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Research Article

Total Polyphenol Content, Antioxidant Potential, Antibacterial and Antifungal Properties of *Ferula* L. Species Growing in Tajikistan

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Abstract

The plants of the genus *Ferula* L. have long been recognized for their significant roles in traditional medicine in the Middle East and Asia, a heritage that we deeply respect and appreciate. A diverse array of biologically active compounds, particularly concentrated in the roots of various *Ferula* species, accounts for their broad range of medicinal properties and applications in folk medicine. Many species within this genus are noted for their high content of phenolic compounds, which exhibit potent antioxidant activity and demonstrate substantial antimicrobial and antifungal properties.

Keywords: Polyphenol content; *Ferula* L; Anti-bacterial and anti-fungal

Introduction

Plantderived phenolic compounds serve as powerful anti-oxidants, a reassuring fact that underscores their potential health benefits. Extensive research has consistently shown that polyphenolic compounds found in many berries and medicinal plants possess anti-inflammatory, antiviral, antibacterial, antifungal, immunomodulatory, anti-oxidant, cardioprotective, anticancer, hypoglycemic, and other properties that may be used in prophylactic and mitigation of a variety of diseases and conditions [1-6].

According to multiple studies many species of the genus *Ferula* are rich sources of phenolic compounds. The total phenol and flavonoid content in medicinal plants, including various *Ferula* species, varies drastically depending on numerous factors such as the plant's collection time and location, stage of development, the specific part or organ, and the method of extraction [7-9].

Phenolic compounds are potent antioxidants due to their ability to donate hydrogen ions from their hydroxyl groups to free radicals, thereby inhibiting the oxidation of organic matter. Numerous species of *Ferula* are characterized by high polyphenol content, which contributes to their significant antioxidant activity [10,11].

Research conducted by authors studying the phytochemical components of *F. communis* and *F. gummosa* in Libya has indicated that the antioxidant activity of different plant parts increases with higher extract concentrations reported that the antioxidant potential of an extract is influenced by the assessment method and the type of extractant used [12-14].

Numerous studies focused on the biological activities of *Ferula* roots and seeds. Limited data are available on the polyphenol content and antioxidant activity of *Ferula* leaves. Notably, Niazmand and Razavizadeh report distinct properties of aqueous-alcoholic extracts from the leaves and gum of *F. asafoetida* in Iran, demonstrating that leaf extracts exhibit superior antioxidant activity compared to gum extracts [15].

Other researchers from Iran have reported that aqueous-alcoholic extracts from the flowers, stems, and leaves of *F. gummosa* demonstrate significant antioxidant and antihemolytic effects, potentially due to their high polyphenolic and flavonoid content.

In addition to antioxidant activity, phenolic compounds confer antibacterial and antifungal properties. Studies have established that biologically active components from both the underground and above-ground parts of *Ferula* species exhibit antimicrobial action against a wide spectrum of pathogenic bacteria (e.g., *Bacillus spp*., *Staphylococcus spp*., *Enterococcus faecalis*, *Salmonella spp*., and *Pseudomonas aeruginosa*) and fungi (e.g., *Candida spp*., *Trichophyton spp*., and *Aspergillus spp*.) [16-21].

F. assafoetida and related species are among the most

extensively studied and widely utilized both in industry and folk medicine. According to Mashael, the antibacterial activity of biologically active components derived from *F. assafoetida* different parts is attributed to compounds such as assafoetidin, conferol, gummosin, neveskone, polyanthinin and samarcandin [22]. While its roots contain asaresinotannol, azulene, bassorine, badrakemin and ferulic acid.

Assessment of the antimicrobial activity of essential oil obtained from the oleo-gum resin and seeds of *F. assafoetida* against four species of oral bacteria (*Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus sanguis*, *Streptococcus salivarius*, and *Lactobacillus rhamnosus*) have shown that the oleo-gum resin oil demonstrated a stronger effect compared to the seed oil [23].

The literature indicates that extracts and gum from *F. ferulaeoides* roots possess strong antimicrobial effects against *Staphylococcus aureus*, *Bacillus subtilis*, and other pathogens Liu et al. isolated terpenoid derivatives from *F. ferulioides* and confirmed their antibacterial activity against drug-resistant *S. aureus* strains [24-26].

