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Influence of extraction methods and solvents on the antimicrobial activity of pickled *Ferula orientalis*

Extraktionsmethoden und Auswirkungen von Lösungsmitteln auf die antimikrobielle Aktivität von eingelegten Ferula orientalis

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Summary

The aim of this study was to determine the antimicrobial effects of the essential oil and various extracts of pickled ***Ferula orientalis*** L. Two Gram (-) bacteria *(**Escherichia coli, Pseudomonas aeruginosa**),* two Gram (+) bacteria *(**Staphylococcus aureus, Streptococcus mutans**),* two yeast *(**Saccharomyces cerevisiae, Candida albicans**),* and two mold *(**Aspergillus niger, Penicillium roqueforti**)* strains were used to determine the antimicrobial effect of the extracts on different cell types. The extracts were prepared using classical and ultrasonic methods with five different solvents (water, ethanol: water, methanol: water, chloroform, and n-hexane). The essential oil was obtained by hydrodistillation. The ***in vitro*** antimicrobial assays showed that the essential oil and extracts were more effective on Gram (+) bacteria and molds. The highest antibacterial activity was exhibited against ***S. mutans*** with ultrasonic ethanol: water extract (31.25 µg mL^{-1}). In addition, the essential oil exhibited strong inhibitory activity against ***A. niger*** (MIC: 62.5 µg mL^{-1}) compared to the other tested microorganisms. The results showed that solvent diversity had an important effect on antimicrobial activity of all the tested microorganisms ($p < 0.01$) and the extraction method (classical or ultrasonic) was effective on ***E. coli, P. aeruginosa, C. albicans, A. niger,*** and ***P. roqueforti*** ($p < 0.05$).

Keywords: antimicrobial activity, essential oil, extraction, ***Ferula orientalis,*** ultrasound

Introduction

Antimicrobial substances extracted from natural sources, especially plants, are used as raw materials for pharmaceuticals and in the preservation of raw or processed foods (Panda et al., 2019). It has been reported that more than 1,340 plants and more than 30,000 components extracted from them that exhibited antimicrobial activity (Tajkarimi et al., 2010). Considering the destructive effects of foodborne pathogenic microorganisms on food spoilage or illnesses and occasionally even death in humans (Penha et al., 2017; Panda et al., 2019), essential oils and extracts should continue to be investigated for use in food and medicinal systems.

The extraction method is critical, especially for the quality and yield of bioactive compounds. Plant-based antimicrobial compounds are obtained by classical (e.g., solid: liquid extraction, soxhlet extraction and hydro, or alcohol or steam distillation) and relatively modern (e.g., ultrasound-assisted, enzyme-assisted, microwave-assisted, pulsed electric field-assisted, and super- and subcritical fluid) extraction methods. One of these methods, ultrasound-assisted extraction, has been proven to increase the solubility and mass transfer rates of bioactive compounds with antimicrobial, antioxidative, and anti-inflammatory properties than maceration and soxhlet extraction without compromising their biological activities (Dzah et al., 2020). It is known that solvent selection, as well as extraction method, is also effective in obtaining a bioactive compound. The purity and activity of extracted bioactive compounds vary depending on the polarity, melting point, boiling point, and density of the solvent (Zou et al., 2014).

Several studies over the past few years have been conducted on plants to determine the antimicrobial activities of the bioactive components obtained using different extraction techniques and solvents (Cetin et al., 2010; Floroian et al., 2017; Majeed et al., 2019). In the past few years, studies by which to determine the antimicrobial activity of different extracts and essential oils from *Ferula* species have increased (Zomorodian et al., 2018; Kahraman et al., 2019; Pavela et al., 2020; Zengin et al., 2020).

