American Journal of Plant Sciences, 2015, 6, 1058-1068
Published Online April 2015 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2015.67110

GC-MS Analysis and *in Vitro* Antimicrobial Susceptibility of *Foeniculum vulgare* Seed Essential Oil

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Received 10 February 2015; accepted 26 April 2015; published 30 April 2015

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Abstract

Essential oil from seeds of *Foeniculum vulgare* was extracted on Clevenger apparatus. Essential oil was analyzed on Gas-Chromatography-Mass spectrometry (GC-MS) from which thirty six components were identified, among which 6 major and 30 minor components having different structural formulae and molecular weight representing total 99.98% of oil. Essential was investigated for its antibacterial and antifungal activity against seven infectious microbial pathogens. Paper disc diffusion and serial micro-dilution assays were performed for the determination of inhibition zone (DIZ) diameters and minimal inhibitory concentration, respectively. The *Foeniculum vulgare* essential oil showed the Diameter of Inhibition Zone (DIZ) ranging from 19.4 ± 0.07 - 26.4 ± 0.09 mm at a concentration level of 28 µg/disc in all the ten strains tested. The minimum inhibitory concentration (MIC) of essential oil against bacterial and fungal strains was obtained in the range of 7.0 - 56 µg/ml. Antibacterial and antifungal activity of *Foeniculum vulgare* essential oil is due to the presence of certain secondary plant metabolites such as terpenoids, steroids and flavonoids, esters and acids which are identified in the essential oil. The oil components can be further studied for their biological activity and overcome the problem of drug resistance in microbes.

Keywords

Antimicrobial Activity, *Foeniculum vulgare*, Essential Oil, GC-MS Analysis, MIC, MBC, MFC, DIZ

1. Introduction

Plant essential oils are aromatic oily liquids, having highly specific volatile odor or flavors and show broad-spectrum antimicrobial activity against diverse groups of pathogens [1]. These are obtained from various plant

parts such as flowers, buds, seeds, leaves, twigs, barks, woods and roots [2]. More than 60 families of angiosperms mainly Lamiaceae, Rutaceae, Geraniaceae, Apiaceae, Aspetaceae, Lauraceae, Fabaceae, and Poaceae possess essential oils in different plant species. Essential oils are complex mixtures of diverse chemical constituents such as lectins, polypeptides, alkaloids, phenols, quinines, flavones, flavonoids, terpenes, tannins, coumarins, benzene derivatives, various hydrocarbons and straight chain compounds. Plant essential oils multiple biological activity such as antibacterial [3], antifungal [4], anti-cancer [5] [6] and anti-oxidant [7]. Essential oils are obtained in pure form or a complex mixture of several components without making any change in their chemical composition [8]. These are used for a wide variety of purposes [9] such as flavoring, perfuming [10] aromatherapy and food preservation [11]-[13]. These are obtained by fractionation or rectification and steam distillation of various plant parts in Clevenger apparatus [14]. Essential oils possess mixed functional groups, too complex in their structure and are highly volatile at a very low temperature. Due to high volatility essential oils easily spread in the environment and medium. These act more efficiently against drug resistant microbes in culture medium due to their fast diffusion and volatile action. Treatment with plant essential oils shows least residual effect in the body, but these severely inhibit growth and metabolism of a variety of infectious pathogens mainly microbes. These are also used alternative medicine in aromatherapy for treatment of cancer, disease pathogens and dermal infections [15] [16].

