Determination of Essential oil Yield, Composition, Antioxidant and Antimicrobial Activity of wild (*Foeniculum vulgare* Mill) from Kurdistan region -Iraq

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Abstract:

The objective of the current study was to determine the chemical makeup of the Shamar plant (Foeniculum vulgare Mill) essential oil extracted by hydro distillation and to assess the antioxidant and antibacterial properties of the Shamar essential oil extracts. Shamar was discovered to have a 0.79% essential oil extract yield. Analysis of the essential oil using Gas Chromatography-Mass Spectroscopy GC/MS revealed the presence of 17 chemicals, with the primary constituents being CELIDONIOL, DEOXY-%15.72, n-Hexadecanoic acid%13.31, 9-Octadecenoic acid, (E)-%10.12, Oleic Acid%7.85, Hexadecane%6.96, and Tetradecane%6.89. Shamar essential oil demonstrated good DPPH radical scavenging activity with an IC_{50} of 330 µg/ml, While the essential oil shown significant antibacterial action against two Gram negative bacteria, E. Coli (ATCC 25922) and B. Cereus (ATCC 14579) and one Gram positive bacteria S. Aureus (ATCC 29213) The MIC and MBC were determined using the microdilution method, and the results showed that the MIC was 1000 µg/ml. We also used inductively coupled plasma ICP-AES to investigate the trace element and heavy metal levels of the Shamar plant, including Pb, Al, Ni, Cu, Mn, Fe, Co, Ca, Cd, Cr, and Ba. Lead, Cadmium, and Nickel were not detected, according to the results, whereas the other metals were found in the following order: Al > Ca > Fe > Ba > Mn >Cr > Cu > Co. According to the findings of the current study, the trace metal levels of Shamar grown in Kurdistan, Iraq, were in the low range. The aforementioned findings suggest that isolated essential oils might be employed in the food industry as a safer substitute for synthetic additives.

Key words: Shamar; Foeniculum vulgare Mill; GC/MS; antioxidant activity; antibacterial activity; ICP-AES.

Introduction:

Finding new antimicrobial drugs that could be utilized against multi-resistant microbes has been a recent research priority for many scientists. The primary source of natural treatments is medicinal herbs and the products they produce, such as essential oils (EOs). They have been employed as the most accessible means of disease treatment from the dawn of mankind. EOs have a variety of biological

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properties, as has been demonstrated numerous times. They have insecticidal, bactericidal, viral, fungal, antiparasitic, and antioxidant properties [1-3]. The membrane and cytoplasm are two common targets for EOs and their constituents' activity, and in extreme situations, they can entirely alter the morphology of the cells [4]. They include a wide variety of intricate and structurally unique chemicals that are physiologically and/or antimicrobial active. The chemical makeup of EOs, functional groups, and potential synergistic interactions amongst constituents are all intimately related to their antimicrobial activity.

Additionally, compared to commercial antimicrobials, which are often based on a single chemical substance, it is more difficult for bacteria to acquire a resistance to these compounds because of the mixture of these active molecules [5]. Understanding the connection between EOs' chemical make-up and possible antibacterial action is crucial for using EOs to treat a variety of bacterial illnesses. It has been discovered that a number of plant-derived substances known as phytochemicals or plant bioactive have the potential to have antioxidant activity. By efficiently removing free radicals, suppressing lipid peroxidation processes, and avoiding additional oxidative damage, a bioactive chemical can sustain cell structure and function [6]. A plant species from the Apiaceae family, Foeniculum vulgare Mill., or "fennel," Fig (1), is native to the Mediterranean region and central Europe. [7,8] especially in areas where the soils had a high salt content [9,10]. Foods like fish, bread, pastries, cake, and cheese were frequently flavored with fennel seeds and their essential oil to improve their flavor and scent. Additionally, they were used as a crucial component in cosmetic and pharmaceutical products [11]. Fennel seeds were frequently employed as an analgesic, anti-parasitic, anti-inflammatory, carminative, and antispasmodic medication [12]. Additionally, fennel essential oil was employed for its noteworthy antibacterial, anti-inflammatory, and antioxidant properties [13,14]. Fennel oil was used to treat intestinal worms and gas. Drinks used to treat fevers included fruit or seed paste. Additionally, seeds are used as a stimulant, to improve libido, to produce more breast milk, to treat venereal illnesses, to ease childbirth, and to relieve coughs. [15] Fennel seed has a long history of usage as an analgesic, diuretic, anti-inflammatory, and antispasmodic plant [16]. Fennel seed methanolic extract contains anti-inflammatory and delayed hypersensitivity reaction-inhibiting properties [17]. Escherichia coli, Bacillus megatrium, and Staphylococcus aureus are just a few of the foodborne pathogens that the essence of fennel seeds has been demonstrated to be effective against [18]. along with Listeria monocytogenes [19]. It is not recommended to employ synthetic antioxidants in food, such as butylated hydroxytoluene (BHT), due to the possibility of carcinogenesis and worries about food safety.