Ferula, a member of the Apiaceae family, contains 180 species (Yaqoob and Nawchoo, 2016) that grow mainly in the arid regions of Eurasia, the Canary Islands, North Africa (Pavlović et al., 2012), and especially Iran and Turkey (Downie et al., 2000). Some *Ferula* plants are known worldwide for their medicinal and biological properties (Khan et al., 2011; Mahendra and Bisht, 2012; Soltani et al., 2018; Zhou et al., 2017). *Ferula orientalis* L. is grows on the rocky slopes of the Eastern Anatolia Region of Turkey at an altitude of 1600-2900 m (Turkish Plants Data Service, 2020). This plant is called "Çaşır" or "Çaşur" by the local people and is made into pickles in April and May. Pickled *F. orientalis* is commonly used as a salad throughout the year.

Some studies have examined the antioxidant, enzyme inhibitory, antimicrobial, and cytotoxic properties of the different solvent extracts of *F. orientalis* (Kartal et al., 2007; Ceylan et al., 2019; Zengin et al., 2020). In addition, some studies have been conducted on the volatile composition, antioxidant, antimicrobial, genotoxic, and antigenotoxic properties of its essential oil (Ozkan et al., 2014; Karakaya et al., 2019). In our previous article, the antioxidant activity, phenolic composition, volatile composition, and *in vitro* neuroprotective activity of pickled *F. orientalis*, its extracts, and essential oil were presented (Topdas et al., 2020); however, there has been no study published in the literature on the antimicrobial activity of pickled *F. orientalis* or its various extracts.

This study aimed to determine the antimicrobial activity of extracts which obtained using two extraction methods (classical and ultrasonic) and five different solvents (water, ethanol: water, methanol: water, chloroform and n-hexane) as well as essential oil of pickled *F. orientalis* against some selected microorganisms.

Material and Methods

Chemicals and media

Dimethyl sulfoxide (DMSO), nutrient broth (NB), sabouraud-2% dextrose broth (SB), potato dextrose agar (PDA), plate count agar (PCA), nutrient broth (NB), and nutrient agar (NA) were purchased from Merck KGaA (Darmstadt, Germany) while amphotericin B and ofloxacin were from Sigma-Aldrich (St. Louis, USA).

Material and test microorganisms

Fresh *F. orientalis* plants (2 kg) were collected from the Eastern Anatolia Region of Turkey (Erzurum) in April 2017. The collected plants were kept at 4±1°C until processing. After the leaves were removed, the plants were washed to purify any dust, soil, or foreign materials. The plants were boiled for 15 min in water and placed in plastic containers. A brine solution with a 9 degrees Baumé was prepared cold water and salt. Then this solution poured onto the plants. The pickled plants were kept in the dark at 20±2°C for 10 d to reach a final degree Baumé of 7. The fresh plant and its pickled form are shown in Figure 1.

The antimicrobial activities of the water, ethanol:water, methanol:water, chloroform, and n-hexane extracts and the essential oil of *F. orientalis* were determined against the following strains: *Escherichia coli* BC 1402, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 29213, *Streptococcus mutans* ATCC 35668, *Saccharomyces cerevisiae* BC 6541, *Candida albicans* ATCC 1223, *Aspergillus niger* ATCC 16888, and *Penicillium roqueforti* BC 111. The test microorganisms were obtained

FIGURE 1: *Ferula orientalis L. (A) collected from 40°29' 20.2''N and 41°21'52.4'' E coordinates and its pickled form (B).*

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from the Food Microbiology Laboratory Culture Collection of Ataturk University, Turkey. Identification of the microorganisms was confirmed using a microbial identification system (Sherlock Microbial Identification System version 4.0, MIDI Inc., Newark, DE, USA), BIOLOG (MicroStationTM ID System, Biolog Inc., Hayward, CA, USA), and classical identification tests from Bergey's Determinative Bacteriology guide (Holt, 1994).