Saunf (*Foeniculum vulgare*) is an annual or biennial plant belonging to family Umbelliferae family. It has sparse, fine, feathery leaves and pinkish/white flowers, which are followed by green seeds. Fennel oil has a sweet, spicy, warm smell, is nearly colorless to pale yellow and has a watery viscosity. Fennel seeds are rich source of essential oil which is extracted by steam distillation and yields 0.8% - 1.0% oil. Due to strong flavor, essential oil extracted from seeds is used for medicinal purposes such as aphrodisiac, antispasmodic, carminative, depurative, deodorant, digestive, fungicidal, lipolytic, stimulant and stomachic substance. It is used in aromatherapy and helps to ease the mind and fight fatigue. *Saunf* oil application warms and calms the digestive system, relieves rheumatism and arthritic pain, muscular spasms and detoxifies the body [9]. It is also used for massage, relieve mental tensionand fatigue. Oil vapors provide freshness and show warming effect on the stomach, relieve wind and cramps, and revitalizing the glandular system. Fennel is famous worldwide as a spice and is regularly used in vegetables, and has multiple medicinal properties as well, such as its digestive and stomachic properties. Fennel seeds are often used as a spice in Indian cuisine and cooked well till the flavor blends with the dish. It has lemony, citrusy flavor when crushed, and it contains pinene and linalool terpenes. Fennel seeds are at their best when used fresh. Often, it is dry roasted and grounded before being added to a dish. Fennel oil improves appetite, regulates endocrinal secretions, cures nausea and eliminates vomiting as a symptom of many conditions. In the present study, essential oil (E.O.) from *Foeniculum vulgare* was isolated from ripe seeds and screened for its antimicrobial activity *in vitro* by applying various bioassays. For evaluation of antimicrobial susceptibility potential various tests and growth inhibitory bioassays were conducted by setting controls and treatments done in seven bacterial strains (*i.e. Escherichia coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Lactobacillus acidophilus*, *Staphylococcus aureus and Streptococcus pneumoniae* and *Micrococcus luteus*) and three isolates of fungi (*i.e. Candida albicans*, *Aspergillusniger*, *Rhizopus stolonifer*). MIC, MBC, MFC, inhibition zone diameters (DIZ) were determined in bacterial and fungal strains were determined in presence of each varying concentrations of essential oil. Antimicrobial susceptibility obtained in Fennel essential oil was compared with broad-spectrum antibiotics drugs.

2. Experimental

2.1. Instrumentation

Gas-Chromatography-Mass Spectrometry (GC-MS)

GC/MS analyses of *Foeniculum vulgare* E.O. was carried out on a Shimadzu GC-MS-QP2010 apparatus equipped with a −5 column (60 m × 0.25 mm i.d., film thickness 0.25 µm). Helium was used as carrier gas at a constant column flow 1.2 ml/min at 173 kpa inlet pressure and injector volume was 1.0 µL. The test was performed according to a set temperature programming which was maintained from 100˚C to 200˚C with constant rise of 5˚C/min and then held isothermal at 200˚C for 6 min. Further, the temperature was increased by 10˚C/min up to 290˚C and again held isothermal at 290˚C for 10 min. The injector and ion source temperatures were 270˚C and 250˚C, respectively. The crude and active bands (AB-1) and (AB-2) (2 mg/ml) were dissolved in methanol (HPLC grade, Merck, India) and are injected with a split ratio of 1:10. Mass spectra were taken at

70 eV; a scan interval of 0.5 s and fragments from 40 to 950 Dalton. The identification of the essential oil components was based on the comparison of their relative retention time (tR) and mass spectra with those of commercial standards (for the main components) and on the GC retention indices (RT) determined rel. to n-alkanes (C8 - C32). The final confirmation of constituents was made by computer matching of the mass spectra of peaks with the Wiley and National Institute Standard and Technology (NIST) libraries mass spectral database. The relative percentages of the essential oil constituents were calculated from the GC peak areas. Further, quantitative analysis was performed by means direct peak area intention technique based on the total ion chromatogram (TIC).

2.2. Plant Material

The seeds of *Foeniculum vulgare* were purchased from local market at Gorakhpur, Uttar Pradesh, India. The identification of plant material was made by plant taxonomist. A voucher specimen is deposited for further confirmation in the departmental laboratory.

2.3. Extraction and Isolation of Essential Oil

Seeds of *Foeniculum vulgare* were grounded by using domestic mixer and powdered material was hydro-distilled in Clevenger apparatus continuously for 5 hrs to yield essential oil [13]. The crude powder was extracted with pure methanol twice and dried residue was dissolved in known volume of fresh solvent (w/v) before testing the antimicrobial activity.