By utilizing disk diffusion methods research has confirmed the antibacterial properties of F. ferulioides, *F. siniangensis*, and *F. siniangensis* leaf extracts. These studies found that ethanol extracts from the leaves of these species effectively inhibit *S. aureus* and *B. subtilis*, with *F. ferulaeoides* exhibiting the strongest activity [27].

In Turkey, researchers reported that essential oil from *F. drudeana* fruit and root extracts displayed variable antibacterial and antifungal activity against many gram-positive and gram-negative bacteria, with petroleum ether root extracts demonstrating notable inhibitory effects against *Candida krusei* and *C. utilis* [28,29].

Studies on the chemical composition and antimicrobial properties of Turkish endemic species such as *F. szowitsiana*, *F. turcica*, and *F. latialata* revealed that the essential oils' main constituent's beta-eudesmol, alpha-eudesmol, and alpha-pinene provide broad-spectrum activity against several pathogens, including *Escherichia coli*, *P. aeruginosa*, *Proteus vulgaris*, *Salmonella typhimurium*, *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus*, and *Candida albicans* [30].

Further studies have confirmed the antibacterial properties of extracts from various *Ferula* species such as *F. drudeana*, *F. lutea*, *F. vesceritensis*, *F. heuffelii*, *F. szowitsiana*, *F. glauca*, *F. assa-foetida*, and *F. hermonis* [31-35].

While significant research has been conducted on the chemical composition, phenolic content, and biological activities of *Ferula* species in many countries, there is a lack of information regarding the biological properties of species native to Tajikistan. This study aims to address this gap by evaluating the potential of endemic and under-researched *Ferula* species in Tajikistan for the development of locally sourced therapeutic and prophylactic preparations.

Objective

To study the total polyphenol content, antioxidant activity, and antibacterial and antifungal effects of three species of plants in the genus Ferula, growing in Tajikistan.

Materials and Methods

This study focused on three species of the genus Ferula: *F. violacea* Kor., endemic to Tajikistan, and *F. kuhistanica* Kor. and *F. gigantea* B. Fedtsch., both endemic to the Pamir-Alai Mountain range. Specimens were collected from diverse natural and climatic zones within the Republic of Tajikistan, including the Varzob Gorge and the Gorno-Badakhshan Autonomous Region (GBAR). Collection sites ranged in altitude from 1,180 to 2,269 meters above sea level (Table 1).

Table 1: Plant material

Species	Local name	The part used	Location	Date of collection	Coordinates
F. viollacea	Kasroof, Roshak	root, seeds	Maykhura, Varzob Gorge	23/07/22	Latitude: 38.48.31.9 Longitude: 68.49.05.23 Altitude: 1180 m
F. kuhista-nica	Kamoli kuhistonī	root	Vanj district, GBAR	21/07/22	Latitude: 37.47.88.7 Longitude: 71.59.68.2 Altitude: 2269 m
F. gigantea	Kamoli buzurg-jusa	root	The southwestern slope of the Shugnan Range, GBAR	21/07/22	Latitude: 37.47887 Longitude: 71.59682 Altitude: 2251 m

Taxonomic identification of the collected plant material was conducted using herbarium voucher specimens from Moscow University [36] and was confirmed by botanical experts affiliated with the Pamir Biological Institute, National Academy of Sciences of Tajikistan.

Method for obtaining dried gum

A novel method was developed for the extraction and drying of gum resins from the studied *Ferula* species. Freshly collected roots were sectioned using a sterile scalpel and left exposed to air on a laboratory bench for 4 hours. Exuded latex (gum) was collected from the cut surfaces every 30 minutes using a sterile scalpel and pooled in Petri dishes. To prevent contamination and undesirable physicochemical changes, the collected gum samples were dried in a vacuum oven (Drier Box DHG–9053A) at 40°C-45°C for 24 hours. The resulting dried gum was stored in airtight vials.

Method for obtaining ethanolic root extract

Freshly collected roots of the study species were macerated and placed in a glass or porcelain vessel. The plant

material was submerged in 70% ethanol at a ratio of 100 g of macerated root to 100 mL of ethanol. Maceration proceeded at room temperature for 24 hours. The mixture was then transferred to a juicer (model SPV-2, produced in Kharkiv, 1984) to facilitate extraction. The resulting extract was collected in Petri dishes and dried in a vacuum oven (Drier Box DHG–9053A) at 40°C-45°C for 24 hours. The dried extract was stored in airtight vials.

