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Chemical Composition and Antimicrobial Efficacy of *Ferula persica* Extracts: A Phytochemical and Bioactivity Assessment in Iraq

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Abstract

The research presented in the tables focuses on analyzing the chemical content of extracts from the Ferula persica plant, a wild species in Iraq with significant medical and therapeutic importance dating back to ancient times. This study involved testing some chemical and physical properties of the alcoholic extract of the plant's aerial parts and evaluating its antimicrobial activity, specifically against Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli). The study is divided into three main sections: Analysis of chemical content, preliminary phytochemical screening, and evaluation of antimicrobial activity. The research reveals the chemical composition of ferula, showing a moderate loss upon drying (3.6% w/w), a high content of crude fiber (19.7%), and a total ash percentage of 7.80%. It also notes the presence of a small percentage of foreign organic matter (3.83% w/w) and the absence of a swelling index. The study provides insights into the phytochemical properties of herb plant extracts using hexane and chloroform. The examination reveals the presence of various compounds, including alkaloids, carbohydrates, glycosides, saponins, steroidal nuclei, phenolic compounds, and tannins, tested using various reagents, such as Mayer, Molisch, Balgit, and ferric chloride tests. The presence or absence of these compounds is indicated by a plus or minus sign, displaying a diverse phytochemical profile. The antimicrobial activity of the herb extracts was also studied. This section evaluates the biofilm inhibition ability of the F. persica extract on S. aureus and E. coli at two concentrations (50 and 100 mg/mL). The results indicate significant inhibition of biofilm formation in both bacteria, with slightly higher efficacy at a concentration of 100 mg/mL. Control readings, presented side by side, highlight the relative potency of the extracts.

Keywords: Chemical, antimicrobial, antioxidant, *Ferula persica*, iraq

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INTRODUCTION

Ferula persica is the third largest genus of the Apiaceae family. It is mainly distributed throughout central and South-West Asia, especially in Iran and the far-East, North India, and the Mediterranean. *Ferula* species are characterized by the presence of oleo-gum-resins. Some of the biochemical characteristics and pharmaceutical and industrial applications of *Ferula* species have been studied, and several biological activities of phytochemicals isolated from various *Ferula* species have been reported, such as antimicrobial, insecticidal, and aphicidal activities. However, specific phenotypic characteristics of *Ferula persica* were not found in the search results [1, 2]. Studies have identified

coumarin sesquiterpenes and sulfur compounds as primary components. These compounds have been found to possess a wide range of pharmacological properties, including antibacterial, anti-inflammatory, and antioxidant activities [3–5]. Traditionally, the plant has been used for various purposes, such as a laxative, carminative, and in treating diabetes, rheumatism, and back pain. The active natural component in *Ferula* responsible for its biological activity is ferutinin, a phytoestrogen derived from *Ferula* plants. Ferutinin has been studied for its various properties, including its antioxidant, anticancer [6–8], and toxicological aspects. Additionally, the oleo-gum resin obtained from the rhizome and roots of the plant, known as asafoetida, contains active compounds, such as alpha-pinene, 2-bornyl acetate, terpinen-4-ol, D-fenchyl alcohol, pinocarveol, and bisulfides [9–11]. These compounds contribute to its biological activities. The essential oil extracted from *F. persica* plants also contains compounds, such as beta-pinene, limonene, and bornyl acetate, which demonstrate antioxidant and antimicrobial activities [12–13]. It is necessary to explore new natural antioxidants to protect the body from reactive oxygen species (ROS) damage. Recent statistics indicate that the health of a high percentage of people is deteriorating because of the secondary effect of antibiotics and their misuse [14–15]. Therefore, it has become necessary to find new medicines from safe wild plants based on natural resources Therefore, *Ferula persica* is indeed a medicinal plant containing active chemical components that have been the subject of extensive research due to their potential therapeutic benefits. The antioxidant properties of *Ferula* are attributed to the presence of phenols and flavonoids in its extracts. Numerous studies have shown antioxidant activities in different parts of the many plants, such as *Ferula* foetida regel, with significant antioxidant and anticoagulant activities in the extracts of its flowers, stems, and leaves [16]. The aqueous ethanol extract of the leaves showed the highest activity in DPPH radical scavenging activity, which may be due to the presence of phenolic compounds and flavonoids. Another study explored the antioxidant properties of *Ferula* orientalis and its essential oil, further supporting the antioxidant potential of *Ferula* plants. Moreover, the plant-derived estrogen ferutinin has been proven to possess antioxidant properties [17–19].