2.4. Identification of Major Constituents

The chemical constituents of essential oil from *Foeniculum vulgare* are listed in **Table 1**. Thirty six components were identified by using GC representing 99.98% of the oil. The main constituents of essential oil were identified 9-octadecenoic acid (18.56%), 8Z)-14-methyl-8-hexadecenal (7.75%), pentad ecanecarboxylic acid (4.25%), o-benzenedicarboxylic acid (14.47%), 1,3,3-trimethyl-2-vinyl-1-cyclohexene (10.77%), 2-methyl-3-oxoestran-17-yl acetate (5.46%), 1H-benzocycloheptene (10.71). The dominant components are 2-hydroxy-1-(hydroxymethyl) ethyl ester (1.84%), and 9-octadecenoic acid (18.56%) but in different ratios [**Figure 1**, **Table 1**] together with few compounds found in minor concentrations. Compounds were identified by comparison of retention time and MS peaks. Both formula and molecular weights were established in each case.

2.5. Determination of Antimicrobial Susceptibility

2.5.1. Source of Microorganisms

Cultures of seven pathogenic bacterial strains each of *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 11778), *Lactobacillus acidophilus* (ATCC 53103), *Micrococcus luteus* (ATCC 9341), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 15380) and *Streptococcus pneumoniae* (ATCC 12755) were maintained in the laboratory in Luria Broth (2% w/v) regularly for four days at 37˚C before use in experiments. For experiments, a portion (100 µl) of the overnight culture was mixed in the tests and control for inoculation. For activity, testing bacterial cultures were stored at 4˚C and sub cultured after every 8th day in solid agar plates. For determination of antifungal activity of plant latex, fungal strains of *Aspergillus niger MTCC* 1344, *Candida albicans MTCC* 227, *Rhizopus stolonifer* MTCC 3789 were grown in the laboratory. Moreover, each test fungi was maintained in strain specific agar medium mainly Sabouroud's Agar and Potato Dextrose Agar and its pure cultures were established by using single spore isolation technique.

2.5.2. Disc Diffusion Assay

The *in vitro* antimicrobial activity of *Foeniculum vulgare* essential oil was evaluated *in vitro* Agar Disc Diffusion Assay. In each assay inhibition zone diameters were measured in presence and absence of essential oil. For treatments essential oil was diluted by applying serial micro-dilution method by adding Luria Broth media separately. Six different concentrations of essential oils (1 - 32 µg) (W/V) were coated on sterile filter paper discs (Whatmann No. 1) of 6 mm size and oil impregnated discs were dried under laminar flow cabinet. Before starting experiments inoculums size was determined and adjusted to prepare a final colony number as 10^8 colony

Table 1. Major and minor constituents isolated from Fennel (*Foeniculum vulgare*) *essential oil.*