Method for obtaining seed juice

Freshly harvested seeds were processed in an electric juicer. The resulting juice was collected in sterile Petri dishes and dried in a vacuum oven at 40°C-45°C for 24 hours. The dried juice residue was stored in airtight vials.

Method for obtaining ethanolic seed extract

The seeds were bisected and placed in a glass or porcelain vessel. One hundred grams of seeds were submerged in 100 mL of 70% ethanol and macerated at room temperature for 24 hours to facilitate the extraction of biologically active compounds. The mixture was then passed through a 1 mm sieve, and the filtrate was transferred to a vacuum oven, where it was dried at 40°C–45°C for 24 hours. The dried extract was subsequently stored in airtight vials.

Preparation of paper disks for bioassays

To assess the antimicrobial and antifungal activities of the extracts, paper disks were prepared following the protocol established by the Ruskin Laboratory at Rutgers University [37]. Whatman GmbH (Germany) paper disks were arranged on a metal sheet, and 90 µL of each test sample was evenly distributed on the disks. The disks were air-dried at room temperature or using a fan before being stored in marked plastic bags.

Test strains of microorganisms

The antimicrobial activity was evaluated using four standard microbial strains: *Staphylococcus aureus* (ATCC 4929), *Escherichia coli* (ATCC 4928), *Pseudomonas aeruginosa* (ATCC 4930), and *Klebsiella pneumoniae* (ATCC 4927). Antifungal activity was assessed using the *Candida albicans* strain from the laboratory's collection.

Culture media and inoculum preparation

Muller-Hinton agar was used for the cultivation of *S. aureus*. *P. aeruginosa* was grown on King A medium, while *K. pneumoniae* was cultured on Klebsiella-5-ASA medium. For *E. coli*, Endo and Levin media were employed, and Sabouraud's medium was used for *C. albicans*. Pure cultures were obtained by streaking single colonies onto slants of the respective media. Inoculum suspensions were prepared from 24-hour cultures adjusted to a McFarland turbidity standard of 10 IU, achieving a final microorganism concentration of 2 CFU/mL × 106 CFU/mL [38].

Assessment of antimicrobial and antifungal properties

Diluted inoculum suspensions were spread on nutrient agar plates. Disks containing the gum and plant extracts were placed on the agar at 1.5 cm-2 cm intervals. Plates were incubated at 37°C for 18 hours–24 hours, after which the inhibition zones around the disks were measured and recorded.

Total polyphenol analysis

The estimation of the Total Phenolic Content (TPC) in the plant extracts was performed using a Folin-Ciocalteu reagent-based method, adhering to a well-established protocol with minor modifications for enhanced reliability [39]. Absorbance was measured at 760 nm using a portable USB-650-VIS-NIR Red Tide Spectrometer, interfaced with SpectraSuite software. The TPC was quantified using a gallic acid standard curve, with dilutions made as necessary to ensure sample concentrations fell within the curve's linear range. Results were reported as micrograms of Gallic Acid Equivalents (GAE) per gram of fresh weight (µg GAE g-1).

Determination of Antioxidant Activity (AOA)

The antioxidant activity of plant extracts was assessed using the ABTS assay, a widely recognized method for evaluating plant-based samples, modified slightly from the procedure described by Walker and Everette [40,41]. Absorbance measurements were performed at 734 nm using a portable USB-650-VIS-NIR Red Tide Spectrometer, coupled with SpectraSuite software. To ensure accuracy, extracts were diluted as needed to fit within the linear range of the Trolox standard curve. Results were expressed in micrograms of Trolox Equivalents (TE) per gram of fresh weight (µg TE g-1).

Statistical analysis

Statistical analysis of the antibacterial, antifungal, TPC, and AOA results was performed using Statistica 10.0 (StatSoft, USA). The normality of data distribution was assessed using the Shapiro-Wilk test. Comparisons among multiple independent groups were conducted using the Kruskal-Wallis H-test. Independent data were compared with the Mann-Whitney U-test, while dependent data were analyzed using the Wilcoxon signed-rank test. Correlations were determined using Pearson's correlation coefficient.

