OnLine Journal of Biological Sciences

Research Article

Phytochemical Composition, Antibacterial, and Antioxidant Properties of *Foeniculum vulgare* Essential Oil

[1]Saif M. Dmour, [1]Saqr Abushattal, [1]Sultan Ayesh Mohammed Saghir, [1]Sulaiman Mohammed Alnaimat, [2]Haitham Qaralleh, [2]Ahmad Mohammed Al-Jaafreh and [3]Eid Musa Alsbou

[1]Department of Medical Analysis, Princess Aisha Bint Al-Hussein College of Nursing and Health Sciences, Al-Hussein Bin Talal University, Ma'an, Jordan
[2]Department of Medical Laboratory Sciences, Mutah University, Mutah, Jordan
[3]Department of Chemistry, Science College, Al-Hussein Bin Talal University, Ma'an, Jordan

Article history
Received: 20-11-2024
Revised: 23-12-2024
Accepted: 26-12-2024

Corresponding Author:
Saif M. Dmour
Department of Medical Analysis, Princess Aisha Bint Al-Hussein College of Nursing and Health Sciences, Al-Hussein Bin Talal University, Ma'an, Jordan
Email: seif.aldmour@ahu.edu.jo

Abstract: This study investigates the phytochemical composition and bioactivity of Fennel (*Foeniculum vulgare*) Seed Essential Oil (FSEO) from Jordan. The essential oil, extracted via hydrodistillation from air-dried samples, yielded 4.42% (v/w). Gas Chromatography-Mass Spectrometry (GC-MS) analyses identified 20 components, constituting 98.5% of the oil. Estragole and Anethole emerged as the predominant oxygenated monoterpenes, comprising 47.15% and 36.2%, respectively. Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) assays were conducted to assess the antimicrobial potential of FEO against various strains, with MIC90 values ranging from 2.13 mg/ml to 2.76 mg/ml. Notably, FEO exhibited significant activity against MRSE and S. aureus. Furthermore, FEO demonstrated substantial phenolic content (39.59 ± 0.062 mg GAE/g dry plant material), flavonoid content (20.95±0.146 mg CE/g dry extract), and tannin concentration (7.15±0.1 mg QE/g dry extract). Antioxidant efficacy, evaluated using DPPH and ABTS assays revealed dose-dependent scavenging activity, with IC50 values of 126.05±1.01 µg/mL and 49.24±1.02µg/mL, respectively. These findings highlight the potential of FSEO as an antimicrobial and antioxidant agent.

Keywords: MRSA, DPPH, GC-MS, MIC, *Foeniculum vulgare*, Estragole

Introduction

Currently, one of major health challenges facing society today include the rising incidence of bacterial infections, the emergence of new infectious diseases, and the growing spread of antibiotic resistance. (Velez and Sloand, 2016). Microbial diseases account for millions of deaths globally each year (Murray *et al.*, 2022). In recent decades, there has been a growing interest in discovering new and safe antimicrobial molecules derived from natural sources, particularly plants. Consequently, scientists have focused on developing natural, safe, and effective antimicrobial agents. Essential Oils (EO) have numerous traditional uses, including their antimicrobial activity as both topical and systemic treatments for bacterial and fungal infections (Girge *et al.*, 2024).

According to the World Health Organization, 88% of the global population utilizes plants for medicinal purposes (Sen *et al.*, 2011). As a result, the demand for these plants has surged, prompting an expansion in their production. In Mediterranean countries, fennel (*F. vulgare*), commonly referred to as Shomar, is a significant medicinal and therapeutic plant from the Apiaceae family. It is renowned for its anti-inflammatory, hepatoprotective, and antioxidant properties (Milenković *et al.*, 2022, Muheyuddeen *et al.*, 2023), antifungal, antithrombotic, anti-tumor (Lauricella *et al.*, 2022), antidiabetic (El-Soud *et al.*, 2011), and antibacterial activity (Belabdelli *et al.*, 2020). *F. vulgare* Seed Essential Oil (FSEO) exhibits these therapeutic potentials due to its volatile components (Milenković *et al.*, 2022).

F. vulgare is extensively cultivated in temperate and tropical regions for its aromatic fruit and EO which are used across various industries as flavoring agents and antimicrobials in food, medicine, cosmetics, and beverages (Alzweiri *et al.*, 2011). All plant parts including stem, fruit, leaves, seeds, and the entire plant, can be utilized medicinally to address different diseases (Sayed-Ahmad *et al.*, 2017). *F. vulgare* is believed to have potential therapeutic effects on various ailments, such as cancer, arthritis, mouth ulcers, hypertension, high cholesterol, liver discomfort, irritable bowel syndrome, and kidney issues (Al-Amoudi, 2017).

Recently, there has been significant exploration into the antimicrobial attributes of EO, uncovering their capacity to impede the growth and spread of antibiotic-resistant pathogenic microorganisms. This antimicrobial activity suggests that EO could serve as an alternative to antibiotic therapy (Zhao *et al.*, 2023). For instance, literatures shows that tea tree oil has demonstrated anti-bactericidal effect against the Methicillin-Resistant *Staphylococcus Aureus* (MRSA), *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *E. coli*, and *L. monocytogenes* (Oliva *et al.*, 2018; Shi *et al.*, 2018). The EO obtained from Apiaceae family is highly regarded due to their abundance of secondary metabolites such as sesquiterpene hydrocarbons, oxygenated sesquiterpenes, phenylpropanoids, and aliphatic compounds (Joshi, 2013). Additionally, these oils contain other compounds such as monoterpene hydrocarbons, oxygenated monoterpenes, aromatic monoterpenes, and oxygenated sesquiterpenes, which exhibit a range of biological activities, including hepatoprotective, antimicrobial, vasorelaxant, apoptosis-inducing, and anti-tumor effects (Ben Abdesslem *et al.*, 2021; Milenković *et al.*, 2022).

Reports indicated that transanethole, estragol, fenchone, and α-phellandrene constitute the primary constituents found in FSEO (Abdelbaky *et al.*, 2023). The extraction method and plant origin significantly influence the variation in chemical content. Volatile compounds can accumulate in different parts of plants, including flowers, fruits, leaves, roots, and stems (Riabov *et al.*, 2020).

Furthermore, limited data exist regarding the antioxidant activity and main components of Jordanian FSEO extract. Therefore, this study seeks to examine the chemical composition and evaluate the antioxidant and antibacterial properties of FSEO from Jordan.