Therefore, the role of *Ferula* in antioxidant activity is associated with the presence of biologically active compounds, such as phenols, flavonoids, and ferutinin, which contribute to its potential health benefits. Due to the prevalence of this plant in Iraq and its local use in some traditional remedies, our study aimed to explore some of its chemical properties and roles as an antioxidant and antibacterial agent against some of the most pathogenic and antibiotic-resistant bacteria, such as *E. coli* and *Staphylococcus*.

MATERIALS AND METHOD

Plant Collection and Identification

Fresh Aerial parts of *F. persica* were collected aseptically in July 2022 from Ramadi City, Iraq, and identified at the Medicinal Botany Section, the Department of Biology, College for Women, University of Anbar.

Preparation of Plant Extract and Its Fractions

The preparation of plant material and fractions employed in our previous work on *F. persica* was adopted with modifications [20]. In this study, one and a half kilograms (1.5 kg) of pulverized sample material was extracted with hexane, chloroform alcohol.

Evaluation Parameters and Preliminary Phytochemical Screening

The evaluation of different parameter bitterness value, loss of drying, foaming index, crude fiber content, total ash, foreign organic matter, swelling index were studied [21–22]. Phytochemical screening was carried out on the crude extracts to detect the presence of plant secondary metabolites: alkaloids, steroidal nucleus, flavonoids, glycosides, tannins, phenolics compounds, terpenoids, and carbohydrates using standard procedures as described in the literature [23–24].

Carotenoid Extraction Methodology

A quantitative extraction method for carotenoids was employed, involving an initial treatment of 10 g of dried plant material with 100 mL of acetone, 1 g of magnesium carbonate, and 5 mg of butylated hydroxytoluene. Homogenization was performed using an Ultra Turrax device, followed by filtration to remove solid residues. Sequential extraction with methanol: acetone (1:1) solution was conducted until depletion of color in the residue, indicative of complete carotenoid extraction. The extract, after separation using diethyl ether and saturated NaCl, was purified, evaporated under nitrogen at 40°C, and reconstituted in 3 mL of water. An overnight alkali treatment with 10% methanol-KOH solution under nitrogen at 25°C was conducted, followed by a final diethyl ether and NaCl extraction, yielding a methanol-resuspended carotenoid extract [25].

Tannin Extraction

The tannin extraction process commenced with the combination of 1.0 g of finely powdered dried plant material with 1.0 mL of plant juice, followed by the addition of 95% ethanol in light-protected vials. Incubation in a rotating water bath at 50°C for 30 minutes facilitated the extraction. Post-incubation, the mixture was filtered using Whatman No. 1 filter paper, and the procedure was repeated thrice. The ethanol in the supernatants was evaporated under vacuum at 40°C. Tannin purification was achieved via chromatography using a Sephadex LH-20 column and elution with 95% ethanol and a 50% acetone solution. The pure tannin extract was obtained post-evaporation of acetone under vacuum at 35°C [26].

Alkaloid Extraction

Following Monsef-Esfahani et al. (2008), 1 g of ground plant material and 1 mL of juice were macerated with 25 mL of methanol. Post-maceration, triple filtration was conducted, and the filtrate was concentrated using a rotary evaporator at 45°C. The dry residue was acidified with 2% HCl, followed by filtration. Petroleum ether was employed for the removal of non-alkaloid impurities. Alkaloid extraction was performed by adjusting the solution to pH 10 using ammonia and extracting with chloroform. The final alkaloid extract was obtained post–evaporation of the chloroform [27].

Phenolic Compound Extraction

Adhering to the methodology of Mattila and Kumpulainen (2002), 1 g of powdered plant material underwent hexane treatment for fat and oil removal. The defatted material was then mixed with 1 mL of juice and treated with 100 mL of an 80 : 20 methanol : water solution. Ultrasonication of the solution for 25 minutes at 25°C enhanced phenolic compound release. Post–centrifugation at 7500 rpm for 15 minutes, the supernatant was treated with activated charcoal to remove impurities. The phenolic-rich extract was finally concentrated using a rotary evaporator under vacuum [28].

