Quercetin 3-(6''-feruloylglucoside) **4** was identified from the needles of *Larix decidua* (Pinaceae) [22]. 9*S*,12*S*,13*S*-Trihydroxy-10*E*-octadecenoic **5** acid was isolated from the tuber of *Pinellia ternata* Breitenbach and was characterized as an effective oral adjuvant for a nasal influenza vaccine [23]. Kuhistanicaol

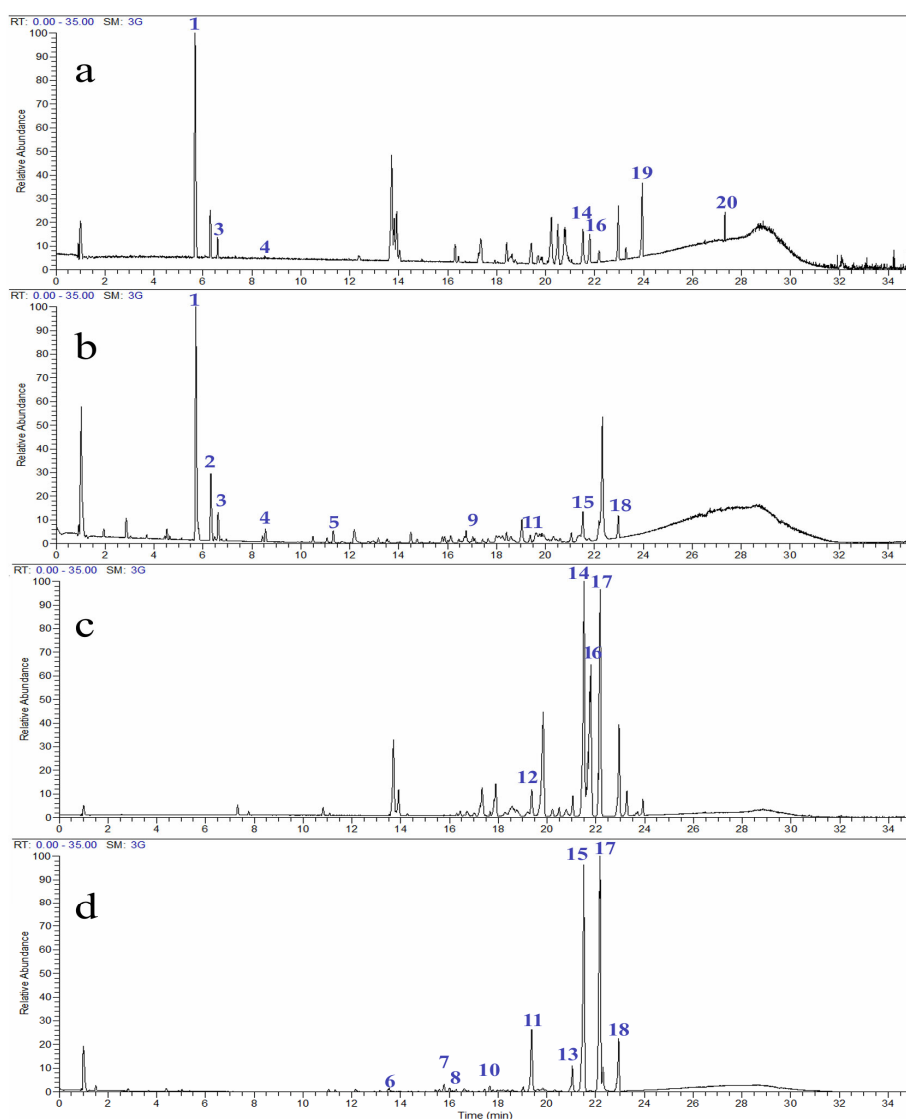


Fig. 1. The base peak chromatogram of the methanol extract of the aerial part and root of *Ferula songarica*: a – ions produced in positive mode (aerial part); b – ions produced in negative mode (aerial part); c – ions produced in positive mode (root); d – ions produced in negative mode (root).

G **6**, kuhistanol D **10**, and 3-farnesyl-*p*-hydroxybenzoic acid **17** were obtained from the roots of *F. kuhistanica* [24–25]. 6-(*p*-Hydroxybenzoyl)lanceroetriol **7** was isolated from roots of *F. elaeochytris* [26]. Akiferidin **11** and akiferidin **13** were isolated from the roots of *F. akitschkensis* [27]. Microferin **14** and microferinin **16** were previously isolated from the roots of *F. microcarpa* and *F. kyzylkumica* [19, 28–29]. Ferutin **15** was characterized from the fruits of *F. kuhistanica*, the roots of *F. elaeochytris* and *F. hermonis* [25, 30–31]. Akichenin **18** was isolated from the roots of *F. akitschkensis* B. Fedtsch. [32]. Furthermore, *p*-coumaroyl jaeskeadiol **19** was

annotated in the roots of *F. communis* using HPLC-DAD-MS analysis. This daucane ester was reported from *F. rigidula* [33].