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In comparison to butylated hydroxytoluene, the acetonic extract and essence of fennel demonstrated high antioxidant activity [20]. Essential oils, which are employed as flavoring agents in many sectors, are the primary phytoconstituents of fennel fruits [21, 22]. Gas chromatography-mass spectrometry (GC-MS) analysis has been used extensively to report on the phytoconstituents of fennel fruit essential oils [23,24,25]. One of the biomarker molecules found in fennel essential oils is fenchone. To measure fenchone in fennel essential oils, the Chiral GC method was used. The GC-MS method was also used to evaluate the presence of fenchone in fennel extract and eight different commercial fenchone formulations [26]. Fennel, or Foeniculum vulgare, is commonly used as a spice and in tea preparation. Three distinct acid mixtures (nitric acid and hydrogen peroxide; nitric acid, hydrochloric acid, and hydrogen peroxide; and nitric acid, hydrochloric acid, and hydrogen peroxide; Three acids (nitric acid, hydrochloric acid, and hydrofluoric acid) were used to assess the fennel's element content. Elements (Al, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Ni, P, S, Se, and Zn) were determined using polarography and inductively coupled plasma optical emission spectrometry (ICP-OES). Under low and high pressure, the extraction was carried out utilizing the microwave digestion process. Lucerna p-alfalfa was employed as reference material to ensure the operations' accuracy and precision. The findings indicate that aqua regia is capable of achieving the digestion of medicinal herbs that contain oil [27].

Fig 1. Foeniculum vulgare

Materials and Methods

Collection and pretreat ment of plant Material

Plant samples (leaves and stems) were collected from wild fennel (Foeniculum vulgare Mill.) in North -East of Kurdistan-Iraq August 2021 and were identified by Prof. Dr. Sirwan Hassan (plant taxonomy) in the University of Sulaimani in Kurdistan-Iraq. The plant material was dried in the shade, then kept at room temperature in paper bags until analysis. At that time, it was chopped and

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ground in a porcelain mortar until uniform-sized particles were produced.

Clevenger-Hydro distillation

For essential oil isolation by Clevenger-type hydrodistillation, disintegrated and homogenized plant material was utilized with a hydro modulus material to water (1:10 m/v) for 120 min [28].

Analysis of the Essential Oil

Gas Chromatography /Mass Spectrometry **analysis**

Shimadzu QP2010 quadrupole Gas Chromatography Mass Spectrometer (GC-MS) apparatus with a carbowax (30 m ×0.25 mm ID; 0.25im film thickness) capillary column (intercut DB5MS. Japan) was used to analyze essential oils using a gas chromatograph. The capillary column received an injection of two microliters of sample. The carrier gas used was helium. Temperatures for the injector and detector were set at 210°C. Split mode injection was carried out (1:30). The column temperature was set to start at 50°C for 1 minute before rising by 3°C each minute to reach a maximum temperature of 210°C. At constant pressure (100 kPa), components were separated, and peaks were found by comparing the mass spectra to the mass spectral database. Based on comparisons of the compounds' mass spectra with those in the NIST Library 2008, the compounds were identified.

Antioxidant Activity

Free radical-scavenger activity assessment (DPPHº assay)

The DPPH assay was carried out using the technique created by Kim et. al. (2002). The technique that was documented was used to calculate the extreme potential for scavenging [29]. The ethanol was used to dissolve the essential oil, and a variety of concentrations were created. 2.5 mL of the produced essential oil solutions were mixed with 1.5 ml of 0.25 Mm of an ethanol solution of the DPPH radical. This mixture was shaken ferociously for a steady condition at room temperature. With a spectrophotometer, the absorbance at 517 nm was measured to determine the degree of DPPH decolorization after 30 minutes. Then, the process of scavenging DPPH radicals was calculated using the following equation. A positive control was ascorbic acid.

