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Examining of Antibacterial Activity of Some Volatile Oil Bearing Plants

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Abstract: *Medicinal plants are considered new resources for producing agents that could act as alternatives to antibiotics in the treatment of antibiotic-resistant bacteria. The aim of this study was to evaluate the antibacterial activity of two plant extracts and oils against Gram-positive and Gram-negative bacteria. The essential oil from coarsely powdered coriander and fennel seeds was obtained using different solvents (water, methanol and chloroform) by Soxhlet and Clevenger extraction and then phytochemical screening by chemical. Coriander and fennel antibacterial activities were performed by using coriander and fennel extracts with the concentrations of 100 mg/mL by agar disc diffusion assay, also antibiotics susceptibility test were done by the disc diffusion method using Muller-Hinton agar medium. And Minimum inhibitory concentrations with four fold serial dilutions (v/v) were prepared 100µl, 75µl, 50µl and 25µl of each dilution prepared. The phytochemical assay was performed for all the extracts and it was observed that the bioactive compounds such as flavonoids, phenols, terpenoids, tannins, steroids and alkaloids were present. Concentrations of coriander and fennel exhibited different inhibition zone against S. aureus and klebisella. The potency of fennel extracts on S. aureus ranging from (13-15) mm diameter zone of inhibition, while that of klebisella ranging from (11-13) mm diameter zone of inhibition, in addition to that the potency of coriander extracts on klebisella ranging from (9-11) mm diameter zone of inhibition, while that of klebisella ranging from (10-12) mm diameter zone of inhibition; however klebisella was more sensitive than aureus. In addition to that the lowest concentration of the fennel and coriander EO which prevents grows of bacteria was 25 µl/ml and 50 µl/ml respectively. However fennel essential oil has higher antibacterial activity than coriander essential oil.*

Key Words*: Extraction, phytochemical screening, and antibacterial activity.*

1. INTRODUCTION:

Medicinal plants contain certain active elements that have been used over the years for the therapy of multiple diseases in traditional medical practice. Plants of reduced and higher categories are examined to generate active chemical substances that they protect against foreign agents, such as invading microorganisms [1].

Sactivum Coriandrum L. (C. s*ativum*) belongs to the Umbelliferae / Apiaceae family and is one of the most helpful vital oil-bearing spices and medicinal plants. The leaves and seeds of the plant are widely used in folk medicine in addition to its use as a seasoning in food preparation. The C. sativum essential oil and extracts possess promising antibacterial, antifungal and anti-oxidative activities as various chemical components in different parts of the plant, which thus play a great role in maintaining the shelf-life of foods by preventing their spoila. [5].

Fennel (*Foeniculum vulgare*) belongs to the Apiaceae family as an annual herbaceous plant. It is commonly grown throughout the world's temperate and tropical areas. In Asian nations, fennel herb is used as a medicinal and economic plant. Survey of published literature disclosed that fennel herbs and seeds efficiently regulate various bacterial, fungal, viral, mycobacterial and protozoan infectious illnesses [6].

1.1 Problem statement

The information on antimicrobial activity of given medicinal plants is poor. Resistance to drugs by microorganisms has increased. This resistance has been attributed to overdose and under dose of drugs due to over counter prescription of drugs, ability of microorganisms to undergo genetic variability (mutation), use of antibiotics in food preservation and general misuse of drugs [8].Hence there is need to come up with sensitive and effective drugs specially using medicinal plants. Therefore, there is need to carry out proper identification of the medicinal plants, their antibacterial activity and know their phytochemical composition in order to use medicinal plants for treatment purpose.

1.2 Aim of Study:

The main aim of this study is to investigate some volatile oils of plant extracts and to test on selected bacteria.

2. LITRATURE REVIEW:

Medicinal and aromatic plants make up a big portion of the natural flora and are regarded an significant resource in the pharmaceutical, flavor and fragrance sectors, perfumery and cosmetics sectors [9].More than 80% of the world's population currently relies on traditional plant-based medicinal products to address multiple human health issues[10].

Coriander (*Coriandrum sativum L.*) is a well-known medicinal / aromatic plant that grows in Mediterranean countries and has many pharmacological activities. All parts of the plant are edible, but the most common parts used in cooking are the fresh leaf and dried seeds. Traditional Indian medicine, C. Sativum is used in the treatment of diaphoretic, diuretic, carminative and stimulant illnesses of the digestive, respiratory and urinary structures. Traditional medicine in Iran, C. For a number of medical issues such as dyspeptic complaints, loss of appetite, seizures, anxiety and insomnia, sativum has been reported [12].

