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Determination of antioxidant and antibacterial properties of *Ferulago angulata* extract obtained by maceration method and ultrasound-assisted

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ABSTRACT

Ferulago angulata is a plant known for its phenolic compounds with antioxidant activity. This study aimed to evaluate the flavonoid content antioxidant, and antibacterial properties of *F. angulata* extract obtained through maceration and ultrasound-assisted extraction methods. Optimal extraction conditions for flavonoid content were determined. The maceration method yielded the highest flavonoid content (276.45 µg QE/ml) with 4 hours of extraction, 250 rpm stirring speed, and 100% ethanol solvent. The ultrasound-assisted method achieved maximum flavonoid content (375.12 µg QE/ml) with 15 minutes of extraction, 140 rpm stirring speed, and 100% ethanol solvent. The maceration extraction method showed the largest inhibition zone diameter, particularly against *Staphylococcus aureus*, a gram-positive bacterium, indicating strong antibacterial activity. The smallest inhibition zone diameter was observed against *Escherichia coli*, a gram-negative bacterium. The extraction method significantly influenced the antibacterial properties of the extracts. These findings contribute to understanding the potential applications of *F. angulata* as a natural source of antioxidants and antibacterial agents.

1. Introduction

Antioxidants are molecular compounds that are able to reduce or prevent oxidation of other molecules. Plant metabolic reactions can produce free radicals that trigger chain reactions and damage cells (1). The health-promoting properties of antioxidants and their role in disease prevention have been a major reason for this increase. In fact, antioxidants prevent the oxidation process, which is a cause of diseases such as cancer, and therefore have an impact on human health (2). Many synthetic antioxidants may be used to control the oxidation of fats and oils and prevent food spoilage. However, due to health concerns, consumer rejection of synthetic antioxidants has limited their use in food products. Given these conditions, currently, the most appropriate way to prevent the use of synthetic antioxidants is to find plant sources that contain high levels of natural antioxidants (3). Phenolic compounds are a large group of natural plant materials, including flavonoids, tannins, anthocyanins, etc., commonly found in fruits, vegetables, leaves, nuts, seeds, roots, and other plant parts. These substances are significant sources in food chemistry, pharmacy, and medicine due to the wide range of desirable biological effects, including antioxidant properties (4). The replacement of natural antioxidants with synthetic compounds has been considered due to their healthier nature and issues such as dissolution in water and oil and the creation of suitable emulsions in the food industry. Extensive studies have also shown the effects of such antioxidants against a wide range of microorganisms and have been used as food preservatives as long as they do not conflict with the taste and smell of the product (5). Plant extracts are rich in antioxidants that are used as a source of nutrients and reduce oxidative