Scavenging activity equation = [A_0 -A_{sample} /A_0]

where A sample is the absorbance of the test compound and A0 is the absorbance of the control reaction, which contains all reagents but the test compound. A graph displaying percentage inhibition against extract concentration was used to determine the extract concentration that provided 50% inhibition (IC_{50}).

Measuring the antimicrobial activity of the ethanolic extract using Microdilution method:

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The bacteria used in this culture procedure were produced at a concentration corresponding to the industry-standard opacity of 0.5 McFarland. A sterile cotton swab was used to evenly inoculate the Mueller Hinton Agar-coated surface of the plate. After that, culture medium was added to the essential oil extract [30]. For 18–24 hours, plates were incubated at 37 °C. The inhibition zone diameter was measured using a ruler after the specified amount of time had passed, and the results were noted. A positive control was also tested using gentamycin (30 mg/disc). The aforementioned procedures were carried out twice for both clinical and reference samples. The extract's minimum bactericidal concentration (MBC) was calculated using the results of the minimum inhibitor concentration.

3- Determination of mineral contents using (ICP-OES).

Fennel pieces weighing around 0.5 g were finely powdered and added to a burning cup along with 15 mL of pure HNO_3. The sample was burned at 600 ^{0}C in an oven. All reagents, standards, and fennel samples were prepared using distilled deionized water and extremely high-purity commercial acids. The samples were filtered through Whatman No. 42 after digestion. Using an ICP-OES (PerkinElmer Optima 2100 DV, USA) equipped with an inductivity coupled plasma optical emission spectrometer, the filtrates were collected in 50 mL Erlenmeyer flasks. The samples' mineral contents were measured against standard solutions with known concentrations while they were being studied simultaneously.

Results and calculation:

Essential oil content

The pertinent information **Table 1** lists the essential oils (EOs) extracted from (Foeniculum vulgare Miller). After 120 minutes of hydro distillation, 0.79 ml/100 gm of fennel essential oil was recovered from the dried plant. The method and length of the extraction period affect the EOs' content. The largest level of EO was obtained in the current study 2 hours later.

Table 1. Yields of essential oil from the wild -grown Shamar obtained after 120min. of hydro distillation (hydro module 1:10 m/v) plant material(dry)

Fennel (Foeniculum vulgare Mill.)	Essential Oil Yield, ml/100 g p.m.
	0.79

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Essential oil composition

Table 2 shows the outcomes of the fennel essential oil's chemical makeup. Overall, seventeen components were detected by GC-MS, accounting for 95.53 % percent of the oil. The primary elements of essential oil evaluated were Ceidoninol-deoxy%15.72, 1-Heptadecene%13.67, n-Hexadecanoic acid%13.31, 9-Octadecenoic acid, (E)-%10.12, Oleic acid%7.85, Hexadecane%6.96, tetradecane%6.89 and 2-Furaldehyde, 5-(hydroxymethyl)-4.40% respectively. The studied fennel essential oil also contains significant amounts of several minor compounds, some of which contributed was < 10%, such as dodecane, octadecane, and others. Linoelaidic acid%2.06, Nonadecane%2.15, Neophytadiene%2.25, Eicosane%1.41, Heneicosane%1.85, Eicosane%1.41, and Thiosulphuric acid (H2S2O3), S-(2%2.89. **Fig. 2** shows the GC-MS (gas chromatography with mass spectra) chromatogram of fennel essential oil.

Table 2. Chemical components identified of fennel essential oil by GC/MS analysis.

Peak	Retention Time	%Area	Chemical Name	Molecular Weight gm/mol	Molecular Formula
1	25.794	1.03	Dodecane	170.33	$C_{12}H_{26}$
2	27.554	4.40	2-Furaldehyde, 5-(hydroxymethyl)	126.11	$C_6H_6O_3$
3	34.927	6.89	Tetradecane	198.39	$C_{14}H_{30}$
4	43.151	6.96	Hexadecane	226.44	$C_{16}H_{34}$
5	50.553	2.81	Octadecane	254.5	$C_{18}H_{38}$
6	51.816	2.25	Neophytadiene	278.5228	$C_{20}H_{38}$
7	52.004	2.15	Nonadecane	268.5	$C_{19}H_{40}$
8	53.136	13.67	CELIDONIOL, DEOXY-	238.4519	$C_{17}H_{34}$
9	53.302	15.72	Tetracosane	282.5	$C_{20}H_{42}$
10	53.987	4.65	Nonadecane	268.5	$C_{19}H_{40}$
11	56.119	13.31	Hexadecanoic Acid	256.42	$C_{16}H_{32}O_2$
12	57.257	1.41	Eicosane	184.1099	$C_{10}H_{16}$
13	60.383	1.85	Heneicosane	296.6	$C_{21}H_{44}$
14	61.486	2.06	Linoelaidic acid	280.45	$C_{18}H_{32}O_2$
15	61.669	10.12	9-Octadecenoic acid, (E)-	282.25	$C_{18}H_{34}O_2$
16	61.812	7.85	Oleic Acid	282.47	$C_{18}H_{34}O_2$
17	62.360	2.89	Thiosulphuric acid ($H_2S_2O_3$), S-(2	114.14	$H_2S_2O_3$), S-