Results

Study of the content of total polyphenols in the roots and seeds of the studied plants

Our studies have shown the content of total polyphenols in the roots and seeds of the same species, as well as in the extracts of similar parts of the 3 species of the genus Ferula, included in the study. Varies in large ranges (Table 2). The total content of phenolic compounds in gum from the roots of the studied samples ranged from 985.7±14.4 µg GAE g^{-1} to 2772.7±57.3 µg GAE g^{-1}. Statistically significant ($P<0.001$) differences in the content of polyphenols were found between gums from the roots of *F. violacea* 2772.7±57.3 µg GAE g^{-1} and *F. gigantea* 985.7±14.4 µg GAE g^{-1}. This value for *F. kuhistanica* (2054.4±384.8 µg GAE g^{-1}) was close to the value of *F. violacea* ($p=0.016$), but significantly greater than the concentration of phenolic compounds in gum from the root of *F. gigantea* ($P<0.001$).

In 70% ethanol extracts from roots, the largest amount of polyphenols was found in *F. kuhistanica* 2176±21.1 µg GAE g^{-1}, which was slightly closer (P=0.033) to the value of the species *F. violacea* 1582.3±21.0 µg GAE g^{-1}, but significantly more than in *F. gigantea* 990.7±4.9 µg GAE g^{-1} (P <0.001).

Table 2: Polyphenols content in the studied plants

The studied samples	*F. violacea*	*F. gigantea*	*F. kuhistanica*	P (df=2)
Gum from the root	2772.7 ± 57.3	985.7 ± 14.4 p1<0.001	2054.4 ± 384.8 p1=0.016 p2>0.05	<0.001
70% ethanol extract from the root	1582.3 ± 21.0	990.7 ± 4.9 p1=0.033	2176.7 ± 21.1 p1=0.033 p2<0.001	<0.001
70% ethanol extract from the seeds	1148.1 ± 28.2	862.5 ± 46.9 p1<0.001	1021.5 ± 21.8 p1=0.033 p2=0.033	<0.001
Seed juice	1592.9 ± 54.2	855.4 ± 32.5 p1<0.001	983.6 ± 20.3 p1=0.033 p2=0.033	<0.001

Note: The results expressed in M ± SD with n=10, p-the statistical significance of the differences in indicators between all plant species (according to the Kruskal-Wallis criterion); post-hoc: p1–the statistical significance of differences in indicators in relation to *F. violacea*; p2–statistical significance of the differences in indicators in relation to *F. gigantea* (post-hoc–according to the Dunn criterion)

A comparative analysis of polyphenol concentrations in seed extracts indicated that the 70% ethanol extracts of *F. violacea* and *F. kuhistanica* contain statistically similar levels of phenolic compounds (p=0.033), with concentrations of 1148.1±28.2 µg GAE g-1 and 1021.5±21.8 µg GAE g^{-1}, respectively. These values are notably higher than the concentration observed in *F. gigantea*, measured at 862.5±46.9 µg GAE g^{-1} (p<0.001). Notably, in the seed juice, only samples from *F. violacea* displayed a high phenolic content (1592.9±54.2 µg GAE g^{-1}), whereas *F. kuhistanica* exhibited a concentration of 983.6±20.3 µg GAE g^{-1}, which is comparable to *F. gigantea* (855.4±32.5 µg GAE g^{-1}, p=0.033), though significantly lower than *F. violacea* (p<0.001).

This study represents the first comprehensive assessment of total polyphenol content in gum and ethanol extracts from the roots and seeds of three *Ferula* species: The country-endemic *F. violacea* and the regional endemics *F. kuhistanica* and *F. gigantea* from the Pamir-Alai range. Elevated polyphenol concentrations were particularly pronounced in the gum and ethanol extracts derived from the roots and seeds of *F. violacea* and *F. kuhistanica*. These levels were significantly higher than those found in the seed juice of *F. kuhistanica* and in all root and seed samples from *F. gigantea*.

Evaluation of antioxidant potential in gum and ethanol extracts from roots and seeds of *Ferula* species

The antioxidant potential of gum and ethanol extracts derived from the roots and seeds of selected *Ferula* species was evaluated. The positive control, Trolox, demonstrated a robust concentration of total antioxidants, confirming both the accuracy of the laboratory equipment and the efficacy of the chemical reagents utilized. This validation underscores the reliability of the study's findings.

Analysis of the antioxidant potential of seed-derived samples revealed that the juice from the seeds of *F. violacea* exhibited the highest antioxidant activity at 83.7±1.3 µg TE g^{-1}, which is significantly greater (p<0.001) than that of *F. kuhistanica* (30.6±1.0 µg TE g^{-1}) and *F. gigantea* (31.5±0.9 µg TE g^{-1}) (Table 3). Additionally, the 70% ethanol extract from *F. violacea* seeds showed notable antioxidant activity, with a concentration of 57.6±0.9 µg TE g^{-1}, further highlighting its potential as a valuable source of natural antioxidants.