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stress, thereby delaying or inhibiting diseases in patients (6). One of these plant extracts is *F. angulata* plant extract. Ferulago, with the scientific name *F. angulata* (Schlecht.) Boiss, locally known as Chavill, Chavir, and Garchi in western Iran, belongs to the *Apiaceae* family and the genus *Ferulago* (7). *F. angulata* is one of the most important medicinal and aromatic plants in Iran and countries such as Turkey, Iraq, Australia, Yugoslavia, Greece, Serbia and Macedonia (8). *F. angulata* extract is a rich source of phytosterols and polyphenolic compounds such as α-pinene, β-pinene, bornyl acetate, ρ-cymene, terpenes, cis-ocimene, flavonoids, especially flavanols, which have antioxidant, anti-inflammatory, and antibacterial activities (9). Conventional extraction methods are based on submerging plant material in a suitable solvent, which is then used to increase the rate of stirring or heating during the process. Common methods of extracting bioactive compounds from plants include soxhlet extraction, distillation, maceration, and percolation (10). Selection of a suitable extraction method for bioactive compounds from a matrix with a minimum of impurities as well as preservation of their biological activity, has been considered in recent years. Extraction can be done traditionally through methods such as Soxhlet extraction and maceration. One of the extraction methods is maceration, which traditionally extracts bioactive compounds (11). Traditional methods of extracting plant material with solvents are mainly based on the correct choice of solvent and the use of heat or agitation, which optimizes mass transfer. One traditional technique for extraction is the maceration method. Long extraction time in maceration increases the extraction efficiency by providing sufficient opportunity to release a suitable volume of solvent into cells and remove antioxidants from their locations (12). It is worth mentioning that ultrasound is one of the new extraction methods in which waves with a frequency higher than 20 kHz penetrate into material, causing successive expansion and contraction in molecules inside the environment, resulting in cavities inside plant material. These cavities are asymmetrically interconnected, causing material to escape from inside cells quickly. In addition, these waves can destroy the cell walls of biological cells and facilitate the release of material (13). The main mechanism of ultrasound extraction relates to the cavitation phenomenon, during which very small bubbles are formed in the liquid mass, grow rapidly to a critical extent, and then explode. The bursting of these bubbles is often accompanied by the release of large amounts of energy that is applied to the environment in the form of shear stress. Extraction by sound waves is cheaper, simpler, and more efficient than conventional methods. The main advantage of using sound waves in solid-liquid extraction is the increase in performance and faster extraction process. Sound waves can lower the process temperature and allow the extraction of unstable compounds against heat (14). Therefore, the extraction efficiency and antioxidant activity of extracted bioactive compounds depend not only on the extraction method but also on temperature, time, and type of solvent used for extraction (15). Due to the importance of extraction conditions and methods, the main purpose of this study was to optimize the extraction of flavonoid, antioxidant, and antimicrobial compounds from *F. angulata* plant extract by maceration and ultrasound.

2. Materials and methods

2.1. Materials

Ethanol (69%), other chemicals including aluminum chloride, potassium acetate solution, Müller Hinton agar culture medium, *Escherichia coli* and *Staphylococcus aureus* from Merck (Germany), and quercetin reagent from Sigma-Aldrich (USA) were prepared.

2.2. Preparation of F. angulata plant

The leaves and stems of *F. angulata* were collected in spring from Kohgiluyeh and Boyer-Ahmad Provinces in Iran. The plant sample was authenticated by researchers at the Herbarium Department of the Institute of Forests and Rangelands of Iran (Alborz Province, Karaj). After cleaning the plant and removing any damaged parts by sieving, the aerial branches were dried in the open air away from direct sunlight. In order to extract the active ingredients of *F. angulata* and prepare its alcoholic extract, the plant was first crushed using liquid nitrogen and an electric mill (Tooskan Khorasan Company, model T8300, made in Iran). Then the resulting powder was passed through a 40-mesh sieve. The prepared powder was stored away from sunlight at 4 °C to prepare the extract (16).

2.3. Extraction by maceration method

About 100 g of powdered *F. angulata* was transferred to a 500 ml Erlenmeyer flask, and ethanol-water solvent ratios (80:20, 20:80, 50:50, 10:90, and 100:0) were added. The mixture was stirred at room temperature using a magnetic stirrer (Mommert, Germany) and mixed with the solvent. Extraction was performed at times (2 and 4h) and stirrer speeds (250 and 100 rpm) at a constant temperature of 25 °C according to Table 1. The solvent-plant mixture was then separated using filter paper. Solvent removal of the obtained alcoholic extracts was carried out in the dark at room temperature. The residue was then squeezed until completely drained to obtain the initial extract. The initial extract was centrifuged at 5000 rpm (Gerber, Germany) for 5 minutes. Then, the supernatant was collected, and the solvent was evaporated using a rotary vacuum distillation apparatus (model RV8, IKA, Germany) at 25 °C for one hour to concentrate the extract to 60°Brix. The extract was later kept in a dark glass container and refrigerated at 4 °C (17).

2.4. Extraction by ultrasound-assisted method

An ultrasonic device from Sonics & Materials, model VCX750, made in the USA with a maximum nominal power of 750 W and a frequency of 20 kHz was used. First, 20 g of dried *F. angulata* powder in a 1:5 ratio was added to ethanol

or water solvents in ratios (80:20, 20:80, 50:50, 10:90, 100:0) according to Table 2. It was then prepared using a stirrer (Mommert, Germany) and subjected to ultrasound. Ultrasound was performed at a constant temperature of 25 °C and at extraction times of 5 and 15 min and stirrer speeds of 80 and 140 rpm.