4-Hydroxy-3-(4-hydroxy-3,7,11-trimethyldodeca-2,6,10-trienyl)benzoic acid **8** and 3-(3,7-dimethyl-4-oxo-oct-6-enyl)-4-hydroxy-5-(4-hydroxy-3-methyl-but-2-enyl)benzoic acid **9** were tentatively identified through online databases (PubChem and COCONUT) spectral matching; however, these compounds did not match any entries in either the Apiaceae or *Ferula* databases. The structures of compounds isolated from the methanol extract of the aerial parts and roots of *Ferula songarica* are presented in Fig. 2.

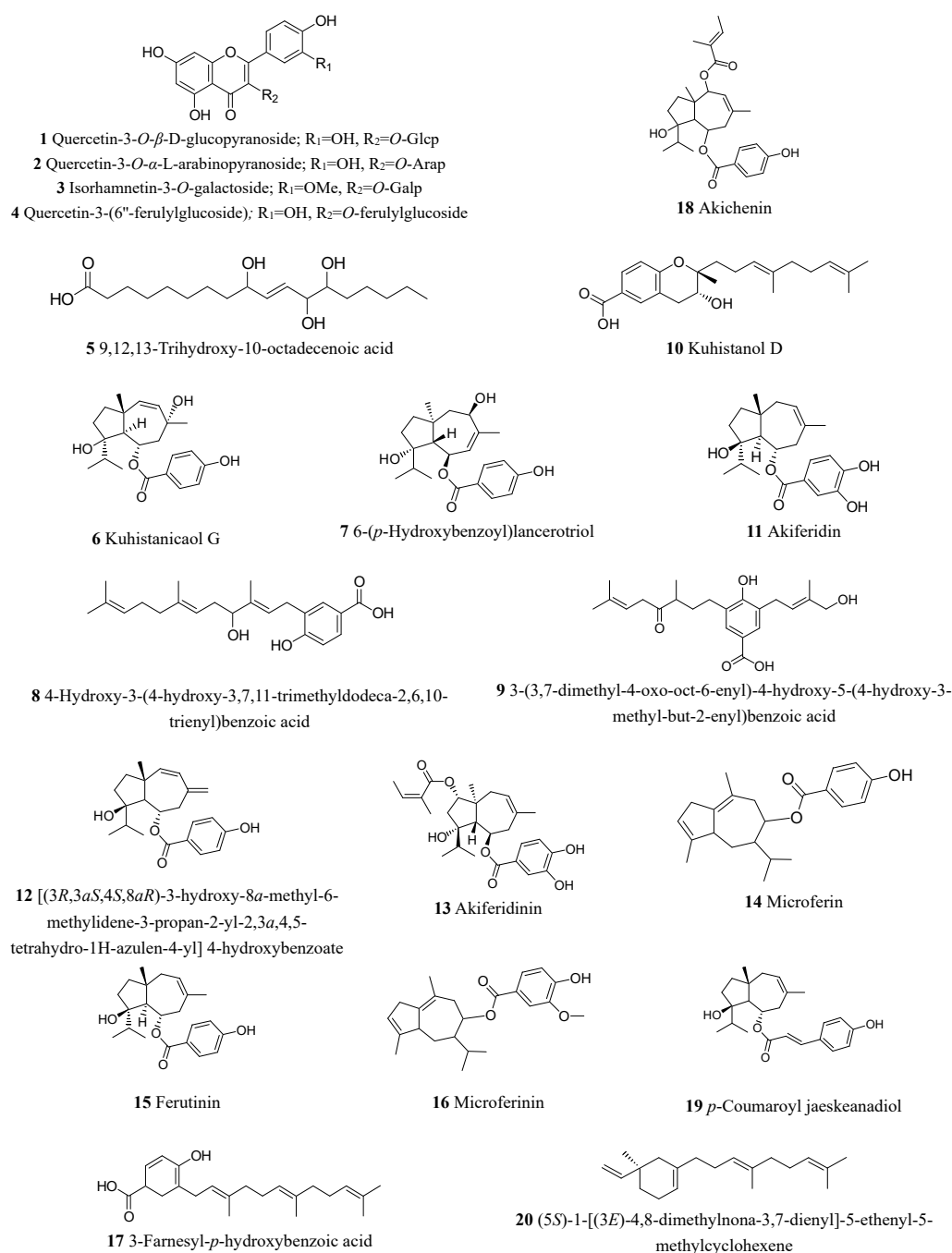


Fig. 2. Structures of the compounds from the methanol extract of the aerial parts and roots of *Ferula songarica*.

