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ORIGINAL ARTICLE

Evaluation of Antimicrobial Activity of Hydroalcoholic Extracts from Different Parts of *Ferula assafoetida* L. in Three Regions of South Khorasan Province

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KEYWORDS

Ferula asafoetida L.; Hydroalcoholic extract; Antimicrobial activity; South Khorasan Province

ABSTRACT: Due to the increased resistance of bacteria to chemical drugs and the low side effects of medicinal plants, using these plants has been considered in disease treatment. *Ferula asafoetida* L., as one of the medicinal and native plants of southern Khorasan province (Iran), is used in traditional medicine to treat many diseases. The present study aimed to investigate the antimicrobial activity of hydroalcoholic extract of different parts of *Ferula asafoetida* L. in three regions of South Khorasan Province.The required chemicals were purchased from Merck and Sigma companies. The microbes were obtained from the Birjand University of Medical Sciences and the Iranian Scientific and Industrial Research Organization. The antimicrobial activity of hydroalcoholic extracts was evaluated by dilution in agar. For this purpose, the plant was collected from three areas: Sarbisheh, Ghayen, and Nehbandan. After identification, drying, and pulverizing, the extracts were prepared by soaking. Some of the extracts showed an inhibitory effect on gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), but none inhibited the growth of Gram-negative bacteria. Moreover, hydroalcoholic extracts of different parts of *Ferula asafoetida* L. of Sarbisheh had no inhibition effect on the growth of *Candida albicans*. The results revealed that the hydroalcoholic extracts of the plant could be used as a suitable substitute for chemical drugs to treat diseases.

INTRODUCTION

Over the past 40 years, several attempts have been made to discover new antibiotics. These efforts have led to producing more than a thousand types of antibiotics [1]. Simultaneous with the production of new drugs and antibiotics, the harmful effects of these drugs appeared gradually. Since the 1950s, scientists have found antibiotic resistance of pathogenic bacteria, which is still expanding [2]. The appearance of antibiotic-resistant bacteria lowered the effectiveness of existing drugs and increased the failure of antimicrobial treatments [3].

Dealing with the issue of drug resistance is of great importance in reducing or limiting the resistance of microbial agents [4]. The increasing expansion of drug resistance of bacteria has led to finding ways to prevent

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resistance occurrence and discover appropriate drugs with less toxicity and fewer side effects. In this respect, medicinal plants have been the subject of intense research [5]. The *Ferula asafoetida* L., with more than 20 types of known chemicals and effective compounds, is one of the medicinal plants that have long been used in diseases treatment [6].

Ferula asafoetida L. is one of the prominent species of Ferula of the Apiaceae family, which is important for pharmaceutical, edible (Figure 1-a), and industrial purposes.

Also, this plat is used as forage for livestock. In the Greek language, theord "Ferula" refers to bludgeon. Foetida also means funkybecause of its sulfuric and funky compounds [7]. It is an herbaceous, downy, perennial, and monocarpic plant with a straight, meaty, and thick root. Also, it has a small little fiber that makes and stores sap that is exploited by scarifying. (Figure1-b).

In the first five years, the plant has several large and meaty leaves without petioles lying on the earth's surface. In the first five years, the plant has several large and meaty leaves without petioles lying on the earth's surface. (Figure 1-c).Gradually, the cylindrical and meaty stems begin to grow, finally turning into complex yellow flowers in the form of compound umbrella inflorescences (Figure 1-d).

Fruits of this plant are dark or light brown oval-shaped schizocarps. These almost flat fruits have 5 distinct lines on each mericarp [8].(Figure 1-e).