Materials and Methods

Plant Material

F. vulgare seeds were purchased from a local herbal shop and visually inspected for quality. The seeds were then ground into a fine powder using a laboratory electric mill, ensuring uniform particle size. The powder was sieved to eliminate any coarse particles and then stored in labeled glass jars with airtight seals to preserve freshness. The jars were kept in a cool, dark environment to ensure the powder's quality until it was needed for further experiments.

Essential Oil Extraction

The FSEO from seeds was obtained as described previously with minor modifications (Shaapan *et al.*, 2021, Kulkarni *et al.*, 2024). In summary, 200 grams of dried *F. vulgare* seeds powder were steam-distilled for 6 h in steam distiller (Modified Clevenger apparatus) to acquire FSEO. The layer of EO on the upper aqueous phase was extracted with n-hexane, dried using sodium sulfate (Na_2SO_4), and stored at 4 °C.

Validation and Identification of EOAH using GC-MS

In the current study, FSEO were analyzed using a Shimadzu QP2010 quadrupole GCMS (Kyoto, Japan) apparatus equipped with split-splitless inlet (S/SL) injector with electron impact (EI, 70 eV) using 40–400 m/z range. The gas chromatography column used was DB-SMS. The injector's temperature was adjusted to 250 °C and its split ratio was 1:10. The temperatures of the detector and transfer line were 160 and 230°C, respectively. To segregate distinct oil components, a temperature gradient was implemented using a linear program. The temperature was set to increase at a rate of 4°C per minute, starting from an initial temperature of 50°C and reaching a final temperature of 290°C. Additionally, the temperature was maintained at 50°C for duration of 5 minutes, resulting in a total runtime of 68 minutes and 25 seconds (Alsohaili and Al-Fawwaz, 2014).

Identification of Compounds Using GC-MS

Utilizing the GC peak area to determine the percentage composition of the essential oil, we identified and characterized isolated compounds from FSEO. The mass spectrum was interpreted by referencing the database of the National Institute of Standard and Technology (NIST).

Compound identification were achieved through retention time, MS were matched with the NIST library and published reports to confirm essential oil constituents. When feasible the specific standard compounds was carried out under the same chromatographic conditions (Adams, 2017).

Assessment the Antimicrobial Activity of FSEO

The effectiveness of the essential oil against five pathogenic bacterial strains was evaluated. Three standard strains included *E. coli* ATCC 25922, *K. pneumonia* ATCC 200603, and S. aureus ATCC 29213, which Jordan University Hospital in Amman, Jordan supplied. Additionally, two clinical isolates, Methicillin-resistant *Staphylococcusaureus* (MRSA, GenBank accession number OQ568766) and methicillin-resistant *Staphylococcus epidermidis* (MRSE, OQ568719), have been incorporated.

Preparation of Bacterial Suspension

The stock cultures of bacteria were sub-cultured onto nutrient agar, and incubation tonight at 37 °C. Subsequently, 3-5 bacterial colonies were introduced into 5 ml nutrient broth and standardized to achieve the desired cell density of 1.5×108 cells/ml using the 0.5 McFarland turbidity standards methods and a spectrophotometer set at 600 nm.

Evaluation of MIC and MBC of FSEO

The MIC and MBC of FSEO were determined by modifying the broth dilution method in the referenced

study (Bostanci *et al.*, 2022).

Determination of Phenolic, Flavonoid and Tannin Contents

Total phenolic content (TPC) in the FSEO was screened by Folin-ciocalteau reagent (mg of gallic acid/g of the FSEO) (McDonald *et al.*, 2001). On the other hand, total flavonoid content (TFC) of FSEO was measured by the modified aluminum chloride technique (Isla *et al.*, 2001; Saghir *et al.*, 2016). Additionally, Total Tannin Content (TTC) was measured as reported previously (Polshettiwar, Ganjiwale *et al.* 2007).

Radical Scavenging Assay using ABTS and DPPH

For the DPPH assay, it was carried out following previously published study with minor changes (Abduh *et al.*, 2023). DPPH was diluted to create 0.94-30µl/mL concentrations and 100µL of each was added to 96 well plate. 100µL of FSEO was added to each concentration. These mixtures were kept at room temperature in darkness. Following a 30-minute incubation period, the absorbance of the mixtures was measured at 517 nm using a spectrophotometer. Gallic acid, known for its robust antioxidant activity, was used as a standard reference. A calibration curve was created using gallic acid concentrations ranging from 10-100µg/ml. Each determination was carried out in triplicate**.** The subsequent equation was utilized to ascertain the DPPH and ABTS radical's percentage inhibition:

$$\%\ inhibition = \frac{Abs\ C - Abs\ S}{Abs\ C} \times 100$$

Where, Abs C = control, and Abs S = sample.

The ABTS test was achieved following Abu-Yamin, *et al.* (2022) (Stratil *et al.*, 2006; Abu-Yamin *et al.*, 2022). Initially, 7 mM aqueous ABTS solution and a 2.45 mM potassium persulfate (K2S2O8) solution at a ratio of 1:136 were mixed and incubated at room temperature for 16 hours. Subsequently, the solution was diluted with phosphate buffer solution until an absorbance of 0.700±0.02 units at 734 nm was achieved. The diluted FSEO or Trolox solution (10–100µg/mL) was then added to 180 µL of the ABTS solution in a total volume of 200 µL. After six minutes, the absorbance was measured at 734 nm using a microplate reader.

Results

Chemical Composition of FSEO

The FSEO was extracted through the hydrodistillation of air-dried samples, resulting in 4.42% (v/w) yield. GC and GC-MS were used for the chemical investigation of the FSEO and the findings are presented in Table 1. A total of 20 components were identified, accounting for 98.5% of the total composition. Estragole, classified as an oxygenated monoterpene, was identified as the predominant component, comprising 47.15% of the oil. Anethole was the second major oxygenated monoterpene detected in the fennel oil, representing 36.2% of the composition. Other components were found to be minor constituents in the essential oil of fennel seeds. Regarding the groups of chemical constituents represented, Estragole (47.15%) and Anethole (36.2%) were the main oxygenated monoterpenes.