Table 3: Antioxidant activity of the studied plants

The sample is examined	*F. violacea*	*F. kuhistanica*	*F. gigantea*	P (df=2)
Seed juice	83.7 ± 1.3	30.6 ± 1.0 P1<0.001	31.5 ± 0.9 p1=0.005 p2>0.05	<0.001
70% ethanol seed extract	57.6 ± 0.9	33.2 ± 1.1 p1=0.033	19.8 ± 0.8 P1<0.001 p2=0.033	<0.001
70% ethanol extract from the root	63.2 ± 0.3	45.6 ± 0.6 p1=0.033	29.7 ± 0.8 P1<0.001 p2=0.033	<0.001
Gum from the root	38.4 ± 0.7	34.0 ± 1.8 p1=0.033	45.7 ± 0.6 p1=0.033 P2<0.001	<0.001

Note: The results expressed in M ± SD with n=10, p-the statistical significance of the differences in indicators between all plant species (according to the Kruskal-Wallis criterion); post-hoc: p1–statistical significance of differences in indicators in relation to *F. violacea*; p2-statistical significance of the differences in indicators in relation to *F. kuhistanica* (post-hoc-according to the Dunn criterion)

It was statistically significantly (P <0.001) superior only to the sample derived from *F. gigantea* (19.8±0.8 µg TE g^{-1}), showing a slightly higher value (p=0.033) than *F. kuhistanica* (33.2±1.1 µg TE g^{-1}). Ethanol extract from the root of this plant species (63.2±0.3 µg TE g^{-1}) was characterized by approximately similar values, which is quite higher (P<0.001) than the values of the sample obtained from *F. gigantea* (29.7±0.8 µg TE g^{-1}) and closer (p=0.033) to those from the root of *F. kuhistanica* (45.6±0.6 µg TE g^{-1}).

The antioxidant potential of gum obtained from the roots of the studied plants is noteworthy. As can be seen from the data in the table above, the gum obtained from the root of *F. gigantea* showed a similar value (p=0.033) of antioxidant activity (45.7±0.6 µg TE g^{-1}) to a similar sample from *F. violacea* (38.4±0.7 µg TE g^{-1}).

For the first time, a comparative assessment of the antioxidant activity of gum, juice and ethanol extracts obtained from the roots and seeds of the *Ferula* species included in the work is given. It has been established that seed juice and 70% ethanol extract from the root of *F. violacea* are characterized by high antioxidant potential.

Study of the antibacterial and antifungal activity of the studied plants

Studies of gum and ethanol extractions of 3 different species of the genus Ferula-*F. violacea*, *F. kuhistanica* and *F. gigantea* showed that gums and ethanol extracts exert different levels of antibacterial action on the test strains of *S. aureus*, *P. aeruginosa*, *K. pneumonia* and *E. coli*, as well as unequal antifungal activity against *C. albicans*.

It should be noted that both here and in other cases, the test strain of *E. coli* did not show sensitivity to the samples studied and, therefore, data on their sensitivity are not shown in the tables. As can be seen from Table 4, the test strain of *S. aureus* with a retention zone diameter of 17.0 mm ± 0.7 mm to 19.6 mm ± 0.5 mm is statistically significantly higher (p<0.0.001) than the same indicator for *P. aeruginosa*, *K. pneumonia*, *C. albicans*-от 8,1 ± 0,6 до 11,5 ± 0,9 mm (p>0,05). The growth restriction zone of the test strain of *C. albicans* around the discs impregnated with gum and ethanol extract from the root, as well as the juice and ethanol extract from the seeds of this plant species, did not exceed 8.5 mm ± 0.5 mm, which indicates their possible fungistatic effect.