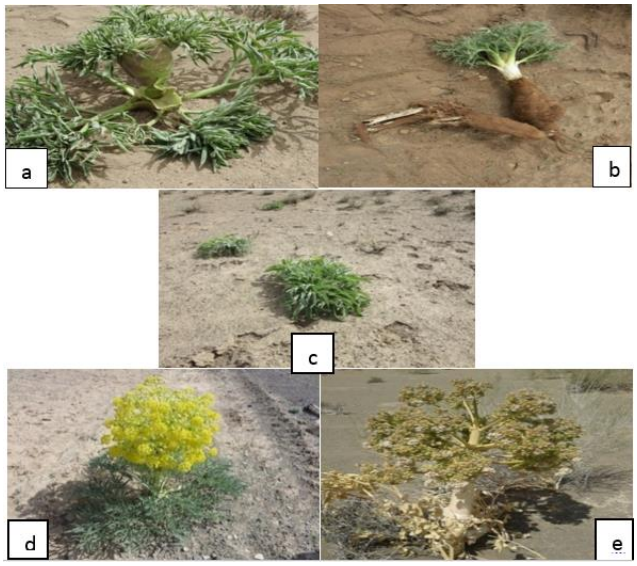

Figure 1. a-Edible part ,b-Root , c-leaves ,d- Flowers ,e-Fruits of *Ferula asafoetida* L.

Vegetative growth starts from mid-March to early April. Flowering begins from early to mid-May and continues until late May. Seeding begins in late May, and seeds are harvested from late June to mid-July. The aerial parts of the plant are green four months of the year, and after drying, the plant's hibernation begins from early July and continues until the end of the year. Plant reproduction takes place only through seeds [9]**.** In an article about the medicinal effects of the root gum of *Ferula asafoetida* [10] and in a systematic review article on the pharmacological effects of this plant [11], the authors have shown the medicinal effects of the plant. *Ferula asafoetida* has antispasmodic, anti-rheumatic, anti-convulsion, anti-coagulation, and anti-worm effects and is a phlegm detergent and appetite enhancer. Moreover, it improves respiratory and digestive diseases with a nervous origin, eliminates intestine indolence and kidney pain, enhances memory, eliminates the harmful effects of fatty foods, and regulates blood pressure [12]. In addition, the antibacterial properties of essential oil and extracts of *Ferula asafoetida* against bacteria such as *Salmonella*, *Shigella* [13], *Staphylococcus aureus*, *Staphylococcus epidermidis* [14], and pathogen

species of *Klebsiella* and *Vibrio Cholera* [15] have been reported.

The antibacterial effect of *Ferula asafoetida* extract on some pathogenic bacteria of food origin was investigated in [16]. In a study on the effects of essential oils and extracts of 50 Iranian medicinal plants, including *Ferula asafoetida*, on the standard strain of *Candida albicans*, the results showed weak anti-candidal effects of the plant's ethanolic and acetone extracts [17]. Also, a study on chemical compounds and antimicrobial activity of methanolic extracts of *Ferula asafoetida* on 4 strains (*Enterobacter cloacae, Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumoniae*) by disk diffusion method showed the significant antimicrobial effect of the extract [18]. The present study investigates the antimicrobial effect of the plant's hydroalcoholic extract. For this purpose, two strains of gram-positive bacteria (i.e., *Staphylococcus aureus* and *Bacillus subtilis*), two strains of Gram-negative bacteria (i.e., *Escherichia coli* and *Pseudomonas aeruginosa*), and *Candida albicans* fungus were used.

MATERIALS AND METHODS

Culture media and Dimethyl sulfoxide were purchased from Merck and Sigma companies, respectively. Standard strains of *Staphylococcus aureus* (ATCC: 29213), *Escherichia coli* (ATCC: 25922), and *Pseudomonas aeruginosa* (ATCC: 27883) were supplied from the microbiology laboratory of Birjand University of Medical Sciences. Standard strains of *Bacillus subtilis* (ATCC: 6051) and *Candida albicans* (ATCC: 10231) were purchased as lyophilization ampules from the Iranian Scientific and Industrial Research Organization. Different parts of the plant were dried in the shade and room temperature and then pulverized. The extracts were prepared by soaking. Antimicrobial activities of hydroalcoholic extracts of the plant were done by diluted method on agar.

Preparation of hidroalcoholic extracts

The antimicrobial effects of different plant parts were evaluated by preparing the hydroalcoholic extract through their soaking. For this purpose, different plant parts collected from Sarbisheh, Ghayen, and Nehbandan were coded according to Table 1.

Table 1. Coding of different parts of *Ferula asafoetida* in Sarbisheh, Ghayen ,and Nehbandan regions

Sample / Region	leaf	edible part	flower	fruit	steam	root
Sarbisheh	S1	S2	S3	S4	S5	S6
Ghayen	G1	G2	G3	G4	G5	G6
Nehbandan	N1	N2	N3	N4	N5	N6

Then, 10 g of the powder of each sample was added to 100 ml of ethanol 95% and was mixed using the shaker for 24 h. Next, the solution was filtered with a Büchner funnel and was evaporated at a temperature of 38℃ with 25 rpm by a rotary device and was transferred to sterilized vials. The vials were placed in a freeze drier to dry. Then dried extracts were weighed and kept in the refrigerator at 4℃.