Peak No.	RT(minutes)	Area (m^2)	Composition%	Name of the compound
1	8.590	2,182,160	0.71	Tetradecane, Hexadecane
2	10.655	6,336,308	2.05	Ethanone, 1-(4-methyl-3-cyclohexen-1-YL)-$$ 1-(4-methyl-3-cyclohexen-1-YL)ethanone, 2-propanone
3	12.989	11,350,176	3.67	H-Benzocycloheptene, 2,4a,5,6,7,8,9, 9a-octahydro-3,5,5-trimethyl-9-methylene-, Longifolene
4	13.767	462,260	0.15	Phenylmethyl ester
5	13.956	6,940,501	2.25	cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H))benzocycloheptene
6	14.215	33,085,809	10.71	1H-Benzocycloheptene,
7	14.762	790,090	0.26	m-Methylacetophenone
8	15.240	663,289	0.21	alpha.-Caryophyllene
9	15.411	425,235	0.14	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-Corylon
10	16.004	1,669,636	0.54	p-Guaiacol
11	16.718	1,489,484	0.48	2-(4a,8-Dimethyl-2,3,4,4a,5,6-hexahydro-naphthalen-2-yl)-prop-2-en-1-ol
12	16.909	2,053,895	0.66	Vetivenene Neoisolongifolene, Aromadendrene
13	17.339	2,775,408	0.90	Anthracene, 1,2,3,4,5,6,7,8-octahydro-1-methyl-
14	17.600	5,370,067	1.74	1-Methyl-6-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptane
15	17.717	3,471,113	1.12	1-hydroxy-2-methoxy-2-methoxy-4-methylbenzene
16	17.821	811,099	0.26	1-(2,3-Dihydroindol-1-yl)-4-phenyl-butan-1,4-dione
17	18.444	815,668	0.26	5,5 Dimethyl-3-vinyl cyclohex-2-en-1-one
18	18.987	1,672,082	0.54	2-Methoxy-4-ethylphenol, 1,2-Dimethoxy-4-methylbenzene
19	19.741	1,151,507	0.37	Bis(4-methylphenyl) methanedisulfonate
20	19.961	977,905	0.32	(-)-5-oxatricyclo[8.2.0.0(4,6)]Dodecane, Cedran-9-one
21	20.173	3,778,176	1.22	2,2-dimethyl-3-phenylpropanoate
22	21.485	890,104	0.29	-Methyl-6-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptane
23	21.699	1,390,963	0.45	2,7-dimethyloct-7-en-5-yn-4-yl ester
24	22.059	6,315,814	2.04	2-Methyl-6-(4-methyl-1,3-cyclohexadien-1-yl)-2-hepten-4-one
25	22.386	9,756,518	3.16	3-Methyl-2-butenoic acid,
26	22.754	16,860,463	5.46	2-Methyl-3-oxoestran-17-yl acetate
27	24.181	2,176,678	0.70	3,3,6-Trimethyl-1-indanone
28	24.394	33,272,538	10.77	1,3,3-Trimethyl-2-vinyl-1-cyclohexene
29	24.795	44,712,526	14.47	o-Benzenedicarboxylic acid,
30	25.715	1,510,861	0.49	1-Isopropyl-1,2,3,4-tetrahydroisoquinoline
31	28.675	612,829	0.20	3,4-Dimethyl-1,5-cyclooctadiene
32	34.362	5,688,743	1.84	2-hydroxy-1-(hydroxymethyl)ethyl ester
33	35.216	13,120,783	4.25	Pentadecanecarboxylic acid
34	39.929	23,958,417	7.75	8Z)-14-Methyl-8-hexadecenal
35	41.407	57,332,536	18.56	9-octadecenoic acid
36	43.290	3,103,542	1.00	2-cis,cis-9,12-Octadecadienyloxyethanol
Total		308,975,183	100.00	99.98