Table 4: Antibacterial activity of *F. violacea* against reference strains of microorganisms

Micro-organism	70% ethanol extract from the root	Gum from the root	70% ethanol extract from seeds	Seed juice	P (df=3)
S. aureus (n=10)	19.6 ± 0.5	19.3 ± 0.5 p1>0.05	19.4 ± 0.5 p1>0.05 p2>0.05	17.0 ± 0.7 p1=0.005 p2=0.005 p3=0.005	<0.001
Ps. Aeruginosa (n=10)	11.5 ± 0.9	9.8 ± 0.3 p1=0.012	11.0 ± 0.7 p1>0.05 p2=0.012	10.5 ± 0.6 p1=0.028 p2=0.043 p3>0.05	<0.001
Kl. Pneumonia (n=10)	9.3 ± 0.9	8.8 ± 1.0 p1 >0.05	9.0 ± 0.8 p1>0.05 p2>0.05	10.3 ± 0.7 p1=0.028 p2=0.012 p3=0.028	=0.002
C. albicans (n=10)	8.5 ± 0.5	8.3 ± 0.4	<0.001	8.1 ± 0.6	>0.05

Note: The result expressed in M±SD with n=10, p-is the statistical significance of the differences in sensitivity indicators between all pathogens (according to the Kruskal-Wallis criterion); post-hoc: p1–statistical significance of differences in indicators in relation to *S. aureus*; p2-is the statistical significance of the differences in indicators in relation to *Ps. aeruginosa*; p3-is the statistical significance of the differences in indicators in relation to Klebsiella (post-hoc–according to the Dunn criterion)

Table 5 presents a comparative assessment of the antibacterial activity of gum from the roots of all 3 studied plants. It was found that gum from the root of *F. violacea* with a diameter of the growth retardation zone around the disc at the level of 19.3 mm ± 0.5 mm was characterized by increased anti-staphylococcal activity (p<0.001), while gums obtained from the roots of *F. gigantea* and *F. kuhistanica* demonstrated the same activity against of this microorganism 12.3 mm ± 0.7 mm and 12.8 mm ± 0.6 mm, respectively. All gums obtained. of the roots of the studied plants, did not differ from each other in the levels of bactericidal effect on the test strain of *P. aeruginosa* from 9.8 mm ± 0.3 mm to 9.9 mm ± 0.9 mm (p>0.05). Low and approximately identical antibacterial activity was characteristic of all the studied samples for *K. pneumonia* ranged from 8.8 mm ± 1.0 mm to 10.8 mm ± 0.9 mm (p=0.002). As we noted earlier, gums from the root of *F. violacea* exhibited fungistatic effects on *C. albicans*. However, gum obtained from the roots of *F. gigantea* showed a slightly increased (10.2 mm ± 0.8 mm) antifungal effect against this test strain (p1<0.001), but a similar value (p1=0.002) to the sample obtained from the roots of species *F. kuhistanica* (9.9 mm ± 0.7 mm).

Table 5: Comparative assessment of the antibacterial activity of gums obtained from the roots of the studied Ferula species relative to reference strains of microorganisms

Micro-organism	*F. violacea*	*F. gigantea*	*F. kuhistanica*	P (df=2)
S. aureus	19.3±0.5	12.3 ± 0.7 p1<0.001	12.8 ± 0.6 p1=0.003 p2>0.05	<0.001
Ps. aeruginosa	9.8±0.3	9.9 ± 0.9	9.9 ± 0.9	>0.05
Kl. pneumonia	8.8±1.0	10.8 ± 0.9 p1=0.002	10.0 ± 0.7 p1>0.05 p2>0.05	=0.002
C. albicans	8.3 ± 0.4	10.2 ± 0.8 p1<0.001	9.9 ± 0.7 p1=0.002 p2>0.05	<0.001

Note: The result expressed in M ± SD with n=10, p-is the statistical significance of the differences in indicators between all plant species (according to the Kruskal-Wallis criterion); post-hoc: p1–statistical significance of differences in indicators in relation to *F. violacea*; p2–statistical significance of the differences in indicators in relation to *F. gigantea* (post-hoc–according to the Dunn criterion)

In our study, juice and ethanol extracts obtained from seeds did not show pronounced antimicrobial activity, i.e. the growth retardation zone around the disks prepared from materials obtained from the seeds of all 3 plants did not exceed more than 8.0 mm ± 1.0 mm, which indicates their low bactericidal effect.

For the first time, a screening study was conducted to evaluate the antibacterial and antifungal activity of the *Ferula* species included in this study. The results indicate that, *in vitro*, the highest antibacterial activity was exhibited by all samples obtained from the gum and seeds of the endemic species *F. violacea*, primarily acting on the test strain of *S. aureus*. These samples showed a modest antibacterial effect against the reference strains *P. aeruginosa* and *K. pneumoniae*, and demonstrated weak fungistatic activity against *C. albicans*. Notably, they exhibited no bactericidal or bacteriostatic effect against the reference strain of *E. coli*.