Preparation of extracts and essential oil

Essential oil

The pickled plant material (50 g) was mixed with 500 mL distilled water (1:10 m:w), put through a blender (Waring HGB2WTS3, USA) and then hydrodistilled using a Clevenger-type apparatus. Glass beads with a diameter of 1 mm were used to prevent any overflow from the system. The essential oil was collected after ~3 h and kept in sealed vials at -20°C until analyses (Ilić et al., 2019).

Extracts

Preliminary: The pickled plant material was cut into small pieces with a steel knife and lyophilized at 10^{-3} mTorr pressure and -86°C (Operon FDU-8612, Korea). After lyophilization, the plants were turned into powder using a blender. Then, 50 g powdered sample was added into the 500 mL water, ethanol: water (1:1 v:v), methanol: water (1:1 v:v), chloroform, and n-hexane solvents.

Classical extraction: The samples were shaken at 90 rpm in a water bath (JSR JSSB-30T, Korea) at 40°C for 20 h. The extracts were filtered using Whatman No. 1 filter paper and centrifuged for 15 min at 4500 rpm. The supernatant was concentrated using a rotary evaporator (Heidolph Laborata 4000, Germany) at 40°C and 150 rpm. The extracts were frozen with liquid nitrogen and lyophilized at 10^{-3} mTorr pressure and -86°C. The lyophilized extracts were stored in the dark at -20°C until analyses.

Ultrasonic extraction: The temperature of the ultrasonic bath (Bandelin Sonorex Super RK 103H, Germany) operating at a frequency of 35 kHz was kept constant at 40 ± 2°C, and the samples were subjected four times to 20 min of ultrasound + 20 min of rest. Next, the extracts were filtered through Whatman No. 1 filter paper and centrifuged for 15 min at 4500 rpm. The supernatant was then concentrated using rotary evaporator at 40°C and 150 rpm. The extracts were frozen with liquid nitrogen and lyophilized at 10^{-3} mTorr pressure and -86°C. The lyophilized extracts were stored in the dark at -20°C until the analyses.

Preparation of bacteria, yeast, and mold isolates

The bacterial isolates stored at -80°C were inoculated on PCA agar, while the pure yeast and mold isolates were drawn onto PDA agar. After inoculation, the bacteria were incubated for 24-48 h at 30°C, the yeast for 24-48 h at 30°C, and the molds for 72-96 h at 30°C. All strains were checked for contamination. The fresh cultures were diluted in 0.85% sterile physiological saline solutions prepared in 9 mL tubes and adjusted to a standard density using a densitometer (Mc Farland, DEN-1 Biosan, Latvia). The density was set to 1 x 10^6 cfu mL^{-1} for the bacteria and 1 x 10^4 cfu mL^{-1} for the yeast and molds, as in Cetin et al. (2010). The specified dose was adjusted to 300 µg/10 µL for the essential oil and other extracts and 5 µg/10 µL for ofloxacin and amphotericin B using DMSO (50%).

Well diffusion method

The agar-well diffusion method was used to analyze the antimicrobial activity of extracts and essential oil (Altundag 2007). First, 100 µL bacteria, yeast, and mold suspensions were transferred to the NA for the bacteria and PDA media for the yeasts and molds using sterile drigalski spatulas. Then, eight wells, with a diameter of 5 mm each, were created in the medium for the extracts, essential oil, and positive and negative controls. 100 µl each of positive control (ofloxacin for bacteria and amphotericin B for yeast and molds), and the negative control (50% DMSO), essential oil, and extracts were loaded into the wells. The petri dishes with the bacteria were incubated at 30°C for 24-48 h, the yeast at 30°C for 24-48 h, and the mold at 30°C for 72-96 h. At the end of the incubation period, the diameters of the clear zones around the wells were measured and recorded in mm. Each assay was repeated three times.