Preparation of the different concentrations of extract

The first 0.5 g of dried extract of each part of the plant was dissolved in 1 ml of DMSO solvent and 4 ml of distilled water. Then, the extracts were filtered by Whatman filter papers and were sterilized by sterile syringe filters of 0. 45 µm.

Preparation of culture media

A certain amount of culture medium (Nutrient Broth, Muller Hinton Agar, and Blood Agar), according to the factory instructions (Merck), was added to 1 L of distilled water and then heated until obtaining a transparent mix. The culture medium was sterilized by an autoclave.

Preparation of final concentrations of extract

The final concentrations of the extract (0.5 1, 2, 3, 4 mg ml^{-1}) were prepared by adding 50 ml of sterile Muller Hinton Agar was added to 0.25, 0.5, 1.5, 2 ml of hyroalcoholic extract.Next, the culture medium containing the extract was transferred to plates and was placed at laboratory temperature to coagulate.

Preparation of microbial suspension

About 0.5 ml of saline solution with Pasteur pipettes was added to the dry matter in the lyophilization ampules and was mixed completely until a homogeneous suspension was obtained. Afterward, the microbial suspension was transferred to 20 ml of the culture medium of nutrient broth. In the next step, the culture media were inoculated, and control plates were put in an incubator for 24 h at 37℃. After that, using a sterile loop, 2 colonies of 24-h culture media in the presence of flame were turned into a suspension in 2 ml of physiological serum. Subsequently, using a sampler, 1 ml of 24-hour microbial suspension was transferred to a tube containing sterile nutrient broth. The opacity of this microbial suspension was compared with 0.5 McFarland standard solutions. Next, 10 µl of microbial suspensions was cultured on Muller Hinton agar containing the extract using a sterilized swab. The combination of DMSO solvent, water, and culture medium was used as a positive control. The plates were incubated for 24 h at 37℃. Then, the antimicrobial activities of different concentrations of the extract were investigated. Eventually, the lowest concentration that inhibited the growth of microbes was considered the minimum inhibitory concentration (MIC) (Figures 2-4).

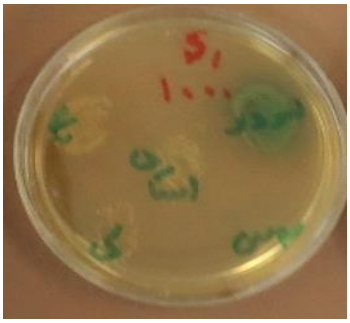

Figure 2. *Staphylococcus aureus* did not grow at a concentration of1500 µg ml^{-1} hydroalcoholic extract of leaf of *Ferula asafoetida* L. in Sarbisheh.

Figure 3. *Bacillus subtilis* did not grow at a concentration of 1000 µg ml^{-1} hydroalcoholic of leaf extract of flower *Ferula asafoetida* L. in Ghayen.

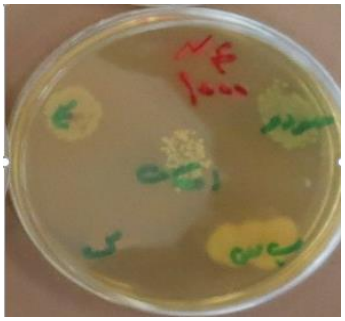

Figure 4. *Candida albicans* did not grow at a concentration of 1000 µg ml^{-1} hydroalcoholic extract of fruit of *Ferula asafoetida* L. in Nehbandan.

RESULTS

According to the results of this study (Table 2) and based on Figures 5-8, the effect of hydroalcoholic extracts of different parts of *Ferula asafoetida* L. on the tested microbes was different in different regions and in each region.