Fennel (*Foeniculum vulgare*) is a significant aromatic plant of the Apiaceae family, long regarded as a herb of medicinal and spice. Fennel oil is widely used as a favored agent in food products and as a component of cosmetic and pharmaceutical products, as well as its antimicrobial characteristics against a broad spectrum of microorganisms [16]. It has been recognized for a long time that some vital plant oils may influence staphylococcal exotoxin expression [17].

2.1 Chemical Constituent

The volatile oil of *C. sativum* contains about α-pinene (1.5%), β-pinene (0.2%), sabinene (2.8%), myrcene (1.6%), α-phellandrene (0.2%), limonene (11.6%), 1,8-cineole (36.3%), γ-terpinene (0.7%), terpinolene (0.5%), linalool (3%), linalyl acetate (2.5%), terpinene (0.9%), α-terpineol (2.6%), α-terpinyl acetate (31.3%), citronellol (0.3%), nerd (0.5%), geraniol (0.5%), methyl eugenol (0.2%) and trans-nerolidol (2.7%) [23].
The major constituents of the essential oil from the fennel were found estragole (61.1%), fenchone (23.5%), limonene+ β-phellandrene + 1, 8-cineole (8.7%) and αpinene (1.2%) [24].

3. MATERIALS AND METHODS

3.1 Plant Material

Coriander and fennel seeds were purchase at local super markets and fresh, Pure and healthy seeds of each variety were used for preparation of crude seed extract in different solvents (water, methanol and chloroform).

3.2 Method of Extracts

3.2.1 Isolation of Essential Oil from Coriander Using Soxhlet

Thirty gram of the cilantro seeds were extracted using 130-150 mL of the solvents at 50-60 oC for 3-4 cycles in a Soxhlet extractor and the crude extract would concentrated by allowing them for evaporation. All the filtered extracts was store at -20°C in an air tight container for further analysis [31].The Coriander fruits (100 g) and 1,000 ml distilled water placed in a round bottom flask and connected to a Clevenger-type apparatus. Hydro distillation was completed for 4h after boiling. Oil yield of the sample was calculated on a moisture free basis. The oil was dried over anhydrous sodium sulphate and kept at 4 °C in the sealed brown vial until required.

$$\text{Essential Oil yield (\%)} = \frac{\text{Volume of essential oil (g)}}{\text{Weight of plant sample (g)}} \times 100\%$$

3.2.2 Isolation of Essential Oil from fennel Using Soxhlet

Hundred gram of dried Foeniculum vulgar seeds was milled to form a course powder and then extracted with methanol (500 ml). The aqueous extract was prepared by 100 g of dried ground seed in 1000 ml distilled water for 6 h. after boiling. Oil yield of the sample was calculated and the oil was dried over anhydrous sodium sulphate and kept at 4 °C in the sealed brown vial until required [32].

$$\text{Essential oil (\%)} = \frac{\text{Volume of the oil}}{\text{Weight of sample}} \times 100\%$$

Detection of Phytochemical Screening: The extracts were subjected to preliminary phytochemical screening for the detection of various plants constituent using the standard procedure described earlier [33-35].

3.2.3 Antimicrobial Activity

A. Disc Diffusion Susceptibility Method: The antibacterial activity of different solvent extracted samples of seed of fennel and coriander was carried by disc diffusion assay as described [35]. Briefly, under aseptic circumstances, two to three sterile paper disks (6 mm in diameter) were lodged, on Mueller Hinton agar Petri dish previously flooded with the appropriate bacterial culture which included Gram +ve (staphylococcus) and Gram -ve (klebisella) bacteria. The discs were aseptically impregnated with 20 μL of Foeniculum vulgare and corandium extract solution (100 mg/mL). These dishes were kept for 15–30 min at room temperature before incubation at 37 oC for 24 hour. The antibacterial activity was evaluated by measuring the zone of inhibition against the tested organism. Chloramphenicol drug was used as a positive control and water was used as negative control.

b. Determination of Minimum Inhibitory Concentration (MIC): MIC means the lowest concentration of the probable antimicrobial agent which prevents growing of bacteria (regardless of killing the bacteria or stopping the growth of them).

The lowest dilution which no gross microbial growth has been seen indicates MIC.The last can be revealed by pouring 50 µl and25 µl of MIC tube and four dilutions before contents on agar plate. In the case, after incubation period. For determination of MIC value, macro broth dilution method was applied. Interpretation of the results was done due to national accepted lett*er* [37].