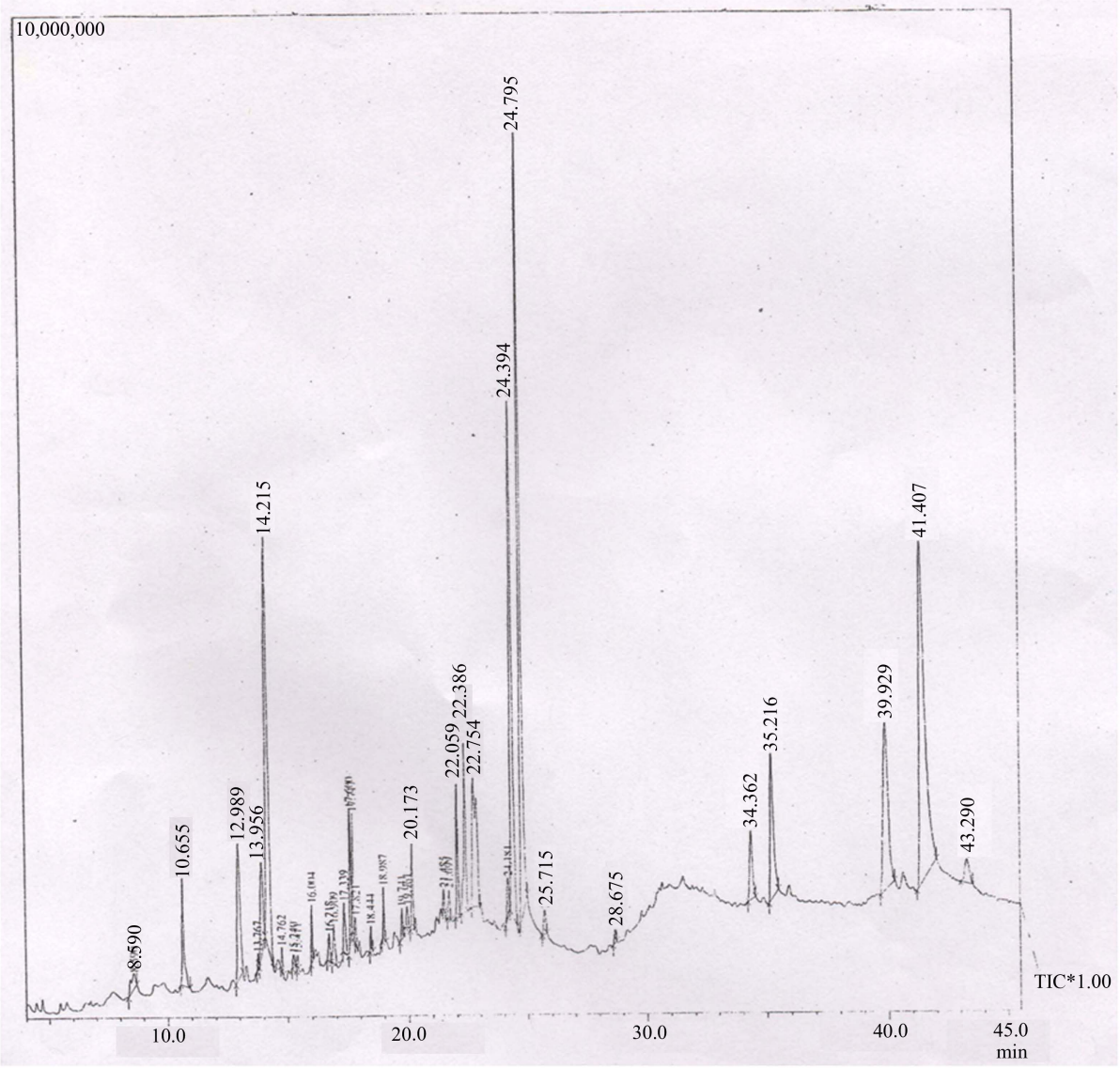

Figure 1. GC-MS spectrum of essential oil obtained from *Foeniculum vulgare*.

forming units (CFU/ml) in sterile agar plates. Bacterial inoculums were spread evenly on to the surface of agar plate by using a sterile rubber pad spreader. After which essential oil coated discs were positioned on the inoculated agar surface in the centre. Essential oil was assayed in triplicate for antibacterial activity testing. All treated and untreated plates were incubated for 24 hrs at 27˚C. DMSO was used as negative control while ampicillin was used as standard (positive control) to compare the bacterial growth and Griseofulvin was used to compare fungal growth in negative control. The radial growth of fungi was measured after 12 hours interval up to 36 hours of initial inoculation. The average percentage inhibition of growth in presence of various essential oils was calculated by using following formula,

$$\text{Inhibition}(\%) = 100\ (C-T)/C$$

where C = diameter of fungus colony in control plates; T = diameter of fungus colony in tested plates.

2.5.3. Minimum Inhibitory Concentration Determination (MIC)

Bacterial growth inhibition was accessed in the presence of different increasing concentrations of essential oil in

Luria Broth culture medium and MIC values were determined for each bacterial and fungal strain. For this purpose, essential oils were diluted in a concentration range from 48 µg/ml to 0.0058 µg/ml by using serial micro dilution method. Essential oil was added to fresh media suspension after following the serial dilutions up to 10^{-10}. Fennel essential oil was assayed in triplicate. Before conducting experiments all the conditions for *in vitro* anti-microbial activity were standardized to determine MIC and MBC values. The MIC values were considered as the lowest concentration of essential oil, in which no turbidity in the culture flask was visualized after 24 hrs of incubation at 37˚C. The turbidity in the culture flasks was considered as visible growth of microorganisms. Further, it was standardized in terms of absorbance at 600 nm in a visible spectrophotometer. For determination of minimum bacterial concentration (MBC) growth inhibitory assays were performed. For this purpose inoculums' size was adjusted to prepare a final colony number as 10^8 colony forming units (CFU/ml in sterile agar plates. The incubation of test and control cultures was also performed at 37˚C for 24 hours. For comparison, both negative and positive control was set and bacterial colony number was counted in all test and control discs. For comparison broad-spectrum antibiotics *i.e.* ampicillin was used as standard to compare the bacterial growth while Griseofulvin for comparison of fungal growth. Results were interpreted by using a standard table that relates to the degree of microbial resistance prescribed by NCCLS (National Committee for Clinical Laboratory Standards). A plot of MIC on a logarithmic scale versus zone inhibition diameters (arithmetic scale) was prepared for essential oil and antibiotic to know the susceptibility level. These plots were used to find the zone inhibition diameters corresponding to the drug concentrations and that of essential oils. The low MIC value was considered as susceptibility of essential oils/drugs to the pathogen, while high MIC value (with a small zone inhibition diameter) was considered as resistant.