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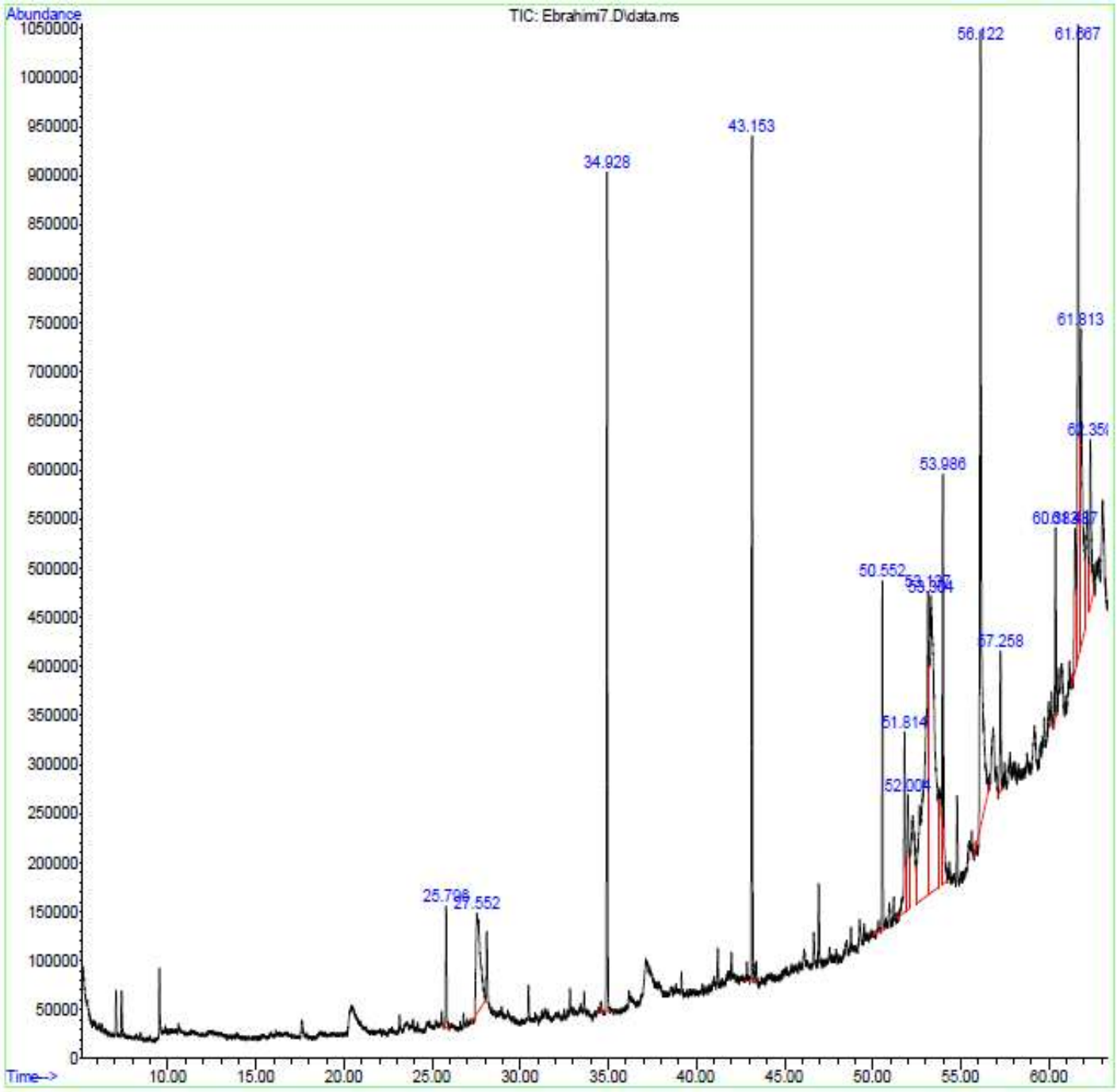