Determining the Antioxidant Activity of *Ferula* Extract

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent was used to prepare an extract solution at a concentration of 1 mg/mL in methanol to measure the free radical scavenging capacity of the oleuropein extracts. A volume of 3 mL of the DPPH solution at a concentration of 0.06 mM was kept in a dark container, to which 0.77 L of the extract solution was added. The sample was placed in a thermostat and left there for 15 minutes. The CARY 50 UV-VIS spectrophotometer was used to measure the absorption at 515 nm. For the control, 0.77 g of methanol was added to the DPPH solution, after which the absorption was measured immediately. The antioxidant activity was calculated as the percentage of inhibition.

Inhibition % of DPPH free radical: (100 X A_DPPH/(Sample A – A_DPPH (where: A_DPPH is the absorbance of the control sample (DPPH + methanol). Sample A is the absorbance of the sample [29].

Antimicrobial Susceptibility Assay

The antibacterial activity of *E. coli*, staphylococci, at five concentrations (50, 100, 125, 250, and 500) mg/ml of the crude extracts was determined by following the agar well diffusion method described by Igbinoza and colleagues [30] with modification. The control treatment was tetracycline antibiotic at a concentration of 100 mg/ml, according to the recommendation of the World Medicines Organization. The bacterial isolates were cultured for 18 hours in nutrient broth and were standardized at .0.5 McFarland criteria (10 6 $cfuml^{-1}$). Two hundred microliters of standard cell suspensions were spread on Mueller–Hinton (Oxide) agar and wells in the agar were drilled using a sterile 6 mm diameter cork drill. About 100 μL of crude extract at 100, 75, 50, and 25 mg mL^{-1} was introduced into the wells, allowed to stand at room temperature for approximately 2 h, and then incubated at 37°C. Controls were prepared in parallel with the solvents used to reconstitute the extract. After 24 hours, the plates were observed for areas of inhibition. The effects were compared with those of ciprofloxacin at a concentration of 5 mg/mL. Antibacterial activity was evaluated by measuring the diameters of growth inhibition zones in triplicate and the results are presented as mean ± SEM.

ANTIBIOFILM ACTIVITY

A stock solution of the dried extract powder is prepared by dissolving 5 grams in 25 ml of dimethyl sulfoxide (DMSO). This solution is vortexed for 15 minutes to ensure proper mixing. This is the primary stock solution, and serial dilutions of 100 and 150 mg/ml are prepared from it using the dilution law. The first dilution volume is 1.5 ml, the second dilution volume is 2 ml, and the third dilution volume is 2.5 ml. Then, a ready-made culture medium of brain-heart infusion is added, resulting in a concentration containing the extract [31]. The method was described by Sepahi et al. [17] as follows: the bacterial suspension was prepared and then placed in a liquid brain-heart infusion medium and incubated at 37°C for 24 hours. Tubes of culture medium containing sub-minimum inhibitory concentrations (MICs) of the ethanolic extract were inoculated with the bacterial suspension and incubated for 24 hours at 37°C. 200 microliters of the culture medium were added to the wells of a 96–well microtiter plate, distributed in three replicates vertically for each isolate, and this was considered the control treatment. 200 microliters of the bacterial culture grown in the culture medium containing the sub-MIC of the Jujube leaf extract was added to each well, and the plates were incubated at 37°C for 24 hours. After the incubation period, all wells were washed with physiological saline to remove non-adherent bacterial cells. The plates were left to dry and stained with 1% crystal violet for 10 minutes. They were then washed three times to remove the stain and left to dry at room temperature. To extract the dye from the adherent bacteria, 160 microliters of glacial acetic acid were added. The adherent bacterial cells' and the dye's optical density (OD) was measured using an ELISA reader at a wavelength of 360 nm to ascertain the isolates' effectiveness in producing biofilms. This was compared to the bacterial cells treated with the plant extract to calculate the percentage inhibition of bacterial adhesion using the following equation [32].

Percentage inhibition of biofilm (%) = 1 – OD (treated)/OD (control) * 100%.