Table 1. Conditions for extracting the extract from *Ferulago angulata* plant by maceration method.

Treatment	Solvent type*	Time (h)	Stirrer speed (rpm)
1	1	2	100
2	1	2	250
3	1	4	100
4	1	4	250
5	2	2	100
6	2	2	250
7	2	4	100
8	2	4	250
9	3	2	100
10	3	2	250
11	3	4	100
12	3	4	250
13	4	2	100
14	4	2	250
15	4	4	100
16	4	4	250
17	5	2	100
18	5	2	250
19	5	4	100
20	5	4	250

1: Ethanol 20% + Water 80 %. 2: Ethanol 80%+ Water20%. 3: 50% Ethanol+ 50% Water. 4: ethanol 100%. 5: water 100%.

Table 2. Conditions for extracting extracts from *Ferulago angulata* plant by ultrasonic method.

Treatment	Solvent type	Time (min)	Stirrer speed (rpm)
1	1	5	80
2	1	5	140
3	1	15	80
4	1	15	140
5	2	5	80
6	2	5	140
7	2	15	80
8	2	15	140
9	3	5	80
10	3	5	140
11	3	15	80
12	3	15	140
13	4	5	80
14	4	5	140
15	4	15	80
16	4	15	140
17	5	5	80
18	5	5	140
19	5	15	80
20	5	15	140

1: Ethanol 20% + Water 80 %. 2: Ethanol 80%+ Water 20%. 3: Ethanol 50%+ Water 50%. 4: ethanol 100%. 5: water 100%.

The initial extract was centrifuged at 5000 rpm (Gerber, Germany) for 5 min. Then, the supernatant was collected, and the solvent was evaporated using a rotary vacuum distillation apparatus (model RV8, IKA, Germany) at 25 °C to concentrate the extract to 60°Brix. The extract was stored away from sunlight (18).

2.5. Measurement of flavonoid content

The total flavonoid content in each extract was determined using the aluminium chloride colourimetric method. 0.5 ml of extract (before drying) was dissolved in 1.5 ml of methanol in a test tube. Then 0.1 ml of 10% aluminium chloride and 0.1 ml of 1 M potassium acetate solution were added. Finally, 2.8 ml of distilled water was added, and the mixture was kept at room temperature for 30 minutes. The absorbance of the resulting solution was then read at 415 nm using a spectrophotometer (Varian Cary 100 Bio UV-Visible Spectrophotometer). Quercetin was used as a standard at concentrations of 6.25, 12.5, 25, 50 and 80 ppm to plot the calibration curve (19).

2.6. Antibacterial effects of F. angulata extracts

The 50, 100, 200 and 400 mg/ml concentrations were prepared from extracts dissolved in 0.5% dimethyl sulfoxide solvent and used in well diffusion tests. Lyophilized *E. coli* (PTCC 1769) and *S. aureus* (PTCC 1917) microbial strains from the Iranian Scientific and Industrial Research Organization were used. The well agar diffusion method was used to investigate the antibacterial effects of aqueous and ethanolic extracts. For this, the mentioned strains were prepared with turbidity equal to 0.5 McFarland and spread in the usual way on Müller-Hinton agar plates. Wells with a diameter of 5 mm and a distance of 2 cm from each other were then created on the surface of the plate. Each well was filled with different dilutions of the mentioned extracts. Streptomycin antibiotic was used as a positive control, and dimethyl sulfoxide was used as a negative control. After finishing, all culture media were incubated for 16-18 hours at 37°C. After this period, bacterial cultures were examined for the presence or absence of growth inhibition zones (measured in millimeters using a caliper). The diameter of the growth inhibition zone indicates the reaction to the test extract concentration. This phenomenon shows a linear relationship between the growth inhibition zone and the logarithm of test extract concentration; by measuring the diameter of the microbial growth inhibition zone and comparing it to a standard, the antimicrobial power of the tested extract was determined (20).