The root of *Ferula foetida* was subjected to tentative annotation using UHPLC-ESI-MS/MS. The annotation of those extracts was acquired using a modified run time of 35 min. The base peak chromatogram of the roots of *F. foetida* is shown in Fig. 3.

A mass accuracy of <5 ppm was achieved. A total of nine metabolites with molecular weights ranging from 327 to 515 g/mol were annotated, including four cinnamic acid derivatives, two fatty acids, and one alkyl glycoside (Table 2). Of these, four compounds were identified using online databases, while three matched the curated family-level (Apiaceae) database.

1,5-Di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, and 9,12,13-trihydroxy-10-octadecenoic acid matched entries in the Apiaceae database. The percentage of similarity was over 74%.

4-Caffeoylquinic acid was isolated from *Foeniculum vulgare* (Apiaceae) [34]. From the ethanolic extract of *Dichrocephala bicolor* (Asteraceae) 5-caffeoylquinic acid was isolated and identified [35]. 1,5-Dicaffeoylquinic acid was isolated from roots of *Ferula elaeochytris* [25]. 3,5-Di-*O*-caffeoylquinic acid was isolated from *Ferula lutea* (Poir.) Maire flowers [36]. 9,12,13-Trihydroxyoctadeca-10,15-dienoic acid (Malyngic acid) has been identified as a major fatty acid in several varieties of the marine blue-green alga *Lyngbya majuscula* [37].

2-[[3,4-Dihydroxy-4-(hydroxymethyl)tetrahydrofuran-2-yl]oxymethyl]-6-isobutoxytetrahydropyran-3,4,5-triol was tentatively identified through online database (PubChem) spectral matching. However, this compound did not match any

entries in either the Apiaceae or *Ferula* databases.

The differences in retention times observed between 4-caffeoylquinic acid and 5-caffeoylquinic acid can be attributed to structural variation and positional isomerism. Although these compounds share the same molecular mass, the position of the caffeoyl moiety on the quinic acid core influences their polarity and interactions with the stationary phase, resulting in distinct retention behaviors. Previous studies have shown that caffeoylquinic acid isomers exhibit different retention times and fragmentation patterns, with separation largely governed by interactions within the chromatographic system. In addition, matrix effects and species-specific composition may influence retention behavior and contribute to the appearance of additional chromatographic peaks [38]. The structures of compounds isolated from the methanol extract of the roots of *Ferula foetida* are presented in Fig. 4.

Only 9,12,13-trihydroxy-10-octadecenoic acid was detected in both *F. songarica* and *F. foetida*. *F. songarica* mainly consists of flavonoids and daucane-types sesquiterpenes while *F. foetida* is dominated by caffeoylquinic acid derivatives. The ionization behavior of the detected metabolites was largely governed by functional group chemistry. Terpenoids and cinnamic acid derivatives preferentially formed $[M - H]^-$ ions in negative mode due to the presence of carboxyl and conjugated phenolic groups that stabilize the resulting anions through resonance. Similarly, flavonoids are ionized efficiently in negative mode via deprotonation of phenolic hydroxyl groups, generating stabilized phenoxide ions.