Table 1: Chemical compositions of essential oil identified from FSEO using GC-MS

Peak	Compound	Ki Cal	KI Let	%
1	β-Pinene	981	973-981	0.32
2	Limonene	1033	995-1035	0.4
3	Fenchone	1088	1086-1092	0.72
4	Camphor	1148	1142	0.44
5	Estragole	1159	1158-1199	47.15
6	Fenchyl acetate	1221	1217-1240	0.52
7	Benzaldehyde, 4-(1-methylethyl)	1233	1226-1257	0.91
8	p-Anisaldehyde	1253	1244-1277	1.25
9	Carvone	1246	1231-1249	3.3
10	Anethole	1282	1247-1284	36.2
11	p-Menth-8-en-3-ol, acetate	1287	1263-1281	0.83
12	Eugenol methyl ether	1405	1395-1415	0.58
13	Caryophyllene	1420	1409-1423	0.86
14	α-Curcumene	1488	1472-1500	0.92
15	β-Bisabolene	1505	1507-1509	0.72
16	β-Sesquiphellandrene	1524	1519-1525	0.7
17	trans-4-Methoxycinnamaldehyde	1570	1569	0.73
18	Apiol	1673	1674-1685	1.04
19	n-Hexadecanoic acid	1977	1970-1977	0.51
20	6-Octadecenoic acid	2070	2073	0.4
	Total			98.5
	Oxygenated monoterpenoids			89.45
	Monoterpene hydrocarbons			0.72
	Sesquiterpene hydrocarbon			3.2
	Other			5.13

Antimicrobial Activity

To assess the antibacterial potential of FSEOs, MIC and MBC values of the FSEO are presented in Table 2. MIC_{90} values for FSEO against 5 strains are ranged from 2.13 mg/mL to 2.76 mg/mL and the controls Cefotaxime from <0.0038 to 0.625 mg/ml and Maxil from <0.0025 to 0.625 mg/ml. Table 2 shows that FEO was the most active against *MRSE* and *S. aureus*, with MIC90 values of 2.13, 2.24 mg/mL, respectively. MBC values of the FSEO varied from 7.5 mg/ml to 30 mg/ml and the controls Cefotaxime from <0.0038 to 0.625 mg/ml and Maxil from 0.019 to 2.5 mg/ml. Specifically, FSEO demonstrated the lowest MIC value of 2.13 mg/mL against MRSE. The MIC and MBC values against MRSE ranged from 2.13 to 30 mg/mL, respectively. Regarding *S. aureus*, FSEO exhibited MIC and MBC values of 2.24 and 30 mg/mL, respectively. For *E. coli*, the MIC and MBC values for FSEO were 2.52 and 7.5 mg/mL, respectively. FSEO displayed MIC and MBC values against MRSA of 2.76 and 30 mg/mL, respectively, while for *K. pneumonia*, these values were 2.76 and 15 mg/mL, respectively.

Table 2: Minimum inhibitory and minimum bactericidal concentrations of FSEO against controls. Data are presented as mean ± SD of three replicates (n=3)

Bacterial Name	Essential Oil (mg/ml)		Cefotaxime (mg/ml)		Maxil (mg/ml)	
	MIC_{90}	MBC	MIC_{90}	MBC	MIC_{90}	MBC
S. aureus	2.24±0.33	30±7.07	0.004±0.00	0.008±0.00	0.003±0.00	0.019±0.01
E.coli	2.52±0.25	7.5±3.54	0.004±0.00	0.004±0.00	0.019±0.01	0.039±0.02
K. pneumonia	2.76±0.34	15±3.54	0.016±0.00	0.125±0.06	0.625±0.06	1.25±0.47
MRSA	2.76±0.55	30±7.07	0. 625±0.07	0. 625±0.25	0.039±0.01	0.078±0.02
MRSE	2.13±0.27	30±7.07	0.1250.02	0.25±0.06	1.25±0.44	2.5±1.12

MIC_{90} concentration (mg/ml) that inhibited the growth of 90% of the strains

Total Phenol, Flavonoid, Tannin Contents and Antioxidant Activities of FSEO

The results indicated that medicinal plants are abundant in secondary metabolites, including flavonoids, phenolic compounds, and tannins. Quantification of these compounds in FSEO was conducted by comparing absorbance values with standard solutions (Table 3). The FSEO exhibited TPC of 39.59±0.062 mg GAE/g and TFC was found to be 20.95±0.146 mg CE/of dry plant material. Moreover, TTC was measured to be 7.15±0.1 mg QE/g dry extract.

The antioxidant potential of FSEO was evaluated *in vitro* using DPPH and ABTS radical scavenging assays. These well-established methods are preferred for their reliability and the stability of the DPPH and ABTS radicals, allowing for a rapid assessment of antioxidant efficacy. Trolox and gallic acid were employed as standard positive controls for the ABTS and DPPH assays, respectively. The essential oils demonstrated significant antioxidant activity, with greater activity correlating with lower half-maximal inhibitory activity (IC_{50}) values. FEO exhibited notable antioxidant efficacy compared to gallic acid and trolox, as determined by the DPPH and ABTS techniques. The scavenging activity increased with higher concentrations of FEO. In contrast to the reference antioxidants gallic acid (IC_{50}=50.91µg/mL) and Trolox (IC_{50}=34.01µg/mL), FEO exhibited potent inhibition of DPPH and ABTS free radical concentrations, with IC_{50} values of 126.05±1.01µg/mL and 49.24±1.02µg/mL, respectively.

The reduction in DPPH absorbance was found to be dose-dependent. At a concentration of 0.1 mg/mL of fennel seed essential oil, the inhibition rates of DPPH and ABTS free radicals reached 44.88% and 63.13%, respectively, while gallic acid and trolox demonstrated reductions of 79.64% and 99.5%, respectively, at the same concentration.

Discussion

This research focused on assessing Jordanian medicinal and aromatic plants to uncover novel bioactive compounds effective against various microbial diseases. In recent studies, researchers have highlighted that certain monoterpene or sesquiterpene hydrocarbons and their oxygenated derivatives, which constitute the primary constituents of essential oils, display promising antimicrobial properties (Naksang *et al.*, 2020). These results strongly align with the outcomes of this study, as the FSEO was also found to encompass these components, affirming its effectiveness as a natural antimicrobial agent. Chromatographic analysis revealed the occurrence of sixteen volatile compounds representing 98.5% of the total amount of extracted essential oil. The main volatile active compounds present in FSEO were estragole (47.15%) and Anethole (36.2%) demonstrated notable antimicrobial activity. Moreover, it demonstrates anti-inflammatory and anti-carcinogenic characteristic (Zhang *et al.*, 2018).