2.7. Determination of MIC and MBC

Dilution tubes were prepared to measure the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) as follows: 1 ml of extract at a concentration of 320 µg was added to the first tube containing sterile Müller-Hinton broth culture. After mixing, 1 ml from the first tube was transferred to the second tube, and this process was continued until the last tube, with 1 ml being discarded from the last tube. Then, 10 µl of bacterial suspension was added to all tubes (McFarland 0.5). A tube containing culture medium and bacteria was prepared as a positive control, and a tube containing only culture medium as a negative control. All tubes were incubated at 37°C for 24 hours, after which the tube showing no turbidity due to bacterial growth was considered the minimum inhibitory concentration, indicating inhibited growth of the

microorganisms. Turbidity in the tubes indicated bacterial growth, while a completely clear tube was considered the MIC. To determine the MBC under completely aseptic conditions, the contents of test tubes incubated for 24 hours, where MIC levels were identified and showed no turbidity, were cultured onto agar plates containing culture medium and incubated at 37°C for 24 hours. Then bacterial growth or no growth was examined. The first concentration showing no growth was considered the MBC (21).

2.8. Statistical analysis

Independent variables in the maceration extraction of *F. angulata* included solvent type (water and ethanol), extraction time (2 and 4 h), and stirrer speed (100 and 250 rpm), and in ultrasonic extraction included solvent type (water and ethanol), extraction time (5 and 15 min) and stirrer speed (80 and 140 rpm). Therefore, 20 treatments were designed in a completely randomized design (full factorial design). One-way analysis of variance (ANOVA - Duncan's test) was performed on the data using Minitab v16 software at a 95% confidence level.

3. Results and Discussion

3.1. Flavonoids in F. angulata extract extracted by the maceration method

Phenolic compounds, including simple phenols, contain an aromatic ring with at least one hydroxyl group. Polyphenolic compounds contain two or more phenolic subunits, including flavonoids (22). Flavonoids are abundantly found in nature, with over 9,000 reported flavonoid structures. Flavonoids contain a basic 2-phenyl-chromone nucleus. An important feature of flavonoids is the presence of the C2=C3 bond, which affects their functional properties and oxidation level (23). The analysis of variance results in Table 3 shows that solvent type, time, and stirrer speed had a significant linear effect on changes in the flavonoid content of *F. angulata* plant extract obtained by the maceration method ($p \leq 0.05$). The interaction effects of solvent type × time, solvent type × stirrer speed, and time × stirrer speed on flavonoid amounts were insignificant ($p > 0.05$). Based on the factor F value, solvent type had the most significant impact on flavonoid content. As shown in Table 3, the modified coefficient of determination (R2-adj) was 99%, indicating a good model fit to experimental data. The flavonoid content of *F. angulata* plant extract extracted by maceration under different conditions is reported in Table 4. Other extraction conditions (time, stirrer speed, and solvent type) significantly affected flavonoid content ($p \leq 0.05$). Flavonoid content ranged from 65.87 to 276.45 (g QE/ml). The highest content of 276.45 (g QE/ml) was obtained with 100% ethanol at 4 hours and 250 rpm stirring, while the lowest of 65.87 (g QE/ml) was at 2 hours, 100 rpm, and 100% ethanol. Increasing extraction time from 2 to 4 hours and stirrer speed from 100 to 250 rpm, along with using ethanol solvent, increased flavonoid extraction. Ethanol solvent resulted in higher extraction than water or ethanol-water mixtures, likely due to flavonoids' polarity.

Table 3. Analysis of variance of solvent effect, time, and stirrer speed on flavonoids extracted from *Ferulago angulata* plant by maceration method.

Source of Changes	Total flavonoid content (µg of QE/ml)	
	F-value	P-value
Block	136.2	0.0001**
Solvent (S)	874.4	0.0001**
Time (t)	24.85	0.0001**
Stirring speed (P)	39.26	0.0001**
Solvent× Time (S×T)	1.26	0.3209
Solvent× Stirring speed (S×P)	1.63	0.2069
Time× Stirring speed (T×P)	0.29	0.5978
S×T×P	0.22	0.9268
Error	-	-
R-sq(r2)	0.99	-

Table 4. Mean comparison results in the amount of flavonoids extracted from *Ferulago angulata* extract in different treatments by maceration method.