Determination of the minimum inhibition concentration

The microwell dilution method was conducted with some modifications using the method of Cetin et al. (2010). All extracts, essential oil, and positive controls (ofloxacin, and amphotericin B) dissolved in 50% DMSO were first diluted to a concentration of 500 µg mL^{-1}, after which a serial twofold dilution was conducted to reach a concentration from 7.8 to 500 µg mL^{-1} comprising NB medium for bacteria and SB medium for yeast and molds. A minimum inhibition concentration (MIC) analysis was conducted using 96-well plates in which 140 µL medium and 10 µL bacteria, yeast, or mold containing inoculum were added to each well. The plates were covered with a sterile plate sealer and shaken for 20 sec at 300 rpm. The plates containing bacteria and yeasts were incubated at 30°C for 24-48 h and those containing the molds were incubated at 30°C for 72-96 h. At the end of the incubation period, microbial growth at 600 nm was measured spectrophotometrically using a microplate reader (Bio Tek Instruments, VT05404-0998, USA). The lowest dose was evaluated using MIC absorbance values. The essential oil and all extracts analyzed in this study were screened three times against each microorganism.

Statistical analyses

Statistical analyses were conducted using SPSS 20.0 (IBM Corp., Armonk, NY, USA). All experiments in the study were carried out in triplicate. The differences among the extraction methods and solvent diversity were evaluated using Duncan's multiple range test. The results were considered statistically significant at $p < 0.01$ and $p < 0.05$.

Results and discussion

The use of different extraction solvents and methods results in the formation of tens of mixtures containing different functional components derived from the same plant. Because of the diversity of the chemical composition of the extracts, it is very important to determine the antimicrobial properties of each mixture among the other properties.

In the present study, ultrasonic extraction, classical extraction and hydrodistillation were used to obtain various fractions of pickled *F. orientalis*. Five different solvents (water, ethanol: water, methanol: water, n-hexane, and

chloroform) with different polarities were used. Water, ethanol, and methanol are polar solvents, while chloroform and n-hexane are non-polar. Thus, the extracts that exhibited the highest antimicrobial activity against the selected strains was tried to be determined. The trial design was created using two Gram (+) bacteria, two Gram (-) bacteria, two molds, and two yeast strains to determine the antimicrobial effects of the extracts on different cell types. The antimicrobial activities of essential oil and various extracts were determined qualitatively and quantitatively by observing the inhibition zone formation, measuring the zone diameter (mm), and determining the MIC values (µg mL^{-1}). The detected antimicrobial properties of the essential oil are shown in Figure 2; the results for the other extracts are presented in Table 1.

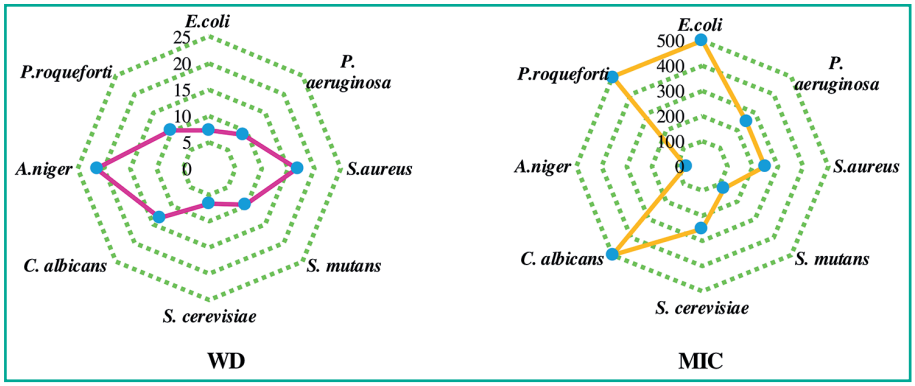

FIGURE 2: *WD and MIC values of F. orientalis essential oil. *WD; inhibition zone (diameter in mm) around the disks (5 mm) filled with 100 µl essential oil.*