Table 2. The MIC values of hydroalcoholic extract of different plant parts on microbes in three regions at micrograms per milliliter

Microbe		*Staphylococcus aureus*	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeroginosa*	*Candida albicans*
Cod	Region					
Sarbisheh	S1	1500	1000	>2000	>2000	>2000
	S2	>2000	1500	>2000	>2000	>2000
	S3	>2000	1000	>2000	>2000	>2000
	S4	>2000	1000	>2000	>2000	>2000
	S5	>2000	1500	>2000	>2000	>2000
	S6	>2000	2000	>2000	>2000	>2000
Ghayen	G1	2000	1000	>2000	>2000	>2000
	G2	>2000	1500	>2000	>2000	>2000
	G3	>2000	1000	>2000	>2000	>2000
	G4	2000	>2000	>2000	>2000	1000
	G5	>2000	2000	>2000	>2000	>2000
	G6	>2000	>2000	>2000	>2000	2000
Nehbandan	N1	1500	1000	>2000	>2000	>2000
	N2	>2000	>2000	>2000	>2000	1500
	N3	>2000	1000	>2000	>2000	>2000
	N4	>2000	>2000	>2000	>2000	1000
	N5	1500	1500	>2000	>2000	>2000
	N6	>2000	2000	>2000	>2000	>2000

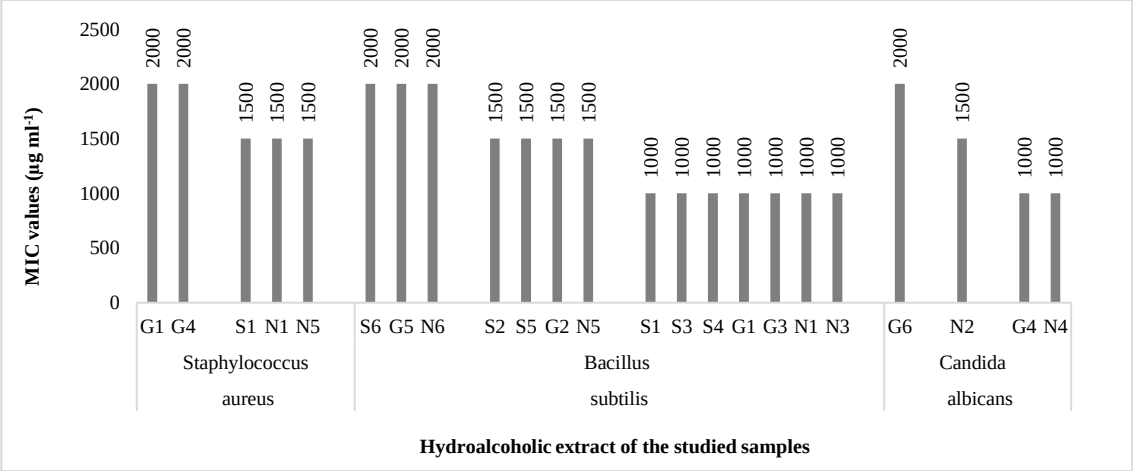

Figure 5. Comparison of the MIC values in the hydroalcoholic extract of the studied samples.

Based on the Figure 5, the hydroalcoholic extracts had an inhibitory effect only on Gram-positive bacteria (i.e., *Bacillus subtilis* and *Staphylococcus aureus*). *Bacillus subtilis* was more susceptible than *Staphylococcus aureus*. None of the extracts inhibited the growth of Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*).

Hydroalcoholic extracts of different parts of *Ferula asafoetida* L. in Sarbisheh did not affect the growth inhibition of *Candida albicans*. In contrast, the growth of *Candida albicans* was inhibited by hydroalcoholic extracts of fruit in Ghayen and Nehbandan, edible parts in Nehbandan, and roots in Ghayen.

The MIC values of hydroalcoholic extracts on the tested microbes was between 1000 and 2000 µg/ml. Extracts at a concentration of less than1000 µg ml^{-1} did not inhibit any microbes' growth.

The concentration of 1000 µg $ml^{-1}$of leaf and flower extract in all areas had the highest inhibitory effect on *Bacillus subtilis*.