Fig. 2. The Fennel essential oil Acquired GC-MS Chromatogram

Antioxidant activity (DPPH Assay)

The most often used technique was the DPPH radical scavenging capacity assay. The scavenging of free radicals (DPPH), hydroxyl radicals, and superoxide anions served as a proxy for antioxidant activity [31]. In this experiment, the presence of hydrogen or electrons supplied by the antioxidant agents of the samples caused the DPPH radical's original purple color to turn yellow. Fennel extract's

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capacity to scavenge DPPH was contrasted with that of ascorbic acid, a well-known benchmark antioxidant. Fig. (3) and (5) display the dosage response curve for the radical scavenging capacity. As indicated in Table (3), the capacity to scavenge free radicals increased as extract concentration increased. As indicated in the conversation diagram in Fig. 3, fennel essential oil extracts demonstrated good radical scavenging action, with IC_{50} values (the extract concentration giving 50% of inhibition) of 330 µg /ml. when measured against the ascorbic acid IC_{50} (21.44 µg /ml), which served as a positive control. As demonstrated in Table 4, Figs. 4 and 6, the ascorbic acid's scavenging action ranged from 11.44% at concentrations of 8.25µg/ml to 80.54% at concentrations of 66 µg/ml. The antioxidant activity of the extract increases with concentration in the current study, which compared the antioxidant properties of the ethanolic extract of fennel with ascorbic acid. At similar concentrations, ascorbic acid has a much stronger antioxidant effect than the ethanolic extract of fennel.

Table 3. Foeniculum vulgare powder's antioxidant potential as measured by DPPH

Conc. (µg/ml)	1250	625	312.5	156.25	78.125	39.0625	19.5312	9.76562	4.8828	Control
OD1	0.212	0.398	0.538	0.689	0.823	0.856	0.921	0.965	0.996	0.992
OD2	0.219	0.386	0.551	0.675	0.811	0.861	0.897	0.956	0.987	0.975
OD3	0.22	0.381	0.529	0.693	0.80	0.847	0.89	0.947	0.993	0.988
Average	0.217	0.388	0.539	0.685	0.8143	0.854	0.902	0.956	0.992	0.985
RSA% 1	78.477	59.593	45.380	30.05	16.446	13.096	6.497	2.030	-1.116	-0.710
RSA% 2	77.766	60.812	44.06	31.472	17.664	12.588	8.934	2.944	-0.203	1.015
RSA% 3	77.664	61.319	46.294	29.644	17.868	14.010	9.644	3.857	-0.812	-0.304
Average	77.969	60.575	45.245	30.389	17.326	13.231	8.358	2.944	-0.710	0.0
S.D.	0.442	0.886	1.122	0.959	0.768	0.7202	1.650	0.913	0.465	0.90

Table 4. Ascorbic acid's antioxidant potential as measured by DPPH

concen. (µg/ml)	66	33	16.5	8.25	Control
	0.171	0.298	0.455	0.785	0.886
	0.173	0.298	0.456	0.784	0.885
	0.173	0.3	0.453	0.784	0.886
Average	0.172	0.298	0.454	0.784	0.885667
RSA1	80.692	66.353	48.626	11.366	-0.03764
RSA2	80.466	66.353	48.513	11.479	0.075273
RSA3	80.466	66.127	48.852	11.479	-0.03764
RSA%	80.541	66.277	48.663	11.441	4.18E-15
S.D.	0.130	0.130	0.172	0.0651	0.065188

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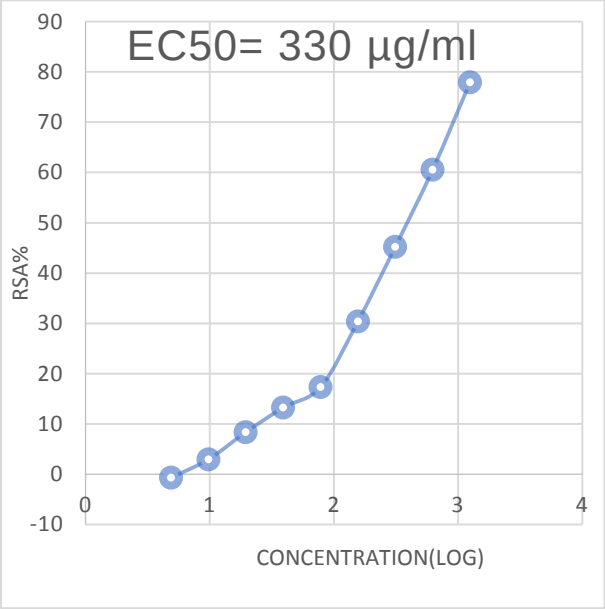