Statistical Analysis

The obtained antioxidant and antibacterial results were expressed in mean ± standard error with observation recorded in triplicates. Analysis of variance for individual parameters was performed based on mean values to determine the significance at $p < 0.05$ using SPPS v20. Regression analysis was deployed to calculate and obtain the IC50 from the regression equation using Excel 2016.

RESULTS AND DISCUSSION

Chemical Content of the Plant

Table 1 presents some of the physical and chemical characteristics of the *F. persica* extract. The moisture content of the plant, indicated by a value of 3.6 ± 0.05% w/w, shows a relatively low

moisture percentage. This aspect is significant as moisture content can affect storage, shelf life, and susceptibility to microbial growth in herbal materials. The crude fiber content, amounting to 19.7%, represents the indigestible part of the plant. This is an important measure for nutritional studies, as fibers play a crucial role in the human digestive process. In the context of medicinal plants, a high fiber content can also affect the extraction and efficacy of active compounds.

The foaming index is a qualitative measure of the saponin content in plants. Saponins are compounds known for their surface tension-lowering properties. The foaming index of less than 100 in *Ferula* indicates a relatively low saponin content, which might influence its potential uses in pharmaceutical formulations, especially in compositions where saponin-derived foam is desired. As for the total ash (%). The total ash content of 7.80 ± 0.09% reflects the overall mineral content of the plant. Ash content is a useful measure of the plant's quality and purity, as it can indicate contamination with soil or other inorganic materials.

Organic matter was measured at 3.83 ± 0.18% w/w, assessing the cleanliness of the herbal material. Foreign organic matter can include parts of other plants or organisms. A lower percentage is preferred as it indicates a higher degree of purity and quality of the herbal samples. The swelling index is a measure of a material's capacity to increase in volume when exposed to liquids. The absence of a swelling index in the plant could be relevant in the context of its use in pharmaceutical formulations, particularly in designing drug delivery systems where swelling properties might affect the release of active compounds.

Each of these parameters offers insight into the physical and chemical properties of *F. persica*, which are crucial for determining its suitability for various applications in the fields of pharmaceuticals [33], nutrition [34], and natural product research. Understanding these properties is essential for standardizing the quality, efficacy, and safety standards of products based on this herb [35].

Table 1. Different evaluation parameters of *Ferula*.

S. No	Parameters	*Ferula*
1	Loss on drying (%)	3.6 ± 0.05 % w/w
2	Crude fiber content (%)	19.7
3	Foaming index (%)	Less than 100
4	Total ash (%)	7.80 ± 0.09
5	Foreign organic matter(%)	% 0.18 ± 3.83w/w
6	Swelling index	Absent

Table 2 presents the results of the preliminary phytochemical screening of various plant extracts using two solvents: hexane and chloroform. The presence of different phytochemical components, such as alkaloids, carbohydrates, flavonoids, glycosides, saponins, steroidal nucleus, and phenolic compounds including tannins were tested. Alkaloids in The Mayer's test indicates the absence of alkaloids in the hexane extract (–) and their presence in the chloroform extract (+).

Carbohydrates in Molisch's test shows the presence of carbohydrates in both extracts (+). Flavonoids in the ammonia test reveals the absence of flavonoids in the hexane extract (–) and their presence in the chloroform extract (+), glycosides indicates the presence of glycosides in the hexane extract (+) and their absence in the chloroform extract (–). Saponins: The foam test shows the absence of saponins in the hexane extract (–) and their presence in the chloroform extract (+). Steroidal Nucleus in the Liebermann–Burchard test indicates the absence of a steroidal nucleus in the hexane extract (–) and its presence in the chloroform extract (+).

The ferric chloride test shows the presence of phenolic compounds and tannins in the hexane extract (+) and their absence in the chloroform extract (–), These results provide valuable information about the chemical composition of the plant extracts and could be beneficial for further applications in pharmacology and natural product research. The differential solubility of these compounds in hexane and chloroform highlights the importance of solvent choice in phytochemical extraction and analysis [36].