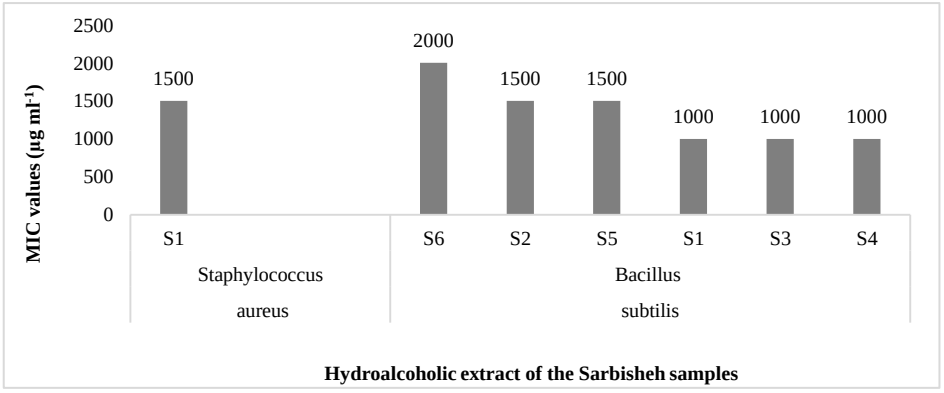

Figure 6. Comparison of the MIC values of microbial growth in the hydroalcoholic extract of the Sarbisheh samples

According to Figure 6, the hydroalcoholic extracts of *Ferula asafoetida* L. in Sarbisheh inhibited the growth of Gram-positive bacteria (i.e., *Staphylococcus aureus* and *Bacillus subtilis*) but did not affect the growth of Gram-negative bacteria and *Candida albicans*. Among the different plant parts, only the leaf extract (at 1500 µg ml^{-1}) inhibited the growth of *Staphylococcus aureus*.

The leaf, flower, and fruit extracts at a concentration of 1,000 µg ml^{-1}, the edible part extracts at a concentration of 1500 µg ml^{-1}, and the stem at a concentration of 2000 µg ml^{-1} had an inhibitory effect on the growth of *Bacillus subtilis*. As a result, the leaf and stem extracts showed the most inhibitory behavior, while the stem extracts showed the least on the bacteria.

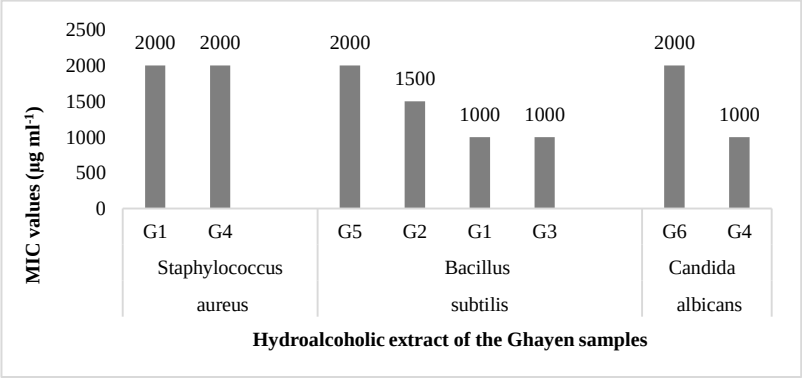

Figure 7. Comparison of the MIC values of microbial growth in the hydroalcoholic extract of the Ghayen samples.

As shown from Figure 7, the hydroalcoholic extracts of *Ferula asafoetida* L. in Ghayen inhibited the growth of Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and *Candida albicans*.

The concentration of 1000 µg ml^{-1} of fruit extract and 2000 µg ml^{-1} of root extract inhibited the growth of *Candida albicans*. The leaf and fruit extracts at a concentration of 2000 µg ml^{-1} prevented the growth of *Staphylococcus aureus*. Therefore, the leaf extract inhibited the growth of two bacteria, and the fruit extract inhibited the growth of a bacterium and fungus.

The growth of *Bacillus subtilis* was inhibited by the extracts of leaf and flower (at 1000 µg ml^{-1}), edible part (at 1500 µg ml^{-1}), and stem (at 2000 µg ml^{-1}). The leaf and flower extracts showed the most inhibitory effect on the growth of *Bacillus subtilis*, and the stem extract had the least inhibitory effect on this bacterium.