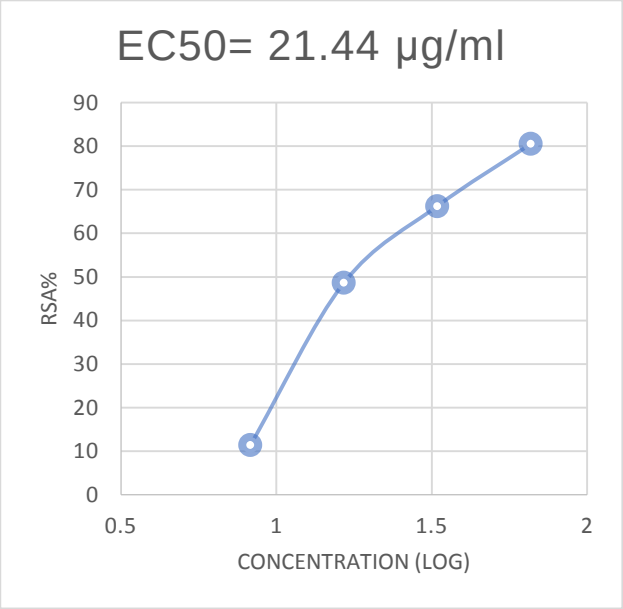
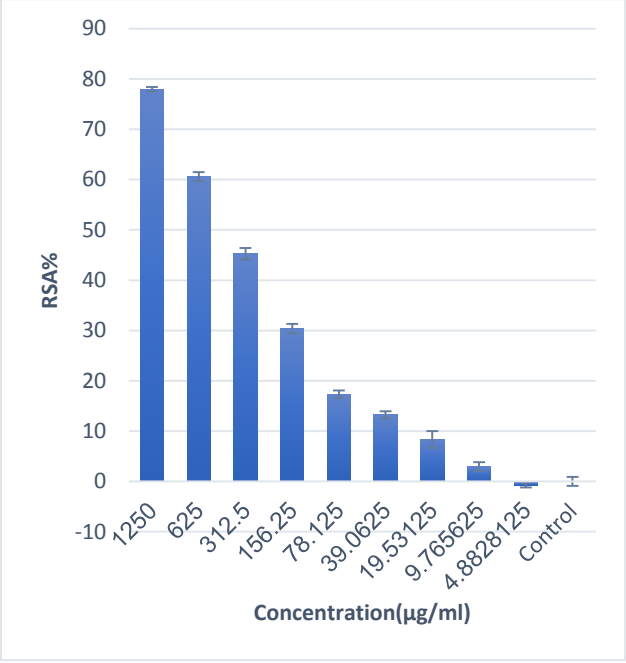


Fig.3. Demonstrate the percentage of Scavenging activity of fennel essential oil on DPPH Radicals

Fig.4. demonstrate the percentage of Scavenging activity of ascorbic acid on DPPH radicals