Solvent	Time and Stirring speed	Total flavonoid content (µg of QE/ml)
Et20/Wt80	2 h+100 rpm	115.76 ± 14.47 ^{k*}
	2 h+250 rpm	142.65 ± 17.18 ^{ij}
	4 h+100 rpm	128.09 ± 21.43 ^{jk}
	4 h+250 rpm	150.23 ± 20.19 ^{i}
Et80/Wt 20	2 h+100 rpm	216.45 ± 27.53 ^{ef}
	2 h+250 rpm	230.93 ± 29.86 ^{de}
	4 h+100 rpm	221.67 ± 15.77 ^{ef}
	4 h+250 rpm	240.17 ± 18.77 cd
Et50/Wt 50	2 h+100 rpm	188.41 ± 19.23 ^{h}
	2 h+250 rpm	195.94 ± 18.77 ^{h}
	4 h+100 rpm	200.17 ± 18.16 ^{gh}
	4 h+250 rpm	211.34 ± 21.61 ^{fg}
Ethanol 100%	2 h+100 rpm	245.98 ± 23.87 ^{c}
	2 h+250 rpm	252.58 ± 23.53 ^{bc}
	4 h+100 rpm	261.86 ± 21.84 ^{ab}
	4 h+250 rpm	276.45 ± 30.97 ^{a}
Water 100 %	2 h+100 rpm	65.87 ± 8.02 ^{m}
	2 h+250 rpm	75.12 ± 8.73 ^{lm}
	4 h+100 rpm	70.95 ± 5.77 ^{lm}
	4 h+250 rpm	81.43 ± 7.33 ^{l}

* Different letters indicate significant differences among the rows ($p < 0.05$).

Extraction factors like material particle size, physical properties, maceration time/solvent, temperature, solvent ratio, and method influence phenolic content (24). Different solvents extract varying amounts due to polarity (25). Saltarelli et al. (26) also found ethanol effectively extracted phenolics like flavonoids and terpenoids from *Ganoderma lucidum* mycelium. In summary, ethanol proved an effective solvent for *F. angulata* flavonoid extraction in this study.

3.2. The amount of flavonoids in extract of F. angulata extracted by ultrasound-assisted

Results of analysis of variance effect of solvent, time, and stirrer speed on flavonoid content of extract obtained from *F. angulata* plant by ultrasonic method are reported in Table 5. According to the results of Table 5, the linear effects of solvent type, time, and stirrer speed on changes in flavonoid content

of *F. angulata* plant extract (extracted by ultrasonic method) were significant ($p \leq 0.05$). The interaction effects of solvent type × time, solvent type × stirrer speed, and time × stirrer speed on the amount of flavonoid changes were not significant ($p > 0.05$). According to factor F, the solvent type had the greatest effect on the flavonoid content of *F. angulata* plant extract extracted by ultrasonic method. As can be seen in Table (5), the value of its modified determination coefficient (R2-adj) was 0.99%, which indicates that the model fits well with experimental data. According to the results of Table 6, as can be seen, different extraction conditions (time, stirrer speed, and solvent type) had a significant effect on the flavonoid content of *F. angulata* plant extract extracted by ultrasound. The amount of flavonoids varied from 107.82 (g of QE / ml) to 375.12 (g of QE / ml). The highest flavonoid content (375.12 g of QE / ml) was obtained with 100% ethanol solvent at 15 min extraction time and stirring speed of 140 rpm, and the lowest flavonoid content (65.87 g of QE / ml) was obtained with 100% ethanol solvent at 5 min extraction time and stirring speed of 80 rpm, and their differences were significant. By increasing the duration of the extraction process by ultrasound from 5 to 15 minutes, increasing stirrer speed from 80 to 140 rpm, and using ethanol solvent, the amount of flavonoids extracted has increased.

Table 5. Analysis of variance effect of solvent, time, and stirrer speed on flavonoids extracted from *Ferulago angulata* plant by ultrasonic method.