4. RESULTAND DISCUSSION:

4.1 Result

Extraction Yield The percentage of extraction yield was calculated by the following formula:-

$$\text{Percentage of Yield (\%)} = \frac{\text{Amount of extract (g)} \times 100}{\text{Amount of dried part used (g)}}$$

Table 1. Extraction Yield of coriander extracts Sample

Amount of dried part used(g)	Solvent	Amount of extract yield	Percentage of yield (%) coriander
120	Chloroform	20ml	16.6%
120	Methanol	14ml	11.6%
1300	Aqueous	16ml	1.2%

Table 2.Extraction Yield of fennel extracts Sample

Amount of dried part used(g)	Solvent	Amount of extract yield	Percentage of yield (%) Fennel
100	Chloroform	22ml	22%
100	Methanol	18ml	18%
600	Aqueous	16ml	2.6%

Phytochemical analysis: The most active extract of each plant was tested for various phytochemicals. Tannins, Flavonoids, Glycosides, cardioglycosides, Terpenoids, Phenols, Coumarins, Teroenoids, Steroids, Saponin, Alkaloids and Phytosterols were predominantly present in active extracts (Table.3and 4).

Table 3. Phytochemical screening of Coriander Sativum extracts

Active constituents	Chloroform extract	Methanol extract	Aqueous extract
Tannins	+	+	+
Flavonoids	-	-	+
Quinones	-	-	
Glycosides	+	+	
Cardio glycosides	+	+	-
Terpenoids	+	-	
Phenols	+	+	
Coumarins	-	+	
Teroenoids			+
Steroids			+
Saponin			+
Alkaloids			+

Table 4. Phytochemical screening of Foeniculum vulgare (fennel) extracts

Active constituents	Chloroform extract	Methanol extract	Aqueous extract
Alkaloids	+	+	+
Flavonoids	+	+	+
Sterols	+	+	+
Terpenod	+	-	
Saponins	-	+	+
Tannis	+	+	-
Phytosterols	+	+	
Glycosids	-	+	
Cardio glycoside			-
Teroenoids			+

Table 5. Antibacterial activity of seed extracts of fennel seed (zone of inhibition in mm)

Name of organism	Zone of inhibition in mm		
	Coriander, EO	Fennel, EO	Chloramphenicol
Staphylococcus aureus	9-11mm	13-15mm	20-23mm
Klebisella	10-12mm	11-13mm	19-21mm

Fig. 1. Results of fennel phytochemical analysis of the chloroform extract; Negative for saponins (5,8), Positive for alkaloids, flavonoids, sterols, Terpenoid,Tannis, phytosterols, glycosides, (1-4,6,7).

Fig. 2. Results of fennel phytochemical analysis of the methanol extract; Negative for terpenod (4), Positive for alkaloids, flavonoids, sterols, saponin, tannins, phytosterols, glycosids(1-3,5-8).

Fig. 3. Results of coriander phytochemical analysis of the methanol extract; Negative for flavonoid (2,3,6), Positive for Tannis, glycosides, cardio glycosides, phenols (1,4,5,7,8)

Fig. 4. Results of coriander phytochemical analysis of the chloroform extract; Negative for flavonoids (2, 3,8), Positive for Tannis, quinines, glycosides, cardio glycosides, terpenoids, phenols (1,4-7)

Fig. 5. Results of coriander phytochemical analysis of the D/W extract; Negative for cardiaco glycosides (3), Positive for Tannins,flavonoids,Teroenoids, steroids, saponin, alkaloids(1,2,4-7).

Fig. 6. Results of fennel phytochemical analysis of the D/W extract; Negative for tannins, cardio glycosides (5, 6), Positive for alkaloids, flavonoids, steroids, saponin, flavonoids, teroenoids (1-4, 7).