Table 2. Preliminary phytochemical screening of *F. persica*

S. No	Plant ConstituentConstituents	Test	Hexane extract	ChloroformChloroform extract
1	Alkaloids	Mayer's reagent	–	+
2	Carbohydrates	Molish's reagent	+	+
3	Flavonoids	Ammonia test	–	+
4	Glycosides	Baljet test	+	–
5	Saponins	Foam test	–	+
6	Steroidal nucleus	Liebermann–Buchard's test	–	+
7	Phenolics compounds	Lead acetate	+	–
8	Tannins	Ferric chloride test	+	–

key: + = present; – = absent

The chemical constituents of secondary metabolites in *F. persica* plants include essential oils, flavonoids, coumarins, sesquiterpene coumarins, sulfur compounds, and various terpenes [37]. These compounds are abundantly present in the roots and rhizomes of this plant. Specific components identified in *F. persica* species include dimethyl trisulfide, dimethyl tetrasulfide, alpha-pinene, lavandulyl 2-methylbutanoate, alpha-terpinyl isobutyrate, alpha-terpinyl n-pentanoate, germacrene, himachalene, carotane, humulene, guaienes, ducane esters, farnesiferol A and B, sinkiangenin C and E, along with numerous monoterpenes, preformed coumarins, and plant estrogens [38–39]. These compounds have been found to exhibit various biological activities, such as antimicrobial, insecticidal, antioxidant, and cytotoxic properties [40]. The diversity of these components contributes to the plant's pharmacological potential, making it a valuable resource for natural product research and drug development. The presence of plant estrogens and other bioactive compounds in *F. persica* suggests potential therapeutic applications, including hormone-related treatments and antioxidant therapies. The identification and characterization of these compounds are essential for understanding the plant's medicinal properties and for exploring its use in various pharmaceutical and therapeutic applications.

The quantitative content of certain chemical compounds in hexane and chloroform extracts is presented in Table 3, which shows the content of phenolic compounds, alkaloids, carotenoids, and tannins in chloroform and hexane extracts from the plant *F. persica*. The phenolic components were 14 ± 0.06 mg/g in the chloroform extract and 15 ± 0.04 mg/g in the hexane extract. As for alkaloids, they were 14 ± 0.01 mg/g in the chloroform extract and 16 ± 0.12 mg/g in the hexane extract. The carotenoids were found to be 10 ± 0.21 mg/g in the chloroform extract and 12 ± 1.03 mg/g in the hexane extract. Tannins were 20 ± 0.07 mg/g in the chloroform extract and 24 ± 0.04 mg/g in the hexane extract. Phenolic compounds are known for their antioxidant activities. The presence of these compounds, albeit at low concentrations in both extracts, indicates the potential for antioxidant properties. However, the relatively large margin of error suggests a need for more precise measurement techniques. Alkaloids, known for their pharmacological effects, are present in higher concentrations in the hexane extract, indicating that hexane may be a more effective solvent for extracting alkaloids from this material. Carotenoids, which are pigments with health-enhancing properties including antioxidant activity, are significantly more concentrated in the hexane extract,

suggesting that hexane is more efficient in extracting these compounds. Tannins, polyphenolic compounds with various biological activities, are present in low concentrations in both extracts, but the very high margin of error makes it difficult to draw definitive conclusions about the efficiency of the solvents in extracting tannins.

Overall, these results suggest that hexane may be a more effective solvent for extracting alkaloids and carotenoids, while both chloroform and hexane show similar efficiency for phenolics and tannins.

Table 3. Test result of phenolic constituting, carbohydrate, saponin, alkaloid content of *F. persica* extract (Mean ± SD)

Constituents	**Hexane Extract**	**Chloroform Extract**
Phenolic constituting	15±0.04	14±0.06
Alkaloids	16±0.12	14±0.01
Carotenoids	12±1.03	10±0.21
Tannins	24±0.04	20±0.07

Based on the research findings, the presence of carbohydrates, saponins, alkaloids, and lipid content in plant extracts of *F. persica* has been reported in various studies. These studies have revealed through phytochemical analyses that herba species contain alkaloids, glycosides, flavonoids, steroids, terpenoids, tannins, and saponins. The primary active components of *Ferula* are its secondary metabolites, including sesquiterpenes, volatile oils, and flavonoids, which are abundantly present in the roots and rhizomes of *Ferula* plants. Several studies have pointed to the potent medical efficacy of *F. persica*, attributing it to these bioactive compounds, which are effective in treating various diseases [41–42].