Source of Changes	Total flavonoid content (µg of QE/ml)	
	F-value	P-value
Block	7.288	0.0001**
Solvent (S)	1599	0.0001**
Time (t)	36.65	0.0001**
Stirring speed (P)	36.14	0.0012**
Solvent× Time (S×T)	13.0	0.9689
Solvent× Stirring speed (S×P)	03.0	0.9985
Time× Stirring speed (T×P)	15.0	0.7012
S×T×P	0.10	0.9813
Error	-	-
R-sq(r2)	0.99	-

3.3. Antimicrobial effects of extracts of Ferulago angulata extracted by maceration and ultrasound-assisted methods

The antimicrobial effects of the extracted extracts of *F. angulata* plant by maceration and ultrasound-assisted, which had the highest amount of flavonoid compounds, were investigated by well diffusion method on *S. aureus* and *E. coli* microorganisms (according to Table 7). The results showed that the highest growth inhibition zone in the maceration method belonged to *S. aureus* at 13.26 mm, and the lowest growth inhibition zone belonged to *E. coli* at 9.19 mm. In the ultrasound-assisted method, the largest growth inhibition zone (12.33 mm) was related to *S. aureus*, and the lowest growth inhibition zone (6.81 mm) was related to *E. coli*. According to the results, the extract obtained from *F. angulata* plant by maceration method had a more significant antimicrobial effect than the ultrasound-assisted method.

Table 6. Mean comparison results in the amount of flavonoids extracted from *Ferulago angulata* extract in different treatments by ultrasonic method.

Solvent	Time and Stirring speed	Total flavonoid content (µg of QE/ml)
Et20/Wt80	5 h+80 rpm	202.47 ± 33.16 ^{k*}
	5 h+140 rpm	211.89 ± 21.03 ^{jk}
	15 h+80 rpm	219.87 ± 36.74 ^{j}
	15 h+140 rpm	224.65 ± 24.52 ^{j}
Et80/Wt 20	5 h+80 rpm	302.54 ± 23.87 ^{f}
	5 h+140 rpm	311.75 ± 15.70 ^{ef}
	15 h+80 rpm	320.65 ± 23.55 ^{de}
	15 h+140 rpm	328.23 ± 22.32 ^{d}
Et50/Wt 50	5 h+80 rpm	247.86 ± 17.61 ^{i}
	5 h+140 rpm	254.76 ±26.83 ^{hi}
	15 h+80 rpm	262.17 ± 25.43 ^{gh}
	15 h+140 rpm	270.45 ± 18.36 ^{g}
Ethanol 100%	5 h+80 rpm	348.94 ± 20.28 ^{c}
	5 h+140 rpm	355.65 ± 17.39 ^{bc}
	15 h+80 rpm	367.16 ± 30.62 ^{ab}
	15 h+140 rpm	375.12 ± 27.68 ^{a}
Water 100 %	5 h+80 rpm	107.82 ± 12.26 ^{n}
	5 h+140 rpm	118.92 ± 15.22 ^{mn}
	15 h+80 rpm	127.68 ± 14.76 ^{lm}
	15 h+140 rpm	134.34 ± 16.35 ^{l}

* Different letters indicate significant differences among the rows ($p < 0.05$).

Table7. The diameter of the Inhibition zone bacteria (mm) at different concentrations extraction of *Ferulago angulata* (mg/ml) by the well distribution method.

Bacterial Strain	Maceration	Ultrasonic
Staphylococcus aureus	13.26 ± 0.253 ^{A}	12.33±0.208 ^{B}
Escherichia coli	9.19 ± 0.144 ^{A}	6.81±0.185 ^{B}

*Different letters indicate significant differences among the rows ($p < 0.05$).

The inhibitory effects of flavonoid compounds depend on surface adsorption of cell membranes' reaction to enzymes, substrates, and metal ions (27). The hydroxyl group in flavonoid compounds binds to the active part of enzymes and inhibits their metabolism. There is a synergist between carvacrol and its precursor para-cymene, which is important; first, para-cymene swells the cell membrane of microorganisms and then facilitates entry of more carvacrol into the cell, and finally, the effect of carvacrol causes destruction of microorganisms (28). Another mechanism that may occur is that these compounds bind to phospholipids in the cell membrane, reducing selective permeability and increasing membrane permeability. In this case, cellular components are removed from the cell, and energy metabolism is damaged. It also alters the uptake of nutrients by microbial cells, their electron transfer, and the synthesis of genetic material (28).