In the study, 4.42% of the essential oil yield was obtained from FSEO. Our finding are higher than those found by (Barrahi *et al.*, 2020; Ghasemian *et al.*, 2020) on fennel from Iran and Morocco, which are characterized by a low EO yield 1.77, 2.82% respectively. Another study reported the yield FSEO from Egypt and China was varied and found to be 1.6% and 1.1%, respectively (Ahmed *et al.*, 2019). The primary source of variability in chemical composition and oil yield among the various populations of FSEO is differences in environmental conditions (Abdellaoui *et al.*, 2020).

Lastly, the EO yields for various species in Tunisian regions exhibit considerable variation depending on the plant's origin which was ranged from 1.2% to 5.06% (Khammassi *et al.*, 2018). The findings of the current study encompass both the lowest and highest reported values within this spectrum.

The yield, content, and chemical composition of EO are all determined by a range of factors, including species, geographical origin, environmental conditions (Piccaglia and Marotti, 2001), extraction techniques, drying methods, cropping practices, and the age of the plant material (Hammouda *et al.*, 2014).

A literature search showed that the EOs content from Morocco FSEO populations ranged from 2.7 to 4% (Abdellaoui *et al.*, 2020). One study of Anwar *et al.*, (2009) founded that the yield of FSEO from Pakistan was 2.81% (Anwar *et al.*, 2009). Similarly, another study in Tunisia reported a yield of 0.54% for the FSEO (Kalleli *et al.*, 2019). Wild fennel from Serbia contained (E)-anethole (66.1–69.0%) and fenchone (13.3–18.8%)

as the main constituents (Radulović and Blagojević, 2010). Yugoslavian FSEO contained trans-anethole methyl chavicol and fenchone. Montenegro, FSEOs were rich in (E)-anethole (75.5%) and fenchone (13.7%) (Šunić *et al.*, 2023).

Researching the antibacterial activity of natural compounds like EOs and synthetic substances such as antibiotics on microbial pathogens holds significant interest. Consequently, In vitro antibacterial studies have been conducted on both commercial antibiotics and FSEO against human pathogens. As shown in Table 2, the antibacterial power of FSEO is demonstrated against *Staphylococcus aureus* ATCC 25293, MRSA, MRSE, *Escherichia coli* ATCC 10536, and *K. pneumonia*. MIC_{90} values for FSEO against 5 strains are ranged from 2.13 mg/mL to 2.76 mg/mL and the controls Cefotaxime from <0.0038 to 0.625 mg/ml and Maxil from <0.0025 to 0.625 mg/ml. This difference may be attributed to several factors. First, essential oils like FSEO are complex mixtures of bioactive compounds, each with its own antimicrobial properties. These compounds often work synergistically, but their collective antimicrobial effectiveness may be less potent than that of purified synthetic antibiotics, which are specifically designed and optimized for antimicrobial activity (Masoud *et al.*, 2022). In contrast, the reference antibiotics (Cefotaxime and Maxil) are single compounds with well-characterized and potent antimicrobial actions, which typically result in lower MIC values. Moreover, factors as the bioavailability, mode of action, and the mechanism by which EOs exert their antimicrobial effects may differ significantly from that of conventional antibiotics. While EOs have been demonstrated to possess broad-spectrum antimicrobial activity, their effectiveness can vary across different bacterial strains due to differences in cell wall structures and resistance mechanisms (Swamy *et al.*, 2016). Furthermore, essential oils like FSEO might require higher concentrations to achieve comparable inhibition, especially in the case of resistant or less susceptible strains (Nagy-Radványi *et al.*, 2024).

Kamilla *et al.*, (2018) found that MIC_{90} for Eucalyptus and Scots pine essential oil against S. pyogenes was 2.82 mg/ml and 1.35 mg/ml, respectively. Also, he showed that Eucalyptus EO agains *S. pneumoniae* was 1.41 mg/ml (Ács *et al.*, 2018). Probst *et al.* (2011) repoted that MIC_{90} value for Ginger EO against *E. coli* and *S. aureus* is 1.1920±0.75 mg/ml and 7.0523±3.13 mg/ml, respectively (Probst *et al.*, 2011).

Natural and cost-effective ways of treating infections can be achieved by using EOs. The use of EOs may be the most effective and cost-efficient method of treating infections. Table 2 presents the results of an investigation into the efficacy FSEO against *E. coli, K. pneumonia, S. aureus, MRSA, and MRSE.* Antimicrobial potential was assessed using the MIC for each sample.

This result indicated that FSEO exhibited strong inhibitory effects against all tested bacteria, encompassing both Gram-positive and Gram-negative bacteria. The results align with previous studies by Ben Abdesslem *et al.* (2021), which showed that FEO exhibited strong inhibitory potential against *E. coli* and *S. aureus* (Ben Abdesslem *et al.*, 2021; Sehar and Khan, 2021).

There are few studies available regarding the antimicrobial properties of FSEO, its ability to inhibit bacteria may be due to the existence of bioactive components, particularly trans-anethole and estragole, as previously documented (Miguel *et al.*, 2010). The antibacterial efficacy of FSEO may be due to its chemical profile, which is rich in major oxygenated monoterpene (estragole 47.15% and Anethole 36.2%) as presented in Table 1. A prior study reported that anethole and estragole were found to be the major components, but with high concentration of anethole (68.53%) and low concentration of estragole (10.42%) compared with the current study (Diao *et al.*, 2014). In addition, other study conducted in Pakistan showed high levels of anethol (69.87%), but low levels of estragole (5.45%) in comparison with the findings of the previous study (Anwar *et al.*, 2009). Moreover, very high concentrations of anethol (75.83) was observed in one study conducted in Italy (Tognolini *et al.*, 2007).

Compounds like these exhibit strong antimicrobial properties, as they interfere with the membrane integrity of bacteria, thereby inhibiting respiration and altering cell permeability (Andrade *et al.*, 2015; Abuskhuna *et al.*, 2020; Jaiswal, 2024).