The existing zones and their sizes in the agar-well diffusion method indicate the sensitivity of microorganism to the extract. A zone of <5 mm indicated no clear zone, while those between 6 and 9 mm and 10 and 14 mm indicated a moderately clear zone and strong clear zone, respectively. The inhibition diameter ≥15 mm indicates a very strong clear zone (Wanja et al., 2020). In the present study, essential oil caused inhibition zones >6 mm against all tested microorganisms (Fig. 2). Among the bacteria, essential oil exhibitied the highest inhibition against *S. aureus* (WD: 16.7 mm, MIC: 250 µg mL^{-1}), while the lowest was against *E. coli* (WD: 7.3 mm, MIC: 500 µg mL^{-1}). The results showed that the essential oil was more effective on Gram (+) bacteria. Similarly, Karakaya et al. (2019) have also indicated that the essential oil obtained from fresh *F. orientalis* is effective against *S. aureus*. The researchers have also reported that the essential oil does not cause any inhibition zone against *E. coli* and *P. aeruginosa*; however, in the present study, a weak antimicrobial effect was observed against *E. coli* and a moderate activity against *P. aeruginosa* (WD: 9.1 mm, MIC: 250 µg mL^{-1}) compared with that of the tested Gram (+) strains. The differences in the results may have been caused by the applied test method or a change in the chemical composition of the essential oil based on soil composition, harvesting time, and plant

TABLE 1: Antimicrobial activity of essential oil and some extracts of *F. orientalis* L.

Sample		*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Streptococcus mutans*	*Saccharomyces cerevisiae*	*Candida albicans*	*Aspergillus niger*	*Penicillium roqueforti*
CWE	WD	0.0±0.0^{E,c}	0.0±0.0^{D,c}	0.0±0.0^{D,c}	12.7±0.2^{D,c}	0.0±0.0^{C,c}	0.0±0.0^{C,b}	0.0±0.0^{I,e}	0.0±0.0^{E,c}
	MIC	–	–	–	125	–	–	–	–
CEWE	WD	0.0±0.0^{E,c}	0.0±0.0^{D,c}	12.7±0.2^{A,a}	16.7±0.3^{B,a}	0.0±0.0^{C,c}	0.0±0.0^{C,b}	14.7±0.2^{B,a}	0.0±0.0^{E,c}
	MIC	–	–	500	31.25	–	–	250	–
CMWE	WD	0.0±0.0^{E,c}	0.0±0.0^{D,c}	12.0±0.1^{B,b}	13.7±0.3^{C,b}	0.0±0.0^{C,c}	0.0±0.0^{C,b}	12.7±0.1^{E,b}	0.0±0.0^{E,c}
	MIC	–	–	500	62.5	–	–	500	–
CHE	WD	10.3±0.3^{A,a}	7.2±0.1^{C,b}	0.0±0.0^{D,c}	0.0±0.0^{F,d}	5.7±0.3^{B,b}	11.1±0.3^{A,a}	12.1±0.1^{F,c}	14.7±0.3^{A,a}
	MIC	500	500	–	–	500	500	500	500
CCE	WD	9.3±0.1^{C,b}	8.2±0.2^{A,a}	0.0±0.0^{D,c}	0.0±0.0^{F,d}	6.1±0.2^{A,a}	0.0±0.0^{C,b}	11.3±0.2^{G,d}	10.7±0.3^{D,b}
	MIC	500	500	–	–	500	–	500	500
UWE	WD	0.0±0.0^{E,c}	0.0±0.0^{D,c}	0.0±0.0^{D,c}	11.7±0.1^{E,c}	0.0±0.0^{C,c}	0.0±0.0^{C,c}	0.0±0.0^{I,d}	0.0±0.0^{E,c}
	MIC	–	–	–	125	–	–	–	–
UEWE	WD	0.0±0.0^{E,c}	0.0±0.0^{D,c}	12.7±0.2^{A,a}	17.5±0.3^{A,a}	0.0±0.0^{C,c}	0.0±0.0^{C,c}	16.3±0.2^{A,a}	0.0±0.0^{E,c}
	MIC	–	–	500	31.25	–	–	125	–
UMWE	WD	0.0±0.0^{E,c}	0.0±0.0^{D,c}	11.7±0.1^{C,b}	13.7±0.3^{C,b}	0.0±0.0^{C,c}	0.0±0.0^{C,c}	13.3±0.2^{C,b}	0.0±0.0^{E,c}
	MIC	–	–	500	62.5	–	–	500	–
UHE	WD	10.0±0.1^{B,a}	8.1±0.2^{A,a}	0.0±0.0^{D,c}	0.0±0.0^{F,d}	6.0±0.1^{A,b}	10.7±0.1^{B,b}	13.0±0.3^{D,b}	13.3±0.2^{B,a}
	MIC	500	500	–	–	500	500	500	500
UCE	WD	8.8±0.2^{D,b}	7.7±0.1^{B,b}	0.0±0.0^{D,c}	0.0±0.0^{F,d}	6.2±0.1^{A,a}	11.3±0.3^{A,a}	10.7±0.2^{H,c}	11.0±0.3^{C,b}
	MIC	500	500	–	–	500	500	500	500
A	WD	14±0.1	32.2±0.3	33.0±0.3	23.3±0.4	8.2±0.1	15.3±0.2	21.3±0.1	11.5±0.2
	MIC	125	7.8	7.8	7.8	62.5	250	62.5	250