3.4. Results of MIC and MBC of Ferulago angulata extract by maceration and ultrasound-assisted

Table 8 shows the MIC and MBC results of optimal extracts of *F. angulata* extracted by maceration and ultrasound-assisted against *S. aureus* and *E. coli*. The MIC and MBC of *F. angulata* extract extracted by maceration were 8 and 10 (µl/ml) for *S. aureus* and 14 and 18 (µl/ml) for *E. coli*, respectively. In ultrasound-assisted extraction, MIC and MBC were

determined to be 10 and 14 (µl/ml) for *S. aureus* and 8 and 10 (µl/ml) for *E. coli*. In fact, due to the difference in the wall of gram-positive bacteria (*S. aureus*) compared to gram-negative bacteria (*E. coli*), gram-positive bacteria have a mucopeptide composition in their cell wall. In contrast, gram-negative bacteria have only a thin layer of mucopeptide, and most of the wall structure is lipoprotein and lipopolysaccharide, so they are more resistant to antibacterial agents. Gram-negative bacteria have an outer membrane around their cell wall, making them more resistant to antibacterial agents (17). Majnuni et al. (29) investigated the antimicrobial effects of aqueous and alcoholic extracts of fenugreek leaves and seeds on different microbial strains. Based on the results, it was found that the inhibitory and antimicrobial effect of fenugreek extracts on gram-positive bacteria is significantly higher than on gram-negative bacteria. In another study conducted by Tabatabaei Yazdi et al. (17), the antimicrobial effect of the ethanolic extract of *F. angulata* was investigated against *S. aureus*, *Bacillus cereus*, and Salmonella typhi. The findings revealed that the MIC of the ethanolic extract of *F. angulata* against *B. cereus*, *S. aureus*, and *S. typhi* was 8, 8, and 32 mg/ml, respectively. Furthermore, the MBC of the ethanolic extract of *F. angulata* against *B. cereus*, *S. aureus*, and *S. typhi* was found to be 16, 32, and 64 mg/ml, respectively.

Table 8. *Ferulago angulata* maceration and Ultrasound-assisted extraction dilutions (µl/ml).

Extraction method	Microorganism	2	4	6	8	10	12	14	16	18
Ultrasound-assisted	*Staphylococcus aureus*	++	++	+	5 colonies	-	-	-	-	-
	Escherichia coli	++	++	++	+	++	++	10 colonies	3 colonies	-
Maceration-assisted	*Staphylococcus aureus*	++	++	++	++	9 colonies	2 colonies	-	-	-
	Escherichia coli	++	++	++	5 colonies	-	-	-	-	-

++represents a highly presence growth of microorganism. + represents a presence growth of microorganisms. - represents a absence of growth

4. Conclusion

The aim of this study was to optimize the extraction of *F. angulata* plant extract by maceration and ultrasound-assisted and to evaluate flavonoid, antioxidant, and antimicrobial properties under different conditions of time, stirrer speed, and solvent type. Based on the obtained results, the highest amount of extraction of flavonoid compounds in the maceration method in extraction time of 4 h and 250 rpm stirrer speed with 100% ethanol solvent and in the ultrasonic pretreatment method in extraction time of 15 min and 140 rpm stirrer speed with 100% ethanol solvent was obtained. The results of the present study showed that the ultrasonic method and use of ethanol solvent in extracting *F. angulata* extracts with the highest amount of flavonoids are more efficient than the maceration method. In contrast, the *F. angulata* extract extracted by the maceration method showed a better antimicrobial effect on gram-positive bacteria (*S. aureus*) compared to gram-negative bacteria (*E. coli*). Due to the high amount of flavonoid and antimicrobial compounds in the extract of *F. angulata* plant, it can be used in food and pharmaceutical industries instead of synthetic antioxidants.

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