Table 3: Total phenolic, flavonoids and tannins and antioxidant activity of FSEO

Test	Values
Total phenols (mg GAE/ g dry extract)	39.59±0.062
Total flavonoids (mg CE/ g dry extract)	20.95±0.146
Tannins (mg QE/g dry extract)	7.15±0.1 mg
DPPH (IC_{50})	126.05±1.01µg/mL
ABTS (IC_{50})	49.24±1.02µg/mL
Trolox	5.8±0.45µg/mL
Quercetin	3.2±0.32µg/mL
Gallic acid	3.65±0.74µg/mL

GAE: Gallic Acid Equivalent, CE: Catechin Equivalents, QR: Quercetin Equivalents

Depending on the plant component used, different amounts of total phenol, tannins and flavonoid content present. There is a wide range of phenolic compounds in plants, such as flavonoids, phenolic acids, and tannins (Tiwari and Patel, 2013; Zhang *et al.*, 2015). Due to their antioxidant, free radical scavenging, and metal chelating characteristics, which may have favorable effects on human health, these substances have drawn attention. Phenols and flavonoids are major antioxidant compounds with therapeutic and protective effects on human health (Samydurai and Saradha, 2016; Hafiz *et al.*, 2022). The use of agro-industry by-products as natural antioxidants and a source of phenolic and flavonoid compounds have

been reported. A total phenol and total flavonoid analysis were carried out on essential oil extracted from *F. vulgare* seeds. TPC, TFC, and tannins content of FSEO extracts are presented in Table 3, it contained high amount of TPC 39.59±0.062 mg GAE/g extract, TFC 20.95±0.146 mg RE /100 g, and TC content was 7.15±0.1 mg GAE/100g, respectively. Our study found that total phenols and flavonoids were higher than what were reported in Tunisia and French (Kalleli *et al.*, 2019).

Table 3 shows the antioxidant activities of FSEO. DPPH radical scavenging activity of Jordanian fennel seeds essential oils (IC_{50}=144.43µg/mL) presented lower antioxidant activities than gallic acid (IC_{50}=50.91µg/mL). Based on this study, FSEO scavenging capacity was 3 times higher than gallic acid. The present study was the first to identify the antioxidant activity of FSEO in Jordan as far as we know.

The antioxidant activity of FSEO in the current study was comparable to the previous study by Christova-Bagdassarian *et al.* (2013), which showed that Bulgarian fennel seed extract had a low antioxidant activity with an IC50 of 113.19 ml/L (Christova-Bagdassarian *et al.*, 2013). In comparison to our findings, both Tunisian and French fennel seed essential oils exhibited lower antioxidant activities. Among these, Tunisian fennel seed essential oils showed stronger antioxidant potential, with IC50 values of 444.2µg/ml for Bizerte, 491.12µg/ml for Korba, and 202.69µg/ml for Djerba. In contrast, French FSEO demonstrated weaker antioxidant activities, with IC50 values of 616.53µg/ml for Marseille and 580.97µg/ml for Villeneuve. A study conducted in Pakistan by Anwar *et al.* (2009) reported higher IC_{50} values for fennel seed (var. Dulce), with an IC50 of 32.32µg/ml, which was higher compared to the antioxidant activity observed in our findings (Kalleli *et al.*, 2019). Ahmed *et al.*, 2019, reported the Chinese FSEO showed high activity in DPPH radical than Egyptian FSEO with IC50 15.66µg/mL and 141.82µg/mL, respectively (Ahmed *et al.*, 2019).

Conclusion

This study focused on evaluating the antimicrobial and antioxidant properties of FSEO while also analyzing its chemical composition. The antimicrobial activity was tested using standardized assays to assess its effectiveness against diverse microbial strains. Antioxidant activities were measured through biochemical methods, including DPPH radical scavenging and ABTS assays, to determine its potential as a natural antioxidant. The chemical composition of the FSEO extract was characterized using GC-MS techniques, revealing the presence of several bioactive compounds with estragole (47.15%) and anethole (36.2%) as the major detected compounds. The results indicate that FSEO possesses notable antimicrobial and antioxidant properties, likely due to its abundant chemical constituents, particularly estragole (47.15%) and anethole (36.2%). These findings underscore the potential of FSEO as a natural antimicrobial and antioxidant agent, offering promising applications in the pharmaceutical and food industries.

Acknowledgment

The authors would like to acknowledge Al-Hussein Bin Talal University for providing facilities to achieve the current work.

Funding Information

No funding was received for conducting this study.

Author's Contributions

Saif Dmour and Saqr Abushattal: Designed the study, developed the methodology, performed the formal analysis, and wrote the first draft of the article.

Sultan Saghir and Sulaiman Alnaimat: Contributed to the methodology, co-wrote the first draft, and reviewed the chemical aspects of the study.

Haitham Qaralleh, Ahmad Al-Jaafreh, and Eid Alsbou: Contributed to methodology development, article selection, and overall manuscript structuring. They also participated in the final revision and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

References

Abdelbaky, A. S., Mohamed, A. M. H. A., Abd El-Mageed, T. A., Rady, M. M., Alshehri, F., El-Saadony, M. T., AbuQamar, S. F., El-Tarabily, K. A., & Al-Elwany, O. A. A. (2023). Bio-organic fertilizers promote yield, chemical composition, and antioxidant and antimicrobial activities of essential oil in fennel (*Foeniculum vulgare*) seeds. *Scientific Reports*, 13, 13935. https://doi.org/10.1038/s41598-023-40579-7

Abdellaoui, M., Bouhlali, E. D. T., Derouich, M., & El-Rhaffari, L. (2020). Essential oil and chemical composition of wild and cultivated fennel (*Foeniculum vulgare* Mill.): A comparative study. *South African Journal of Botany*, 135, 93-100. https://doi.org/10.1016/j.sajb.2020.09.004

Abduh, M. S., Saghir, S. A. M., Al Hroob, A. M., Bin-Ammar, A., Al-Tarawni, A. H., Murugaiyah, V., & Mahmoud, A. M. (2023). Averrhoa carambola leaves prevent dyslipidemia and oxidative stress in a rat model of poloxamer-407-induced acute hyperlipidemia. In *Frontiers in Pharmacology* (Vol. 14). https://doi.org/10.3389/fphar.2023.1134812

Abuskhuna, S. M., Zeglam, T. H., Fhid, O. N. R., & Jebril, A. O. (2020). Antibacterial Activity of Hydroxyimidazole Derivatives. *Asian Journal of Pharmacy and Technology*, 10(1), 7-10. https://doi.org/10.5958/2231-5713.2020.00002.1

Abu-Yamin, A.-A., Abduh, M. S., Saghir, S. A. M., & Al-Gabri, N. (2022). Synthesis, Characterization and Biological Activities of New Schiff Base Compound and Its Lanthanide Complexes. *Pharmaceuticals*, 15(4), 454. https://doi.org/10.3390/ph15040454

Ács, K., Balázs, V. L., Kocsis, B., Bencsik, T., Böszörményi, A., & Horváth, G. (2018). Antibacterial activity evaluation of selected essential oils in liquid and vapor phase on respiratory tract pathogens. *BMC Complementary and Alternative Medicine*, 18, 1-9. https://doi.org/10.1186/s12906-018-2291-9

Adams, R. P. (2017). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*.