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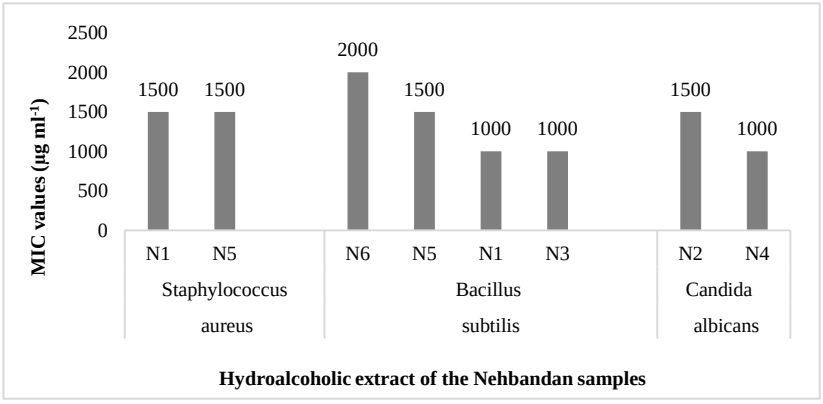

Figure 8. Comparison of the MIC values of microbial growth in the hydroalcoholic extract of the Nehbandan samples.

According to Figure 8, the extracts of *Ferula asafoetida* in Nehbandan prevented the growth of Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) and *Candida albicans*.

The growth inhibition of *Bacillus subtilis* was performed by the leaf and flower extracts (at 1000 μg ml^{-1}), the stem (at 1500 μg ml^{-1}), and the root (at 2000 μg ml^{-1})

The stem and leaf extracts of the plant, at a concentration of 1500 μg ml^{-1}, inhibited the growth of *Staphylococcus aureus.*

The concentration of 1000 μg $ml^{-1}$of fruit extract and the concentration of 1500 μg ml^{-1} of the edible part extracts prevented the growth of *Candida albicans*.

DISCUSSION

Increasing the number of multi-drug resistance pathogenic microbes in humans and unwanted side effects of certain antibiotics have encouraged enormous interest to search for new antimicrobial drugs of plant origin [19].

This study showed that hydroalcoholic extracts of different parts of the plant had antimicrobial effects on gram-positive bacteria and *Candida albicans*.

In different studies, the sensitivity of gram-positive bacteria was studied concerning herbal extracts. For instance, researchers studied the antibacterial effects of 39 methanol extracts of 25 Australian herbs against two gram-positive bacteria (i.e., *Bacillus cereus* and *Bacillus subtilis*) and two gram-negative bacteria (i.e., *Pseudomonas aeruginosa* and *Aeromonas hydrophila*) by disk diffusion method. The results showed the sensitivity of gram-positive bacteria [20]. In addition, a study was conducted on the antibacterial effects of different extracts of India and Nepal on 10 important human pathogenic bacteria. The results showed that the extracts were more effective in gram-positive bacteria compared to gram-negative bacteria. The most sensitive bacteria were *Bacillus subtilis* and *Staphylococcus aureus,* and the most resistant bacteria were *E. coli*, *Shigella disenteriae*, *Klebsiella pneumoniae* and *Salmonella Typhimurium* [21].

Studying the methanolic extracts of 10 herbal medicines in Pakistan revealed that gram-positive bacteria are more sensitive [22]. The results of a study on the antibacterial effects of *Ferula asafoetida* L. and *Carom capsicum* on *Staphylococcus aureus*, *Escherichia coli,* and *Salmonella typhimurium* revealed that *Ferula asafoetida* L. had the least inhibitory effect. The hydroalcoholic extract of Ferula prevented the growth of *Staphylococcus aureus* sensitive and resistant to methicillin while having no effect on the growth of *E. coli* and *Salmonella typhimurium* [23]. This finding confirms the results of the present study.

The results of a study on the chemical compounds and antimicrobial and antioxidant properties of the essential oil and extract of *Ferula asafoetida L*. in Neyshabur (Iran) showed that the MICs of the essential oil of the plant against *Staphylococcus aureus, Escherichia coli, Salmonella Typhimurium, Shigella Dysentery*, and *Bacillus* were 62.5,62.5,125,125 and 500 mg ml^{-1}, respectively [24]. Compared to this study, the plant extract had stronger antimicrobial activities on *Staphylococcus aureus* and *Bacillus.*

In a study on antibacterial activity of ethanolic extract of *Ferula asafoetida* L., in disc diffusion method, *Staphylococcus aureus* and *Proteus mirabilis* were the most sensitive of tested bacteria. In addition, *Bacillus subtilis*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* were resistant to ethanolic extracts [25]. Also, the antimicrobial effect of *Ferula asafoetida* resin on *Streptococcus pyogenes* and *Streptococcus pneumoniae* strains has been reported in the literature [26].