Result of Antibacterial Activity of Two Plant Extract and Zone of Inhibition against the tested organism

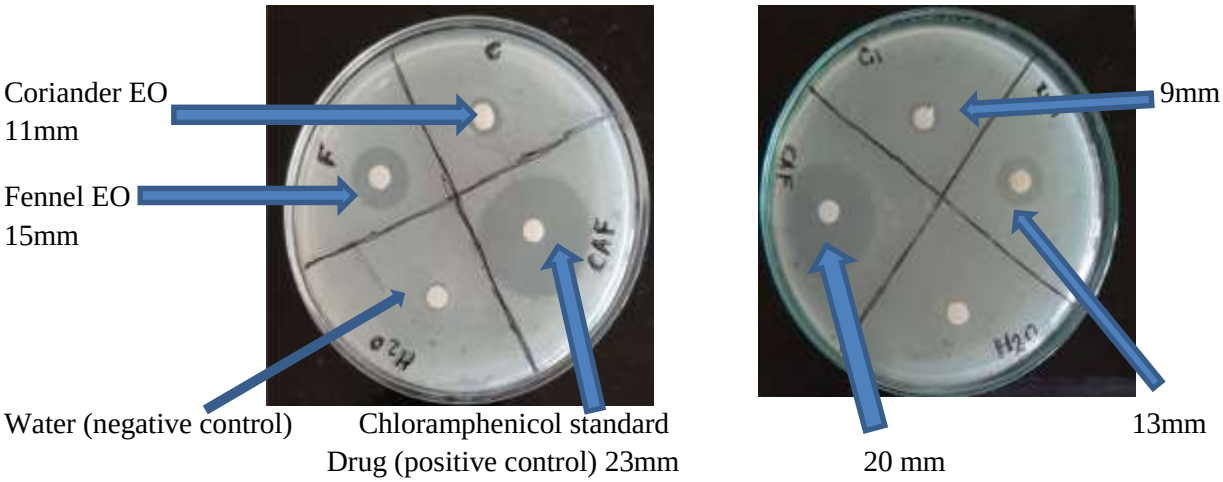


Fig. 7.Zones of inhibition produced from extracted Coriander and Fennel EO and test on S. aureus Organisms.

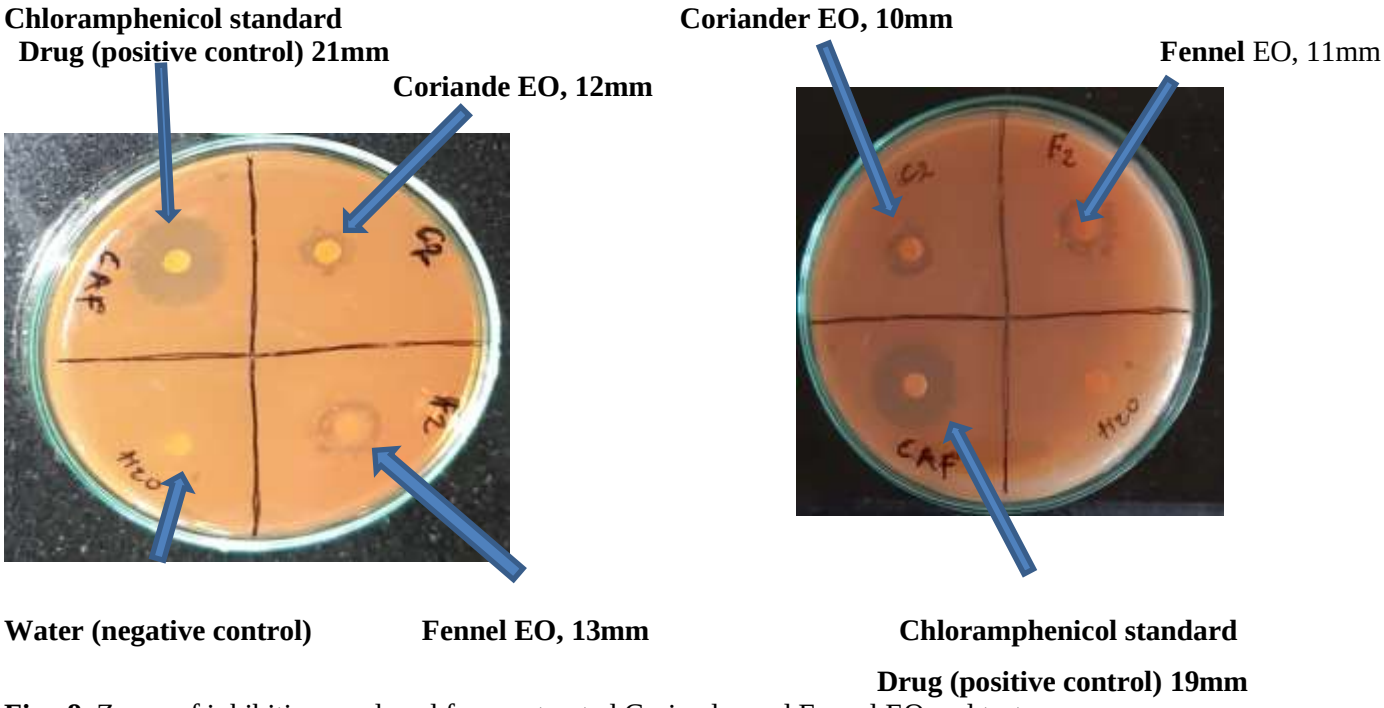
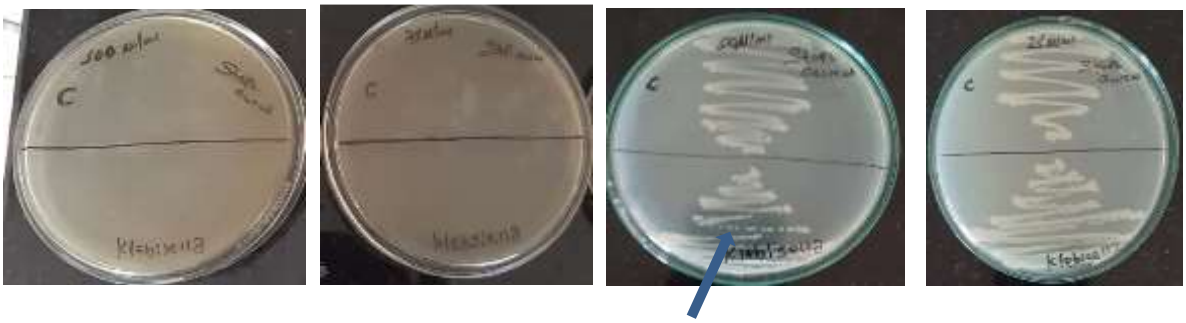