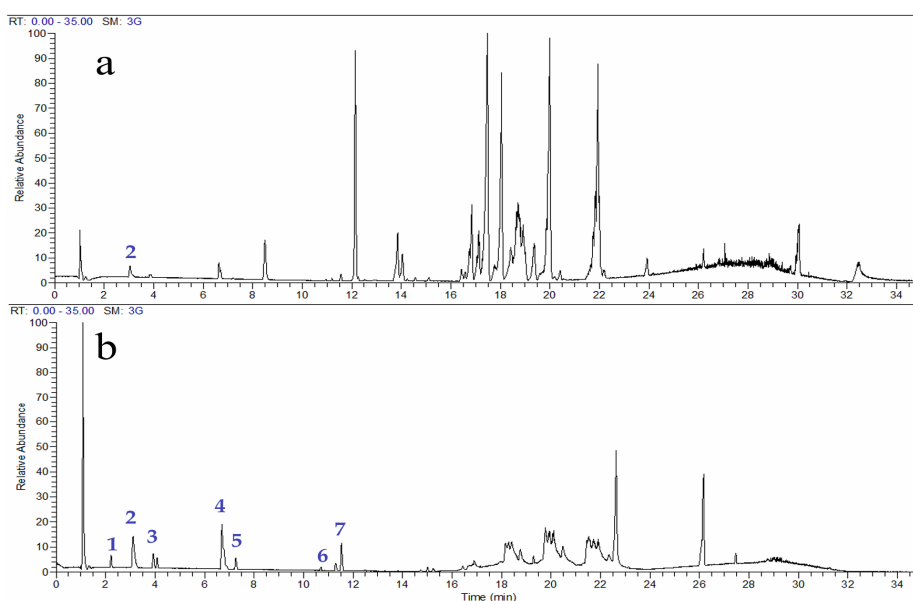


Fig. 3. The base peak chromatogram of the methanol extract root of *Ferula foetida* (a – ions produced in positive mode; b – ions produced in negative mode).

Table 2. Compound annotation in the methanol extract of roots of *Ferula foetida* by UHPLC-ESI-MS/MS

No.	Tentative identification	RT (min)	Molecular formula	[M+H] ⁺	[M–H] [–]	[M+H] ⁺ Fragment ions	[M–H] [–] Fragment ions	Ref.
1	2-[[3,4-dihydroxy-4-(hydroxymethyl) tetrahydrofuran-2-yl]oxymethyl]-6-isobutoxy-tetrahydropyran-3,4,5-triol ^{b,c}	2.22	C ₁₅ H ₂₈ O ₁₀	ND	367.1593	ND	235.1176, 61.0445, 143.0341, 101.0234, 83.0128, 73.0285	No match for this compound was found in either the Apiaceae or <i>Ferula</i>
2	5-Caffeoylquinic acid ^{b,d}	3.05 ^a 3.11 ^b	C ₁₆ H ₁₈ O ₉	355.1017	353.0864	193.0503, 163.0387	191.0553, 179.0342, 161.0235	Lin et al., 1999 [35]
3	4-Caffeoylquinic acid ^{b,d}	3.92	C ₁₆ H ₁₈ O ₉	ND	353.0861	ND	191.0553, 179.0342, 161.0240	Parejo et al., 2004 [34]
4	1,5-Di-O-caffeoylquinic acid ^{b,d}	6.70	C ₂₅ H ₂₄ O ₁₂	ND	515.1173	ND	353.0865, 335.0760, 191.0553, 179.0343, 173.0450, 161.0238	Alkhatib et al., 2008 [26]
5	3,5-Di-O-caffeoylquinic acid ^{b,d}	7.26	C ₂₅ H ₂₄ O ₁₂	ND	515.1173	ND	354.0896, 353.0861, 191.0550	Znati et al., 2014 [36]
6	9,12,13-Trihydroxyoctadeca-10,15-dienoic acid ^{b,f}	10.70	C ₁₈ H ₃₂ O ₅	ND	327.2161	ND	291.1956, 229.1424, 211.1323	Cardellina & Moore, 1980 [37]
7	9,12,13-Trihydroxy-10-octadecenoic acid ^{b,f}	11.54	C ₁₈ H ₃₄ O ₅	ND	329.2320	ND	229.1433, 211.1324, 209.1170, 183.1377, 171.1011, 127.1120	Nagai et al., 2002 [23]