*^{A,B,C}: Different uppercase superscript letters in the same column indicate that the means differ significantly ($p < 0.05$) in all extracts. ^{a,b,c}: Different lowercase letters in the same column indicate that the means differ significantly ($p < 0.05$) in the classical and ultrasonic extract groups themselves. ** CWE; classical water extract, UWE; ultrasonic water extract, CEWE; classical ethanol: water extract, UEWE; ultrasonic ethanol: water extract, CMWE; classical ethanol: water extract, UMWE; ultrasonic methanol: water extract, CHE; classical n-hexane extract, UHE; ultrasonic n-hexane extract, CCE; classical chloroform extract, UCE; ultrasonic chloroform extract, EO: essential oil, WD; inhibition zone (diameter in mm) around the disks (5 mm) filled with 100 µl extracts. *** –; Not active. ****A; Antibiotics: Ofloxacin (µg mL^{-1}) was used as the reference antibiotic for bacteria, amphotericin B (µg mL^{-1}) was used as the reference antibiotic for fungi. *****MIC values are given in µg mL^{-1}.

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processing methods. Some studies have shown that some *Ferula* species, such as *F. glauca, F. heuffelii,* and *F. assafoetida*, also exhibit antimicrobial activity against Gram (+) bacteria (Maggi et al., 2009; Pavlović et al., 2012; Samadi et al., 2016). In previous reports, it was determined that Gram (-) bacteria are less sensitive to essential oils than Gram (+) bacteria (Burt, 2004; Sahin et al., 2004; Hussain et al., 2010), which is mainly related to the structure of the cell walls of Gram (-) bacteria. These cell walls contain lipoprotein and lipopolysaccharides that act as a barrier against hydrophobic compounds (Inouye et al., 2001).

The antifungal potential of the essential oil is presented in Figure 2. Compared with the size of the amphotericin B zone (the reference antibiotic for fungi used as a positive control), the inhibition zone of essential oil is smaller for *P. roqueforti* (WD: 10.3 mm, MIC: 500 µg mL^{-1}) and similar for *A. niger* (WD: 21.3 mm, MIC: 62.5 µg mL^{-1}). Early studies have reported the antimicrobial activity of some *Ferula* species against *A. niger* (Al-Ja'fari et al., 2011; Kavoosi and Rowshan, 2013; Divya et al., 2014). In addition, the essential oil caused the larger inhibition zone against the pathogenic yeast *C. albicans* (WD: 13.3 mm) than against the probiotic yeast *S. cerevisiae* (WD: 6.7). These findings are consistent with those of Karakaya et al. (2019), Zomorodian et al. (2018), and Iranshahi et al. (2008), who reported anti-*Candida* activity of essential oils from fresh *F. orientalis*, *F. assafoetida* oleo-gum-resin, and *F. latisecta* fruits, respectively.