Ahmed, A. F., Shi, M., Liu, C., & Kang, W. (2019). Comparative analysis of antioxidant activities of essential oils and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Egypt and China. *Food Science and Human Wellness*, 8(1), 67-72. https://doi.org/10.1016/j.fshw.2019.03.004

Al-Amoudi, W. M. (2017). Protective effects of fennel oil extract against sodium valproate-induced hepatorenal damage in albino rats. *Saudi Journal of Biological Sciences*, 24(4), 915-924. https://doi.org/10.1016/j.sjbs.2016.10.021

Alsohaili, S. A., & Al-Fawwaz, A. T. (2014). Composition and antimicrobial activity of Achillea fragrantissima essential oil using food model media. *European Scientific Journal*, *10*(30), 156-165.

Alzweiri, M., Sarhan, A. A., Mansi, K., Hudaib, M., & Aburjai, T. (2011). Ethnopharmacological survey of medicinal herbs in Jordan, the Northern Badia region. *Journal of Ethnopharmacology*, *137*(1), 27-35. https://doi.org/10.1016/j.jep.2011.02.007

Andrade, T. C. B., Lima, S. G. D., Freitas, R. M., Rocha, M. S., Islam, T., Silva, T. G. D., & Militão, G. C. G. (2015). Isolation, characterization and evaluation of antimicrobial and cytotoxic activity of estragole, obtained from the essential oil of croton zehntneri (euphorbiaceae). *Anais Da Academia Brasileira de Ciências*, *87*(1), 173-182. https://doi.org/10.1590/0001-3765201520140111

Anwar, F., Ali, M., Hussain, A. I., & Shahid, M. (2009). Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* Mill.) seeds from Pakistan. *Flavour and Fragrance Journal*, *24*(4), 170-176. https://doi.org/10.1002/ffj.1929

Barrahi, M., Esmail, A., Elhartiti, H., Chahboun, N., Benali, A., Amiyare, R., Lakhrissi, B., Rhaiem, N., Zarrouk, A., & Ouhssine, M. (2020). Chemical Composition and Evaluation of Antibacterial Activity of Fennel (*Foeniculum vulgare* Mill) Seed Essential Oil Against Some Pathogenic Bacterial Strains. *Caspian Journal of Environmental Sciences*, *18*(4), 295-307. https://doi.org/10.22124/cjes.2020.4276

Belabdelli, F., Piras, A., Bekhti, N., Falconieri, D., Belmokhtar, Z., & Merad, Y. (2020). Chemical Composition and Antifungal Activity of *Foeniculum vulgare* Mill. *Chemistry Africa*, *3*(2), 323-328. https://doi.org/10.1007/s42250-020-00130-x

Ben Abdesslem, S., Boulares, M., Elbaz, M., Ben Moussa, O., St-Gelais, A., Hassouna, M., & Aider, M. (2021). Chemical composition and biological activities of fennel (*Foeniculum vulgare* Mill.) essential oils and ethanolic extracts of conventional and organic seeds. *Journal of Food Processing and Preservation*, *45*(1), 15034. https://doi.org/10.1111/jfpp.15034

Bostanci, M. T., Bulbul, A. S., Celik, I. S., Kocabas, Y. Z., Burhan, H., Bayat, R., Sen, F., Zakariae, N., Esmaeili, R., Jafari, H., Karimi, F., & Karimi-Maleh, H. (2022). Investigation of antibacterial, antifungal, antibiofilm, antioxidant and anticancer properties of methanol extracts of Salvia marashica İlçim, Celep & Doğan and Salvia caespitosa Montbret & Aucher ex Benth plants with medicinal importance. *Chemosphere*, *288*, 132602. https://doi.org/10.1016/j.chemosphere.2021.132602

Christova-Bagdassarian, V., Bagdassarian, K. S., & Atanassova, M. (2013). Phenolic profile: antioxidant and antibacterial activities from the Apiaceae family (dry seeds). *Mintage J. Pharm. Med. Sci*, *2*(9), 26-31.

Diao, W.-R., Hu, Q.-P., Zhang, H., & Xu, J.-G. (2014). Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (Foeniculum vulgare Mill.). *Food Control*, *35*(1), 109-116. https://doi.org/10.1016/j.foodcont.2013.06.056

El-Soud, N. A., El-Laithy, N., El-Saeed, G., Wahby, M. S., Khalil, M., Morsy, F., & Shaffie, N. (2011). Antidiabetic Activities of *Foeniculum vulgare* Mill. Essential Oil in Streptozotocin-Induced Diabetic Rats. *Macedonian Journal of Medical Sciences*, *4*(2), 139-146. https://doi.org/10.3889/mjms.1857-5773.2011.0173

Ganjiwale, R., Wadher, S., Yeole, P., & Polshettiwar, S. (2007). Spectrophotometric estimation of total tannins in some ayurvedic eye drops. *Indian Journal of Pharmaceutical Sciences*, *69*(4), 574. https://doi.org/10.4103/0250-474x.36949

Ghasemian, A., Al-Marzoqi, A.-H., Mostafavi, S. K. S., Alghanimi, Y. K., & Teimouri, M. (2020). Chemical Composition and Antimicrobial and Cytotoxic Activities of *Foeniculum vulgare* Mill Essential Oils. *Journal of Gastrointestinal Cancer*, *51*(1), 260-266. https://doi.org/10.1007/s12029-019-00241-w

Girge, S. N., C. Kardile, V., M. Kawade, S., & A. Thorat, K. (2024). Evaluation of Anthelmintic Potential in Ethanolic and Methanolic Extracts of Saraca asoca Roxb. Bark. *Research Journal of Science and Technology*, *16*(3), 171-175. https://doi.org/10.52711/2349-2988.2024.00025

Hafiz, I., Novilia, L., Luthvia, L., Husna, N., & Fauzan Lubis, M. (2022). Antibacterial Activity of Water, Ethyl Acetate and n-Hexane Fractions of Pagoda leaves (Clerodendrum paniculatum L.) against Propionibacterium acnes. *Research Journal of Pharmacy and Technology*, *15*(6), 2521-2524. https://doi.org/10.52711/0974-360x.2022.00421