The results of a study on the antimicrobial effect of the essential oil of *Ferula ovina* on *Enterococcus faecalis* and *Staphylococcus epidermidis* (Gram-positive) and *Escherichia coli* (Gram-negative) showed that essential oil was more sensitive to gram-positive bacteria, especially *Enterococcus faecalis* [27].

The gram-negative bacteria are more resistant to chemical agents than gram-positive species [28]. In another research, the antioxidant, anti-cancerous and antimicrobial effects of the essential oil and extract of the leaves, fruits, and stems of *Ferula ovina* Boiss in the Azaran area of Kashan (Iran)

were investigated. The results showed that the extracts and essential oils of this plant, in addition to the antioxidant and anti-cancerous activities, had a weak antimicrobial activity against microorganisms [29]. In the Ferula genus, strong antibacterial effects of *Ferula kuhistanica* against methicillin-resistant *Staphylococcus aureus* were observed [30].

Various studies have shown the higher sensitivity of the cell wall of the gram-positive bacteria to many antibiotics, antimicrobial chemical compounds, and even many plant medicines than gram-negative bacteria [31].

Also, research has shown that gram-positive bacteria are more sensitive to plant extracts than gram-negative bacteria [32,33], probably due to the inherent tolerance of gram-negatives and the nature of plants' compounds [34]. In all studies, gram-positive bacteria (due to having a monolayer cell wall) were more sensitive to hydroalcoholic extracts of the plant. Gram-negative bacteria, unlike gram-positive bacteria, in addition to the peptidoglycan layer, have an outer membrane and a periplasmic space in their cell wall.

The hydrophilic surface of this membrane is rich in lipopolysaccharide molecules and acts as a barrier against antibiotics. It also prevents the penetration of hydrophilic molecules into bacteria. Due to enzymes' presence, periplasmic space decomposes molecules coming from outside. However, in gram-positive bacteria, the cell wall and the cytoplasmic membrane are destroyed easily by antimicrobial agents, leading to cytoplasm leakage and coagulation [35,36].

Therefore, the lipopolysaccharides layer and periplasmic space of gram-negative bacteria are the reasons for the relative resistance of gram-negative bacteria [4].

An additional contribution to intrinsic resistance in Gram-negative bacteria is provided by efflux pumps that actively pump out a broad spectrum of compounds (e.g., antibiotics, toxins, β-lactamase inhibitors, dyes, detergents, lipids, and molecules involved in quorum sensing) from the periplasm to the outside of the cell. The overexpression of efflux pumps (e.g., Resistance, Nodulation, and Division efflux pumps) is recognized as a major component in developing the multi-drug resistance phenotype in Gram-negative bacteria [37,38]. The ineffectiveness of plant compounds toward Gram-negative pathogens is strongly related to efflux pumps as the combination of plant antimicrobials with efflux pumps inhibitors leads to a striking increase in antimicrobial activity [39].

Generally, the antimicrobial effects of herbs depend on their secondary metabolites [4].

The antimicrobial activity is due to various secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, polypernoids, aromatic compounds, and sesquiterpenes that have antimicrobial effects on a wide range of bacteria due to the presence of phenolic groups in their structure [22, 40].

Overall, even at 10 mg ml-1 concentration, all the plant extracts inhibited the growth of tested bacteria compared to the control containing no extract. It can be found that the antimicrobial agents are presented in the extracts. Therefore, the phenolic compounds found in extracts are more than that of essential oils [41].

The inhibition of microbes' growth is related to the phenolic compounds of the extracts [42, 43].

The phenolic compounds are an important factor in the antibacterial activities of the extract of medicinal plants. These compounds are widely found in different parts of plants such as roots, leaves, buds, seedlings, and skin [44].

A study on extracts of several medicinal plants revealed a significant relationship between antibacterial activities and polyphenolic compounds of the studied plants [45]. At higher concentrations of phenolic compounds, due to the increase in the number of hydroxyl groups, the probability of hydrogen donation to free radicals and the extract's inhibitory strength increases [46].