The ultrasonic and classical water, ethanol: water, methanol: water, n-hexane and, chloroform extracts of pickled *F. orientalis* demonstrated varying antibacterial and antifungal activity, as shown in Table 1. The solvent diversity and extraction method (ultrasonic or classical) was statistically effective on the inhibition zones of Gram (-) bacteria at $p < 0.01$ and $p < 0.05$ levels, respectively. Chloroform and n-hexane extracts showed an antibacterial effect against tested Gram (-) strains (*E. coli* and *P. aeruginosa*), even though at low levels (with 500 µg mL^{-1} MIC values); however, none of the classical and ultrasonic water, ethanol: water or methanol: water extracts caused inhibition zones against this group of bacteria. The results do not agree with those of studies that reported weak antimicrobial activity of *F. orientalis* methanol extracts against *E. coli* and *P. aeruginosa* (Ceylan et al., 2019; Zengin et al., 2020). The differences between our data and those of the above-mentioned studies may be explained by changes in the bioactive compositions of the extracts based on two reasons. First, although the same plant was studied, the collection time, the collection area, and the procedures applied before the extraction processes were not the same. Second, 50% methanol: water was used as a solvent in the present study, while absolute methanol was used in the others.

The chloroform and n-hexane extracts were not effective on Gram (+) strains (Table 1). Ethanol: water and methanol: water extracts showed moderate antimicrobial activity against *S. aureus*. The highest inhibition zone was 12.7 mm in the classical and ultrasonic water: ethanol extracts. Solvent diversity significantly affected the antimicrobial activity of Gram (+) strains ($p < 0.01$) but the extraction method (classical or ultrasonic) was ineffective ($p > 0.05$). Several studies have proved the moderate or strong inhibitory activity of different extracts of *Ferula* species, including *F. orientalis*, against *S. aureus* (Bhatnager et al., 2015; Pavlović et al., 2015; Ceylan et al., 2019; Kahraman et al., 2019).

The effect of extracts on *S. mutans* is quite impressive. Among all test microorganisms, classical and ultrasonic water extracts formed inhibition zones (12.7 and 11.7 mm, respectively) against only *S. mutans*. The highest antimicrobial effect exhibited against *S. mutans* belongs to ultrasonic ethanol: water extract (WD: 17.5 mm and MIC: 31.25 µg mL^{-1}). *Streptococcus mutans* is the most important bacteria in the human oral cavity because it produces acid by sticking to the tooth enamel and causing the ambient pH to drop to <5.5. The oral acidic pH leads to demineralization of dental enamel and subsequent tooth decay (Cakir et al., 2010). The high antimicrobial activity of even the water extract of pickled *F. orientalis* (MIC: 125 µg mL^{-1}) suggests that those who consume the pickle may have less tooth decay. Unlike the present study, Fani et al. (2015) have found that water and ethanol extracts of *F. assafoetida* are effective against *S. mutans* at a low level (MIC: 12.5 mg mL^{-1}) , while Shamshad and Chaurasia (2016) have found that methanolic extracts are effective at a moderate level (4–10 mm).