Fig. 8. Zones of inhibition produced from extracted Coriander and Fennel EO and test on Klebisella organisms.

Result of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration of fennel is 25µl/ml

Fig. 9. Results s, aureus streaking on MHA medium for MIC test Caption a) Dose extract 100% b) Dose extract 75% c) Dose extract 50% d) Dose extract 25%..



Minimum Inhibitory Concentration of fennel is 50µl/ml

Fig. 10. Results klebisella streaking on MHA medium for MIC test Caption a) Dose extract 100% b) Dose extract 75% c) Dose extract 50% d) Dose extract 25%.

4.2 Discussion:

Resistance growth in bacteria is one of the natural adaptation mechanisms for the existence of an antimicrobial agent that inhibits sensitive organisms and chooses resistant ones. The issue of antibiotic resistance, which restricted the use of inexpensive and old antibiotics, required a continuous search for new antimicrobial compounds.

The result of the present study indicates different concentrations of coriander and fennel extract exhibited inhibition zone against S. aureus, and klebisella (Table 5).The potency of coriander extracts on S. aureus ranging from (9-11) mm diameter zone of inhibition, while that of klebisella ranging from (10-12) mm diameter zone of inhibition.as well the potency of fennel extract on S. aureus ranging from (13-15) mm diameter zone of inhibition, while that of klebisella ranging from (11-13) mm diameter zone of inhibition.

The lowest concentration of antimicrobial agent that completely inhibits growth of the organism in micro-dilution wells or tubes as detected by the unaided eye is called minimum inhibitory concentration (MIC) [43]. The most appropriate bioassays for the determination of MIC value are these dilution methods, as these bioassays offer the possibility of estimating the concentration of the tested antimicrobial agent in the agar (agar dilution) or broth medium (macro dilution or micro-dilution).according to this theory our studies shows the lowest concentration of coriander EO was 50µl/ml, and 25µl/ml the lowest concentration of fennel EO. There for fennel essential oil higher antibacterial effects than coriander essential oil.

5. CONCLUSION:

The antimicrobial activity of coriander and fennel extract indicates that there suitability for being used as important certain medicines, so there is active to serve as anti-bacterial agents against multi-drug resistant bacteria. Among the studied essential oils, coriander and fennel essential oils were the most effective. Moreover, chloramphenicol was the most effective antibiotics against almost all the studied bacteria. Therefore, coriander and fennel could act as bactericidal agents against Grame- positive and Gram-negative bacteria. The minimum inhibitory concentration (MIC) extract of coriander and fennel EO of the bacterium aureus 25% and klebisella obtained at doses 75%. There for, fennel essential oil higher antibacterial effects than coriander essential oil and both are effective of antibacterial activity.

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