Discussion

In recent years, research in medicine, pharmacy, and pharmacology has increasingly centered on identifying biologically active compounds from wild medicinal plants. Extracts and gums derived from both aerial and subterranean parts of various *Ferula* species have historically been used in traditional medicine in regions where these plants are indigenous. Today, these plant-based compounds serve as the basis for numerous bioactive supplements and pharmacological products manufactured and distributed both domestically and internationally [42-45].

Studies have shown that many Ferula. species contain high levels of phenolic compounds, which are recognized for their potent antioxidant, antimicrobial, and antifungal properties [46,47]. Despite extensive research on the genus, there remains a gap in specific data on species native to the Republic of Tajikistan, particularly the endemic *F. violacea*, as well as the regional endemics *F. kuhistanica* and *F. gigantea*.

The primary objective of this study was to quantify total polyphenols and assess the antioxidant potential of gum and ethanol extracts obtained from the roots of these selected *Ferula* species. Analytical methods followed protocols established by the Ruskin Laboratory at Rutgers University, USA. Analysis of phenolic content in the roots and seeds of the studied species revealed substantial variability in total phenolic concentrations, expressed as gallic acid equivalents, across different plant organs. This finding aligns with other studies that report similar variations in polyphenol concentrations across roots, leaves, flowers, and seeds of *Ferula* species [48].

Our data show that the highest concentrations of total polyphenols were found in the gums and ethanol extracts of *F. violacea* and *F. kuhistanica*, with moderately lower levels in *F. gigantea*. Antioxidant capacity was standardized using Trolox as a control. Results indicate significant variation in antioxidant potential based on plant parts and species, with the highest antioxidant activity recorded in samples from the roots and seeds of *F. violacea*, which had the highest phenolic content. This correlation between polyphenol concentration and antioxidant activity concurs with previous findings [49].

This study presents the first data on the polyphenol content and antioxidant activity of *F. violacea*, *F. kuhistanica*, and *F. gigantea*, thus limiting direct comparison with existing literature.

Bacterial infections are a significant concern in human and veterinary health, often presenting zoonotic risks. Though antibacterial and antifungal agents are commonly used for treatment, their administration can result in adverse side effects, such as allergic reactions, dysbiosis, bone marrow suppression, and neurotoxicity affecting auditory and optic nerves [50,51]. These limitations highlight the need for new, effective therapeutic agents with a lower risk profile.

Accordingly, this study also evaluated the antibacterial and antifungal properties of the examined *Ferula* species. *In vitro* assays tested the bactericidal activity of the gum and ethanol extracts against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *Candida spp*. Previous research has demonstrated that different *Ferula* species exhibit varying antimicrobial efficacy [52]. For example, gums, sap, and

ethanol extracts from the roots and seeds of *F. violacea* showed pronounced inhibitory activity against *S. aureus*, while showing comparatively lower activity against *P. aeruginosa* and *K. pneumoniae* and only minimal fungistatic activity against Candida species.

Comparative analysis revealed that gum extracted from the roots of *F. violacea* exhibited superior anti-staphylococcal activity relative to the gums from *F. kuhistanica* and *F. gigantea*. However, all root-derived gums from the studied species demonstrated comparable bactericidal effects against *P. aeruginosa* and *K. pneumoniae*. The antimicrobial and antifungal properties of seed-derived samples across all species were generally weaker. Notably, none of the tested extracts affected *E. coli*, suggesting that potential therapeutic agents derived from these *Ferula* species would not disrupt the normal intestinal flora, thereby reducing the risk of dysbiosis and providing a significant advantage over conventional synthetic treatments.

Conclusion

The findings of this study provide the first comprehensive data on total polyphenol content, antioxidant activity, and the antimicrobial and antifungal properties of gum, sap, and extracts from the roots and seeds of three *Ferula* species: The endemic *F. violacea* and the regional endemics *F. kuhistanica* and *F. gigantea*. The lack of prior research on the bioactivity of these specific *Ferula* species underscores the novelty and significance of this work. These results lay a foundation for identifying new natural sources that offer a combination of antioxidant activity, pronounced antibacterial effects, and fungistatic properties, presenting promising alternatives for safer therapeutic and prophylactic applications.

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Ethics Declarations

Ethics approval and consent to participate are not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

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