Hammouda, F. M., Saleh, M. A., Abdel-Azim, N. S., Shams, K. A., Ismail, S. I., Shahat, A. A., & Saleh, I. A. (2014). Evaluation Of The Essential Oil Of *Foeniculum vulgare* Mill (Fennel) Fruits Extracted By Three Different Extraction Methods By Gc/Ms. *African Journal of Traditional, Complementary and Alternative Medicines*, *11*(2), 277-279. https://doi.org/10.4314/ajtcam.v11i2.8

Isla, M. I., Nieva Moreno, M. I., Sampietro, A. R., & Vattuone, M. A. (2001). Antioxidant activity of Argentine propolis extracts. *Journal of Ethnopharmacology*, *76*(2), 165-170. https://doi.org/10.1016/s0378-8741(01)00231-8

Jaiswal, S. (2024). Antibacterial activity of Cinnamomum cassia against multiple drug resistant Klebsiella pneumoniae. *Research Journal of Pharmacology and Pharmacodynamics*, *16*(3), 256-259. https://doi.org/10.52711/2321-5836.2024.00044

Joshi, R. K. (2013). Artemisia capillaris: medicinal uses and future source for commercial uses from western himalaya of Uttrakhand. *Asian Journal of Research in Pharmaceutical Science*, *3*(3), 137-140.

Kalleli, F., Bettaieb Rebey, I., Wannes, W. A., Boughalleb, F., Hammami, M., Saidani Tounsi, M., & M'hamdi, M. (2019). Chemical composition and antioxidant potential of essential oil and methanol extract from Tunisian and French fennel (*Foeniculum vulgare* Mill.) seeds. *Journal of Food Biochemistry*, *43*(8), e12935. https://doi.org/10.1111/jfbc.12935

Khammassi, M., Loupassaki, S., Tazarki, H., Mezni, F., Slama, A., Tlili, N., Zaouali, Y., Mighri, H., Jamoussi, B., & Khaldi, A. (2018). Variation in essential oil composition and biological activities of *Foeniculum vulgare* Mill. populations growing widely in Tunisia. *Journal of Food Biochemistry*, *42*(3), e12532. https://doi.org/10.1111/jfbc.12532

Kulkarni, V., Jain, N., Shinde, R., & Kamble, S. (2024). Formulation and Evaluation of Antimicrobial Gels using Essential Oils. *Asian Journal of Pharmaceutical Research*, *14*(2), 129-132. https://doi.org/10.52711/2231-5691.2024.00022

Lauricella, M., Maggio, A., Badalamenti, N., Bruno, M., D'Angelo, G., & D'Anneo, A. (2022). Essential oil of*Foeniculum vulgare*subsp.*piperitum*fruits exerts an anti-tumor effect in triple-negative breast cancer cells. *Molecular Medicine Reports*, *26*(1), 243. https://doi.org/10.3892/mmr.2022.12759

Masoud, W., Sharoba, A., Eldesouky, A., & Osheba, A. (2022). Antimicrobial Activity of Nanomaterial and Essential Oil against Foodborne Pathogen on Fish Fillets during Cryopreservation. *Egyptian Journal of Food Science*, *50*(2), 173-189. https://doi.org/10.21608/ejfs.2022.150252.1135

McDonald, S., Prenzler, P. D., Antolovich, M., & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry*, *73*(1), 73-84. https://doi.org/10.1016/s0308-8146(00)00288-0

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., Han, C., Bisignano, Catherine, Rao, P., & Wool, E. (2022). Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis. *The Lancet*, *399*(10325), 629-655. https://doi.org/10.1016/S0140-6736(21)02724-0

Miguel, M. G., Cruz, C., Faleiro, L., Simões, M. T. F., Figueiredo, A. C., Barroso, J. G., & Pedro, L. G. (2010). *Foeniculum vulgare* Essential Oils: Chemical Composition, Antioxidant and Antimicrobial Activities. *Natural Product Communications*, *5*(2). https://doi.org/10.1177/1934578x1000500231

Milenković, A., Ilić, Z., Stanojević, L., Milenković, L., Šunić, L., Lalević, D., Stanojević, J., Danilović, B., & Cvetković, D. (2022). Essential Oil Yield, Composition, Antioxidant and Microbial Activity of Wild Fennel (*Foeniculum vulgare* Mill.) from Monte Negro Coast. *Horticulturae*, *8*(11), 1015. https://doi.org/10.3390/horticulturae8111015

Muheyuddeen, G., Husain Rayini, S., Yadav, P., & Kumar Gupta, S. (2023). In vivo Analgesics and in vitro Antioxidants Activity of Newly Synthesized Mannich Bases of Lawsone. *Asian Journal of Pharmaceutical Research*, *13*(1), 11-17. https://doi.org/10.52711/2231-5691.2023.00002

Nagy-Radványi, L., Ormai, E., Koloh, R., Ángyán, V. D., Kocsis, B., Bencsik-Kerekes, E., Szabó, P., Csikós, E., Farkas, Á., Horváth, G., Kocsis, M., & Balázs, V. L. (2024). Biofilm Inhibition Activity of Fennel Honey, Fennel Essential Oil and Their Combination. *Microorganisms*, *12*(11), 2309. https://doi.org/10.3390/microorganisms12112309

Naksang, P., Tongchitpakdee, S., Thumanu, K., Oruna-Concha, M. J., Niranjan, K., & Rachtanapun, C. (2020). Assessment of Antimicrobial Activity, Mode of Action and Volatile Compounds of Etlingera pavieana Essential Oil. *Molecules*, *25*(14), 3245. https://doi.org/10.3390/molecules25143245

Oliva, A., Costantini, S., De Angelis, M., Garzoli, S., Božović, M., Mascellino, M., Vullo, V., & Ragno, R. (2018). High Potency of Melaleuca alternifolia Essential Oil against Multi-Drug Resistant Gram-Negative Bacteria and Methicillin-Resistant Staphylococcus aureus. *Molecules*, *23*(10), 2584. https://doi.org/10.3390/molecules23102584

Piccaglia, R., & Marotti, M. (2001). Characterization of Some Italian Types of Wild Fennel (*Foeniculum vulgare*Mill.). *Journal of Agricultural and Food Chemistry*, *49*(1), 239-244. https://doi.org/10.1021/jf000636+