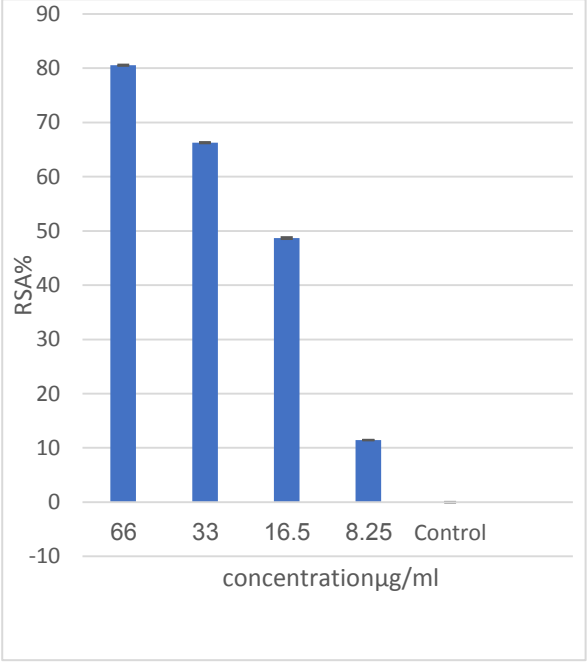


Fig.5.The DPPH Radical Scavenging Activities of Foeniculum vulgare

Fig. 6. The DPPH Radical Scavenging activity of Ascorbic acid

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Antimicrobial activity

The bioactive components of fennel (Foeniculum vulgare Mill.) extract were chosen to test the plant's antimicrobial activity because they have the potential to lower the risk of inflammation and seasonal diseases through their antimicrobial, antioxidant, anti-inflammatory, and free radical-scavenging properties. Through the measurement of MIC and MBC, organic extracts of F. vulgare have been found to have antibacterial activity against E. coli (ATCC 25922), S. aureus (ATCC 29213), and B. cereus (ATCC 14579), which are presented in Table 5. In agar, the essential oil of F. vulgare demonstrated antibacterial effectiveness in the range of $1000\mu.ml^{-1}$ or below. For E. Coli (ATCC 25922), S. Aureus (ATCC 29213), and B. Cereus (ATCC 14579), the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were found to be identical at $>1000\mu.ml^{-1}$. These findings corroborate a study that found monoterpenes hydrocarbon and its oxygenated derivatives, which are the main compounds in essential oils, have strong antibacterial properties. Gram-negative bacteria seem to be more resistant to EOs than Gram-positive bacteria in general [32]. Our findings corroborate this since, as indicated in Table 5, Gram-positive bacteria were more susceptible to the tested EOs than Gram-negative bacteria. It's likely that the active ingredients in EOs make it simpler to break crucial connections (peptidoglycan) in Gram-positive bacteria's cell walls. Gram-positive bacteria's cell walls are designed in a way that makes it simple for hydrophobic chemicals to enter the cells and act on both the cell wall and the cytoplasm. The reactive components of the essential oil can enter the cell's interior after the cell wall has been damaged, further damaging the DNA. The EOs also contains phenolic chemicals, which often exhibit antibacterial effect against Gram-positive bacteria. However, Gram-negative bacteria have far more complicated cell walls, which is one of the reasons why they are more resistant to physiologically active substances (EOs) [33]. Researchers have been inspired to study F. EO's antibacterial properties and employ nanoparticles in the food industry as safe, non-chemical alternatives. The Kurdistan region's ethanolic fennel extract has antibacterial and antioxidant properties, and the effective ratios of these chemicals can be employed as both an antimicrobial material and a culinary taste.

Table 5. Antimicrobial activity of fennel essential oil

Sample	Strains					
	E.Coli (ATCC 25922)		S. Aureus (ATCC 29213)		B. Cereus (ATCC 14579)	
	MIC	MBC	MIC	MBC	MIC	MBC
S1	>1000 µg/ml	>1000 µg/ml	>1000 µg/ml	>1000 µg/ml	1000 µg/ml	>1000 µg/ml

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Determination of mineral contents:

Utilizing an (ICP-OES) Inductivity Coupled Plasma Optical Emission Spectrometer, researchers were able to assess the mineral composition of fennel (Foeniculum vulgare Mill.). Major (Ca, Al, Fe, and Mn), trace essential (Cu, Zn, Cr, and Co), and trace nonessential (Ni, Cd, and Pb) elements were examined in the fennel fruit. Tables 6 provide the mean concentrations and standard deviation of the triplicate analysis. Additionally, Fig.7's graphic portrayal of the mineral contents of foeniculum vulgare powder.The following levels of metallic elements were established in that order: Al>Ca>Fe>Ba>Mn>Cr>Cu in Fennel (Foeniculum vulgare Mill.),The concentration of calcium in F. vulgare is 13981.119 g/L, which is the second highest concentration, while aluminum is significantly richer at 20884.9 g/L. The samples had an iron concentration of 1201.38 /L. Similar to chromium, there was less variation in copper content, with fennel (Foeniculum vulgare Mill.) having the lowest levels. Lead, Nickel, and Cadmium were not found in F. vulgare. Due to its potential toxicity to biota at low doses, it is of particular concern, though [34]. The majority of the cadmium in food comes from numerous environmental pollution sources. Organization for World Health [35]. declared that 300 µg kg^{-1} of cadmium is the permissible quantity in medicinal plants. Some trace heavy elements, such as iron, copper, zinc, and manganese, are important micronutrients with one or more structural or functional functions for living organisms even though they are only needed in very small quantities [36,37]. Iron is the micronutrient that plants require the most of all. Many enzyme systems depend on copper, manganese, and zinc [38]. These substances can, however, become poisonous if used in excess [39,40,41]. For iron, copper, manganese, and zinc, the recommended daily allowances (RDA) are, 18, 2, 5, and 15 mg d-1 person-1, respectively [33]. The observed values for these elements in the current study were generally in lower ranges compared to prior investigations on the trace metals Cu, Fe, Mn, and Zn in herb and spice plants from different areas of the world. Additionally lower than the values listed above by the Food and Nutrition Board of the National Academy of Sciences-United States were the levels of Cu, Fe, Mn, and Zn [42].