a – ions produced in positive mode

b – ions produced in negative mode

c – alkyl glycoside

d – cinnamic acid and derivative

e – terpenoid

f – fatty acid

ND – not detected

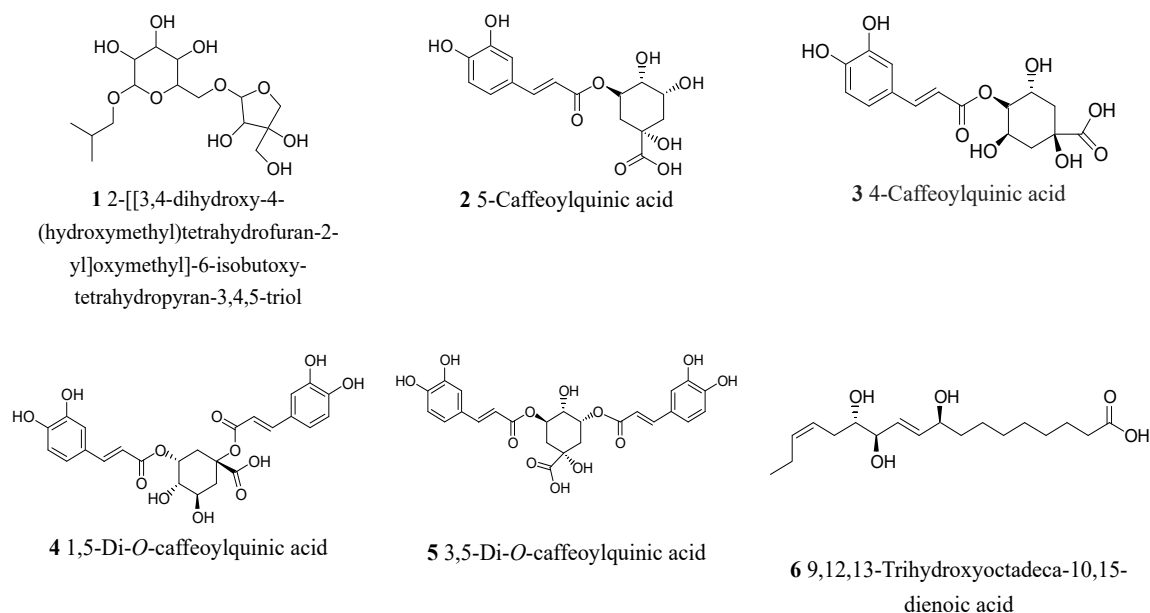


Fig. 4. Structures of the compounds from the methanol extract of the roots of *Ferula foetida*.

The flavonoid composition of *F. songarica* was dominated by flavonol glycosides. Initial cleavage occurred at the C-3 *O*-glycosidic bond via heterolytic dissociation, releasing sugar moiety and generating the deprotonated aglycone. This process is facilitated by charge delocalization across the flavanol backbone. Subsequent fragmentation involved dehydration, likely arising from rearrangement and cleavage across the C-4 carbonyl system, yielding product ions in the m/z 300–301 (Table 1).

The terpenoid was mainly daucane-type sesquiterpenes [39] which displayed characteristic fragmentation initiated at the lactone moiety. Cleavage of the ester bond within the lactone ring proceeds via charge-directed dissociation, generating a stabilized carbocation/anion fragment at m/z 204, typical of the daucane scaffold. Subsequent fragmentation involves sequential cleavage of the side chain through homolytic or heterolytic pathways, producing smaller, resonance-stabilized ions at m/z 136 and 137 (Table 1). These ions likely arise from rearranged hydrocarbon fragments retaining conjugated systems, consistent with established fragmentation mechanisms for sesquiterpene lactones.

For caffeoylquinic acid derivatives, fragmentation was dominated by charge-driven cleavage at the ester linkage and adjacent sp^2 -hybridized carbons. Deprotonation typically occurs at the carboxylic acid group, followed by intramolecular rearrangement and cleavage to yield the quinic acid-derived ion at m/z 191 (Table 2). This ion arises from stabilization of the deprotonated quinic acid through multiple hydroxyl

groups and is a well-established diagnostic fragment. The cleavage of the caffeoyl moiety can generate complementary phenolate ions, reflecting the conjugated π -system of the cinnamic acid derivative.

4. Conclusion

This study provides the first comprehensive UH-PLC–ESI–MS/MS-based characterization of *Ferula songarica* and *F. foetida* from Kazakhstan, revealing distinct yet complementary metabolite profiles. The chemical profile of *F. songarica* was characterized predominantly by daucane-type sesquiterpenes and flavonol glycosides, together with prenylated benzoic acids and a fatty acid, whereas *F. foetida* was characterized mainly by caffeoylquinic acid derivatives and fatty acids. Only 9,12,13-trihydroxy-10-octadecenoic acid was detected in both species.

The distinct distribution of these metabolite classes provides a chemical basis for comparative characterization of the two species and may be relevant to future chemotaxonomic studies within the genus *Ferula*. The occurrence of compounds previously reported from other *Ferula* species further contributes to understanding the chemical diversity of the genus. The predominance of different metabolite classes in the two species also supports further investigation of their constituent compounds as potential sources of structurally diverse natural products. Isolation of the annotated compounds and subsequent evaluation of their biological activities will be important for assessing their pharmacological relevance.

Overall, these findings expand the phytochemical knowledge of regional *Ferula* species and provide a foundation for future isolation, bioactivity evaluation, and chemotaxonomic studies.

CRediT authorship contribution statement

Tatyana Khosnutdinova: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Hidayatul Atiqah Abd Karim:** Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Anis Irfan Norazhar:** Visualization, Investigation, Formal analysis, Data curation. **Sharinah Ideris:** Methodology, Investigation. **Serik Kubentayev:** Resources. **Che Puteh Osman:** Writing – review & editing, Writing – original draft, Supervision, Resources. **Nor Hadiani Ismail:** Supervision, Resources, Conceptualization. **Nurgul Sultanova:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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