DPPH Assay (In-vitro Antioxidant Activity)

Figure 1 demonstrated the antioxidant efficacy of hexane and chloroform extracts by providing the required concentration to inhibit 50% of DPPH radicals. This method measures the ability of antioxidants to scavenge free radicals. Higher ratios indicate stronger antioxidant activity. The activity is concentration-dependent, as shown by increasing values across higher concentrations. Ascorbic acid shows the highest DPPH scavenging activity, which is expected given its well-known antioxidant properties. The hexane extract generally shows higher antioxidant activity compared to the chloroform extract. This suggests that the compounds responsible for antioxidant activity are more effectively extracted by hexane. However, both extracts show a significant increase in activity with increasing concentration, indicating a dose-dependent effect. Ascorbic acid has a much lower IC50 value (4.51 µg/ml) compared to hexane (74.1 µg/ml) and chloroform extracts (74.6 µg/ml). This indicates that ascorbic acid is a strong antioxidant, requiring a lower concentration to achieve 50% inhibition of DPPH radicals.

The similar IC50 values for hexane and chloroform extracts indicate overall antioxidant efficacy among these two solvents, despite varying percentages of DPPH scavenging activity at individual concentrations. These results are valuable for understanding the antioxidant properties of the tested material. The effectiveness of different solvents in extracting antioxidant compounds can guide further research and extraction methods

Results of the i*n vitro* antioxidant assay (in IC50) of the crude extracts of *Ferula* are given in Figure 1 below. The Hex extract showed relatively *in-vitro* DPPH scavenging activity compared to CHCI extract.

The studies suggest that extracts of *F. persica* may possess in vitro antioxidant properties, which could be beneficial in preventing or treating various diseases. The activity may vary when using

different solvents for traditional extraction. Extraction using non-polar solvents, such as hexane and ether benzene, provides better antioxidant properties than methanol or acetone. This is primarily due to the chemical substances released during extraction, including phenolics, flavonoids, and other secondary metabolites, which enhance immunity and aid in healing certain diseases. The choice of solvent for extraction is a critical factor in isolating bioactive compounds [43–44]. Using solvents with different polarities expands the possibilities for extracting a wider range of phytochemicals from plant materials.

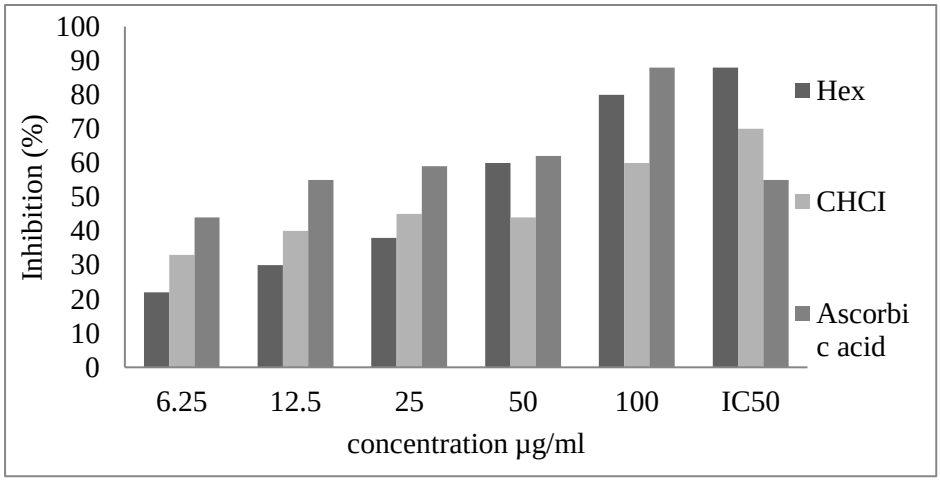

Figure 1. DPPH assay: IC50 extrapolation graph of nHex, CHCl3 extracts of *F. Persia*.