The phenolic compounds can act at two levels: the microorganisms' cell membrane and cell wall [47]. They can also penetrate bacterial cells and coagulate cell content [48]. These compounds create their effects through creating toxicity for microbes, including surface absorption and destruction of the cell wall, interference in the activities of Plasma membrane and the membrane proteins, cytoplasmic coagulation, intracellular content leakage, reaction with enzymes, reduction of metal ions needed by bacteria, and the death of the bacteria's cell [49].

The secondary metabolites usually act through cell lysis, triggering the leakage of cellular contents and consequently cell death [50]. The interaction with genetic material and protein synthesis is also a possible factor in promoting therapeutic action. In this case, when there is contact with the genetic material, the compound can promote changes in the genetic machinery, resulting in ineffective transcription and disturbance of vital functions for the cell [51, 52].

Since the ecosystems and different conditions play an important role in the biosynthesis of secondary metabolites, the secondary metabolites are different. Environmental factors are important in producing secondary metabolites of herbal drugs. Factors such as temperature, precipitation, light intensity, and altitude, which determine the climate of a region, affect the accumulation of secondary metabolites [4]. It has been proved that different levels of active ingredients in herbal drugs are affected by climate. However, the accumulation and distribution of secondary metabolites are not equal, and the differences in results of studies may be due to the differences in climate effects of different variations of herbs.

Metabolites can be diverse due to habitat differences and weather conditions [53].

In the present study, the hydroalcoholic extracts of the plant showed an anti-Candida effect, which confirms the results of researchers. The alcoholic extract of *Ferula gumosa* roots had anti-fungal effects on *Candida albicans* [54].

In a study on the anti-fungal activities of *Ferula narthex* on *Candida albicans* and *Aspergillus flavus*, the results showed that essential oil was ineffective on *Candida albicans* and inhibited 20% of the growth of *Aspergillus flavus* [55]. The results of a study on the antimicrobial effect of essential oil and extracts of *Ferula asafoetida* L. on standard strains of candida using the disk diffusion method demonstrated the weak anti-Candida property of the ethanolic and acetonic extracts of plant gum [56].

Studying anti-fungal activities of aqueous extracts of aerial parts of *Ferula asafoetida* on *Candida albicans* using broth microdilution method, concentrations of 0.273 and 4.4 mg ml^{-1} were determined as MIC_{50} and MIC_{90}, respectively [57]. However, in the present study, the MIC values of this fungus by fruit extract of *Ferula asafoetida* L. was 1 mg ml^{-1}, indicating the stronger and more effective anti-fungal properties of this extract. In this respect, the extraction method, the used organ, and the studied areas can be the reason for the difference in the obtained results.

The essential oil of *Ferula asafoetida* L. was used to study the antimicrobial effect on the species of Mucormycosis and rhizopus [58]. The results showed the plant gum has a stronger anti-fungal effect than the extracts of leaves, edible parts, flowers, fruits, stems, and roots of the plant. The difference in the type of studied fungi, parts of the plant, and the material extracted from the plant (extract or essential oil) can explain the difference in the results.

The difference in reported amounts is due to various methods and used strains in the experiments [59, 60]. Species of used plants are effective in differences in the results. The drying method of the plant, the method of extraction, solvent type (water, methanol, or ethanol), culture medium, and the extract concentration are other important and effective factors in the difference of the results [61,62]. The measurement method of phenolic compounds is another factor to explain the differences in the results of the studies [63].

Plant types, conditions, culture methods, and even the collection time of plant organs are effective factors in the antimicrobial activities or the type and amounts of the plant effective materials [64].

Therefore, the difference in the MIC values in the present study with other studies can be attributed to the difference in the plant species and studied regions (causing a change in the plant metabolites), types and strains of studied microbes, the chemical combinations of the extract, harvest season, extraction method of extracts, and the method of determining the antimicrobial effect.

CONCLUSIONS

The inhibitory effect of the hydroalcoholic extract of *Ferula asafoetida* L. on the growth of *Candida albicans, Staphylococcus aureus*, and *Bacillus subtilis*, the undesirable effects of chemical compounds on human health, and the resistance of bacteria to antibiotics show that using the products of medicinal plants as antibacterial

agents is a suitable method for controlling pathogenic bacteria. Therefore, the extract of this plant can be used in the food industry as a natural preservative and in the pharmaceutical industry as a suitable alternative to chemical drugs.

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Conflict of interests

The authors confirm that this article content has no conflicts of interest.

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