Probst, I., Sforcin, J., VLM, R., Fernandes, A., & Fernandes Júnior, A. (2011). Antimicrobial activity of propolis and essential oils and synergism between these natural products. *Journal of Venomous Animals and Toxins Including Tropical Diseases*, *17*(2), 159-167. https://doi.org/10.1590/s1678-91992011000200006

Radulovic, N., & Blagojevic, P. (2010). A note on the volatile secondary metabolites of *Foeniculum vulgare* Mill. (Apiaceae). *Facta Universitatis - Series: Physics, Chemistry and Technology*, *8*(1), 25-37. https://doi.org/10.2298/fupct1001025r

Riabov, P. A., Micić, D., Božović, R. B., Jovanović, D. V., Tomić, A., Šovljanski, O., Filip, S., Tosti, T., Ostojić, S., Blagojević, S., & Đurović, S. (2020). The chemical, biological and thermal characteristics and gastronomical perspectives of Laurus nobilis essential oil from different geographical origin. *Industrial Crops and Products*, *151*(1), 112498. https://doi.org/10.1016/j.indcrop.2020.112498

Saghir, S. A. M., Sadikun, A., Al-Suede, F. S. R., Majid, A. M. S. A., & Murugaiyah, V. (2016). Antihyperlipidemic, Antioxidant and Cytotoxic Activities of Methanolic and Aqueous Extracts of Different Parts of Star Fruit. *Current Pharmaceutical Biotechnology*, *17*(10), 915-925. https://doi.org/10.2174/1389201017666160603013434

Samydurai, P., & Saradha, M. (2016). Effects of Various Solvent on the Extraction of Antimicrobial, Antioxidant Phenolics from the Stem Bark of *Decalepis hamiltonii* Wight and Arn. *Asian Journal of Research in Pharmaceutical Science*, *6*(2), 129-134. https://doi.org/10.5958/2231-5659.2016.00018.7

Sayed-Ahmad, B., Talou, T., Saad, Z., Hijazi, A., & Merah, O. (2017). The Apiaceae: Ethnomedicinal family as source for industrial uses. *Industrial Crops and Products*, *109*, 661-671. https://doi.org/10.1016/j.indcrop.2017.09.027

Sehar, S., Amiza, A., & Khan, I. H. (2021). Role of ZnO Nanoparticles for improvement of Antibacterial Activity in Food Packaging. *Asian Journal of Pharmaceutical Research*, *11*(2), 128-131. https://doi.org/10.52711/2231-5691.2021.00024

Sen, S., Chakraborty, R., & De, B. (2011). Challenges and opportunities in the advancement of herbal medicine: India's position and role in a global context. *Journal of Herbal Medicine*, *1*(3-4), 67-75. https://doi.org/10.1016/j.hermed.2011.11.001

Shaapan, R. M., Al-Abodi, H. R., Alanazi, A. D., Abdel-Shafy, S., Rashidipour, M., Shater, A. F., & Mahmoudvand, H. (2021). Myrtus communis Essential Oil; Anti-Parasitic Effects and Induction of the Innate Immune System in Mice with Toxoplasma gondii Infection. *Molecules*, *26*(4), 819. https://doi.org/10.3390/molecules26040819

Shi, C., Zhang, X., & Guo, N. (2018). The antimicrobial activities and action-mechanism of tea tree oil against food-borne bacteria in fresh cucumber juice. *Microbial Pathogenesis*, *125*, 262-271. https://doi.org/10.1016/j.micpath.2018.09.036

Stratil, P., Klejdus, B., & Kubáň, V. (2006). Determination of Total Content of Phenolic Compounds and Their Antioxidant Activity in VegetablesEvaluation of Spectrophotometric Methods. *Journal of Agricultural and Food Chemistry*, *54*(3), 607-616. https://doi.org/10.1021/jf052334j

Šunić, L., Ilić, Z. S., Stanojević, L., Milenković, L., Stanojević, J., Kovač, R., Milenković, A., & Cvetković, D. (2023). Comparison of the Essential Oil Content, Constituents and Antioxidant Activity from Different Plant Parts during Development Stages of Wild Fennel (*Foeniculum vulgare* Mill.). *Horticulturae*, *9*(3), 364. https://doi.org/10.3390/horticulturae9030364

Swamy, M. K., Akhtar, M. S., & Sinniah, U. R. (2016). Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: An Updated Review. *Evidence-Based Complementary and Alternative Medicine*, *2016*(1), 3012462. https://doi.org/10.1155/2016/3012462

Tiwari, P., & Patel, R. K. (2013). Estimation of total phenolics and flavonoids and antioxidant potential of drakshasava prepared by traditional and modern methods. *Asian Journal of Research in Chemistry*, *6*(3), 204-208.

Tognolini, M., Ballabeni, V., Bertoni, S., Bruni, R., Impicciatore, M., & Barocelli, E. (2007). Protective effect of *Foeniculum vulgare* essential oil and anethole in an experimental model of thrombosis. *Pharmacological Research*, *56*(3), 254-260. https://doi.org/10.1016/j.phrs.2007.07.002

Velez, R., & Sloand, E. (2016). Combating antibiotic resistance, mitigating future threats and ongoing initiatives. *Journal of Clinical Nursing*, *25*(13-14), 1886-1889. https://doi.org/10.1111/jocn.13246

Zhang, D.-Y., Yao, X.-H., Duan, M.-H., Wei, F.-Y., Wu, G.-H., & Li, L. (2015). Variation of essential oil content and antioxidant activity of Lonicera species in different sites of China. *Industrial Crops and Products*, *77*, 772-779. https://doi.org/10.1016/j.indcrop.2015.09.048

Zhang, S., Chen, X., Devshilt, I., Yun, Q., Huang, C., An, L., Dorjbat, S., & He, X. (2018). Fennel main constituent, trans-anethole treatment against LPS-induced acute lung injury by regulation of Th17/Treg function. *Molecular Medicine Reports*, *18*(2), 1369-1376. https://doi.org/10.3892/mmr.2018.9149

Zhao, A., Zhang, Y., Li, F., Chen, L., & Huang, X. (2023). Analysis of the Antibacterial Properties of Compound Essential Oil and the Main Antibacterial Components of Unilateral Essential Oils. *Molecules*, *28*(17), 6304. https://doi.org/10.3390/molecules28176304