EFFECT OF PLANT EXTRACT ON THE GROWTH OF PATHOGENIC BACTERIA AND BIOFILM FORMATION

The results in Figure 2 show the effect of various concentrations (50, 100, 125, 150, 200, 250, 500 mg/ml) of the ethanol extract of *Ferula* on *S. aureus* and *E. coli*. The study focused on these bacteria due to their importance in medical microbiology, involvement in numerous infections, and tendency to form biofilms, which can lead to antibiotic resistance. For *S. aureus*, the highest inhibition at 500 mg/ml concentration was 22 mm in diameter, followed by 250 mg/ml with 18 mm. The lowest concentration (50 mg/ml) showed 11 mm inhibition. Interestingly, all concentrations demonstrated positive effects in inhibiting Staphylococcus aureus growth, indicating the extract's effectiveness in killing these bacteria. This aligns with previous research supporting this plant's efficacy in bacterial eradication.

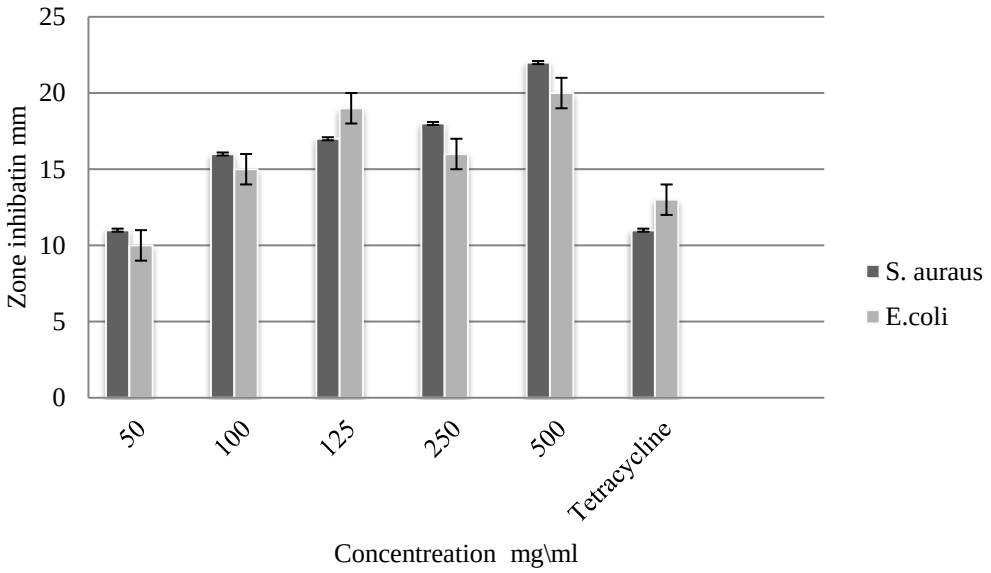

Figure 2. Antimicrobial activity (mm) of *Ferula* crude extract on *S. auraus* and *E. coli* mean ± SD of three replicates.

For *E. coli*, the best concentration was 500 mg/ml with an inhibition diameter of 20 mm, followed by 250 mg/ml with 18 mm, and the lowest at 50 mg/ml with 11 mm. These concentrations were compared with the antibiotic tetracycline, which showed 11 mm inhibition for *Staphylococcus aureus* and 13 mm for *E. coli*. The chemical active substances, such as alkaloids, phenolics, and saponins in the plant, adhere to the cytoplasmic membranes of Gram-negative bacteria, causing membrane rupture and leakage of bacterial contents, ultimately leading to bacterial death. These substances also prevent the production of bacterial cell walls by interacting with sulfur groups in essential proteins and generate reactive oxygen species, leading to bacterial death. The small particle size and large surface area allow for a stronger interaction with the bacterial surface. Overall, the results indicate that secondary metabolites from plant extracts are effective substances.

The results in Table 3 show the effect of the alcoholic extract on the biofilm formation of Staphylococcus aureus and *E. coli*. Two concentrations, 50 mg/ml and 100 mg/ml were used with biofilm inhibition rates of 0.062% and 0.072%, respectively, to evaluate the efficacy of *F. persica* extract. The dual concentration approach is useful to understand dose-dependent effects of the extract. The results indicated a biofilm formation rate of 61.96% for *Staphylococcus aureus* and 63.54% for *E. coli* at the lower concentration (100 mg/ml), suggesting the presence of bioactive substances from secondary metabolites and biofilm formation mechanisms, where higher concentrations increase effectiveness.