Solvent diversity had a very significant effect on yeasts at $p < 0.01$. Nonpolar extracts (chloroform and n-hexane) showed moderate activity against tested yeast strains at 500 µg mL^{-1} MIC. These extracts caused inhibition zones within the range of 10.7–11.3 mm for *C. albicans* and 5.7–6.2 mm for *S. cerevisiae*. *C. albicans* is the most sensitive yeast strain to nonpolar extracts. The ultrasonic chloroform extract resulted in the highest inhibition zone against this strain, while no inhibition was observed in its classical extract. This effect may be the result of the different compound(s) extracted using ultrasound; however, further analyses are needed to clarify this presumption. All extracts, except those prepared with water, exhibited a strong antifungal effect (WD: 10.7–16.3 mm and MIC: 125–500 µg mL^{-1}) against *A. niger*; whereas, only chloroform and hexane extracts caused an inhibition zone between 10.7 and 14.7 mm (MIC: 500 µg mL^{-1}) against *P. roqueforti*. Amphotericin B was active against all fungal strains with the inhibition zones and MIC values of 8.2–21.3 mm and 62.5–250 µg mL^{-1}, respectively.

Several studies on the antifungal properties of extracts from different *Ferula* species against the tested strains have been published; however, their results are quite different from each other. Bashir et al. (2014) have reported that methanolic extracts of roots, aerial parts, and subsequent fractions of *F. narthex* had no effect on *C. albicans*. According to Kahraman et al. (2019), the chloroform and methanol extracts of *F. caspica* were effective against *C. albicans* at 256 µg mL^{-1}, while the ethanol extracts exhibited an inhibition at 128 µg mL^{-1}. Al-Ja'fari et al. (2013) have detected antifungal activity in hexane, dichloromethane, methanol, water, and decoction extracts of *F. hermonis* against *S. cerevisiae*, *C. albicans*, and *A. niger* at >256 µg mL^{-1}. Zia-Ul-Haq et al. (2011) have stated that ethanol extracts of *F. assafoetida* show moderate antimicrobial activities against *A. niger* and *S. cerevisiae*. Ceylan et al. (2019) have reported that methanol extracts of *F. orientalis* leaves and stems show high antifungal effects against different Candida spp. (e.g., *C. albicans*, *C. glabrata*, and *C. krusei*) (MIC: 125 µg mL^{-1}). The methanol extracts of *F. rigidula* and *F. orientalis* had inhibitory effects against *A. niger* at MIC levels of 0.37 and 0.75 mg mL^{-1}, respectively (Zengin et al., 2020). According to Ibraheim et al. (2012), the antifungal activity exhibited by some *Ferula* species may be related to compounds of phenols, flavonoids, and sesquiterpenes in the extracts.

Conclusions

The aim of the present study was to determine the antimicrobial effects of extracts obtained from pickled *F. orientalis* using different solvents and different extraction methods on eight microorganisms consisting of Gram (+) and Gram (-) bacteria, yeasts, and molds. Our results showed that the cell wall structures of the microorganisms, solvents, and extraction methods were important factors in antimicrobial activity. The water, ethanol: water and methanol: water extracts were more effective on Gram (+) bacteria, while the chloroform and n-hexane extracts inhibited higher levels of Gram (-) bacteria. The essential oil created inhibition zones at different levels against all tested microorganisms, with *A. niger* being the most sensitive strain. The solvent diversity had a significant effect on the inhibition zones of all microorganisms ($p < 0.01$), while the extraction method (ultrasound or classical) was effective on *E. coli*, *P. aeruginosa*, *P. roqueforti*, *A. niger*, and *C. albicans* ($p < 0.05$). The extracts and essential oil exhibited varying levels of antimicrobial activity on all tested microorganisms, while their effects against *S. mutans* was interestingly higher. In particular, the ethanol: water extracts more significantly inactivated *S. mutans* than the other extracts. We suggest that this property of the plant, determined under *in vitro* conditions, be studied under in vivo conditions as a natural antibiotic to prevent tooth decay.

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Conflict of interest

The authors declare no conflict of interest with respect to the research, authorship, and/or publication of this article.

Credit author statement

Elif Feyza Topdas: Investigation, methodology, writing – original draft, review & editing. **Memnune Sengul:** Supervision, project administration, writing – review & editing. **Bulent Cetin:** Guiding, review & editing.

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