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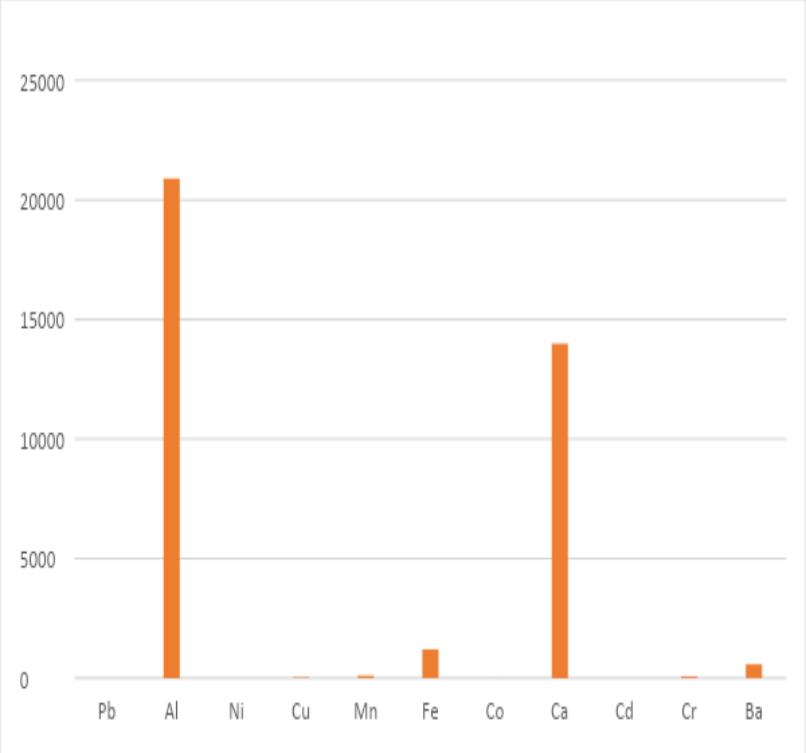
Table 6. Analysis of Foeniculum vulgare

Elements	ppb = µ/L
Al	20884.90513
Ni	< LOD
Cu	36.2133
Mn	103.130
Fe	1201.38
Co	2.890
Ca	13981.114
Cd	< LOD
Cr	68.3192
Ba	262.0298
Pb	< LOD

Powder's Elements Using (OES-ICP Perkin Elmer 2100)



elements

Fig.7. Graphical Representation of Foeniculum vulgare Powder's mineral contents

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Conclusion

Numerous herbal therapeutic formulations include F. vulgare E.O., which is well known for its medicinal benefits. In the past, the F. vulgare plant and its essential oils were utilized to cure minor disease symptoms. The findings of this study showed that fennel essential oil extract had potent antioxidant and free radical-scavenging properties. Fennel essential oils have the potential to be a natural source of antioxidants that might replace synthetic antioxidants in food products and stop the oxidative deterioration of those products. Due to the high antibacterial activity of fennel essential oil and the composition of E.O s, as well as its notable antimicrobial activity, these oils have a lot of promise for use in the future as natural antimicrobials or food preservatives. The Kurdistan fennel fruits can be used as a healthy supplement for people, especially for children and pregnant women. They are rich in Al and Ca as well as other necessary elements (Fe, Ba, and Mn). The fennel fruit does not contain the harmful elements Cd or Pb. Fennel fruits from Kurdistan are therefore suitable for human consumption. According to a research review, fennel seeds and ginger have a lot of phytochemicals and minerals that may be the cause of numerous pharmacological effects. These results demonstrated that ginger and fennel seeds are excellent sources of nutrients necessary for the health of the human body. The findings of this study will offer scientific justification for the use of these spices in conventional medicine. More research in other scientific fields will demonstrate its potential.

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Authors' Declaration:

Conflicts of Interest: The authors declare no conflict of interest

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