The control group results are crucial in understanding the natural tendency of these bacteria to form biofilms. The significant reduction in biofilm formation in the presence of the extract, especially at 50 mg/ml, highlights the potential of the herba plant extract as an anti-biofilm agent. The notable inhibition of biofilm formation could be due to various mechanisms, such as disrupting quorum sensing pathways, altering the bacterial cell surface, or interfering with the biofilm matrix. Further studies are needed to clarify these mechanisms.

Table 3. Effect of *F. persica* extract on biofilm formation at two concentrations of 50 and 100 mg/ml (LSD 55 = 0.022).

Bacteria Isolates	Biofilm Inhibition %	Concentration of 50 mg\ml	Concentration of 100 mg\ml	Control
S. aureus	61.96	0.062	0.072	0.163
E. coli	63.54	0.070	0.081	0.192

The antimicrobial activity of *Ferula* species has been the subject of numerous studies. Research has shown that methanol and ethyl acetate extracts from certain *Ferula* species exhibit antimicrobial activity against *S. aureus, C. albicans,* and *E. coli.* Additionally, essential oils from various *Ferula* species have shown antibacterial activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) and other bacteria. These findings suggest that extracts of the herba plant may possess antibacterial properties against *S. aureus* and *E. coli*. [45–46]. A study found that antimicrobial and antibiotic activities were observed in *Ferula* sp. Collected from Erzurum region. Antimicrobial activity of *Ferula* sp. It has been observed against *S. aureus*, *E. coli*, and *C. albicans*. It has also been found to have anti-biofilm activity through increasing concentrations against *S. aureus* and *C. albicans*. This is the first study to demonstrate the antibiotic activity of *Ferula* sp. [47], showed that essential oils extracted from the aerial parts of *F. orientalis* obtained from Erzurum were effective against *S. aureus*. Antimicrobial effects have also been observed on *E. coli*.

Ferula is a good source of bioactive compounds, such as sesquiterpenes, coumarin terpenoids, and sulfur-containing compounds. The most important of these compounds are the antimicrobial properties of the plant [48–49], combination of antibiotics with essential oils of *Ferula* species, such as *Ferula* iliensis Krasn, the previous. Corofen has been proposed as a potential alternative treatment option for bacterial infections, including those caused by multidrug-resistant bacteria. Different species of the herb and its extracts may exhibit different levels of antibacterial activity due to geographical differences. For example, the chemical composition of essential oils from *F. oopoda* and *F. Badghysi*, both grown in Iran, was found to have different retention factor values, indicating differences in their chemical composition, it has been used in traditional medicine for its antibacterial properties. For example, *Ferula* assa-foetida oleo-gum-resin has been used as an anti-inflammatory agent in Kazakhstan [44]. *Ferula persica* and other *Ferula* species have shown potential as antibacterial agents due to their extracts and essential oils [50–51], the presence of bioactive compounds, antibacterial properties of essential oils, and potential to combat multidrug–resistant bacteria make *Ferula* a promising candidate for further research and development of new antibacterial therapies. However, it is necessary to consider geographical differences in the chemical composition of herb species and potential differences in antibacterial activity [52–53].

CONCLUSION

This study provides a comprehensive analysis of *Ferula*, revealing its significant phytochemical content and antimicrobial properties, particularly against *S. aureus* and *E. coli*. The findings indicate potential applications of herbal plant extracts in developing natural antimicrobial agents, although further research and validation are necessary to fully understand their efficacy and potential uses in medical or pharmaceutical contexts. The study also underscores the importance of focusing on evaluating the antioxidant activity and potential therapeutic applications of these extracts. The study presents compelling evidence of the biofilm inhibitory properties of *Ferula* plant extracts against *S. aureus* and *E. coli*, especially at a concentration of 50 mg/ml. However, the unexpected lower efficacy at higher concentrations highlights the need for more research to understand the underlying mechanisms and optimize the use of herba extract for potential therapeutic application. Overall, this body of research opens new horizons for the use of natural compounds in combating microbial resistance. It emphasizes the need to continue exploring plant-based antimicrobials, which could play a crucial role in addressing the global challenge of antibiotic resistance. Further studies, particularly

those focusing on isolating and characterizing specific bioactive compounds within these extracts, will be essential for translating these findings into clinical applications.

Conflict of Interest
The authors declare no conflict of interest.

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