2.5.4. Statistical Analysis

All statistical calculations are expressed as mean ± SE of three replicates. Data were analyzed by one way ANNOVA to locate significant variations in oil activity in various bacterial and fungal strains followed by the Duncan's multiple range tests.

3. Results

Chemical Composition of Oil

Fennel essential oil is extracted from the seeds of coriander with the help of steam distillation. The scientific name of Fennel (Saunf) is *Foeniculum vulgare*. Plant origin natural products are known to have more antimicrobial activity against drug resistant microbes. This activity could act as chemical defense against pathogenic diseases. However, in the present time, both the traditional and folk medicines have been considered as alternatives of synthetic drugs for healthcare to the patients. However, for screening pharmaceutical and therapeutic potential of these natural products various bioassays are developed to detect and confirm the anti-pathogenic effects and establish a good correlation with disease pathogens. For obtaining broad-spectrum drugs, essential oils are found to be good therapeutic-targeting molecules. The present study emphasizes the composition of essential oil isolated from *Foeniculum vulgare* and its effect on inhibition of bacterial and fungal growths.

Few major components are identified as 2-hydroxy-1-(hydroxymethyl) ethyl ester (1.84%), and 9-octadecenoic acid (18.56) but in different ratios [**Table 1**] together with few compounds found in minor concentrations. The dominant components are 2-hydroxy-1-(hydroxymethyl) ethyl ester (1.84%), and 9-octadecenoic acid (18.56%) but in different ratios [**Table 1**] together with few compounds found in minor concentrations. Disc diffusion assays were conducted with *Foeniculum vulgare* essential oil to measure growth inhibition zone diameter and screen anti-microbial potential. The essential oil of *Foeniculum vulgare* has shown higher range of inhibition zone diameter 20.2 ± 0.18 - 26.5 ± 0.14 mm at a concentration level of 24 µg/disc. The positive control has shown diameter of inhibition zone (DIZ) ranging from 15.1 ± 0.30 - 18.8 ± 0.37 mm at concentration of 24 µg/disc. All DIZ corresponding to test organisms are mentioned in **Table 2**. The results of MIC obtained against all the bacterial strains have been given in **Table 3**. Lower MIC values presented have shown very high antimicrobial susceptibility of *Foeniculum vulgare* to *E coli*, *Bacillus cereus*, *L. acidophilus* and *S. pneumoniae* are in a range of 6 - 48 µg/ml.

Lower MIC values presented have shown very high antimicrobial susceptibility of *Foeniculum vulgare* to *E coli*, *Bacillus cereus*, *L. acidophilus* and *S. pneumoniae* are 6, 12.0, 24, 24 µg /ml respectively.

Table 2. Zone of inhibition of essential oil from methanolic extract of *Foeniculum vulgare*.

S. No.	Name of the organism	Essential oil (IZD in mm)	Negative control (mm)	Positive control (mm)
1	*Escherichia coli*	24.63 ± 0.21	--	18.8 ± 0.37
2	*Bacillus cereus*	26.5 ± 0.14	--	16.8 ± 0.28
3	*Lactobacillus acidophilus*	25.9 ± 0.16	--	15.1 ± 0.30
4	*Micrococcus luteus*	24.1 ± 0.19	--	17.4 ± 0.41
5	*Staphylococcus aureus*	21.6 ± 0.28	--	17.3 ± 0.11
6	*Klebsiella pneumoniae*	23.2 ± 0.28	--	18.3 ± 0.43
7	*Streptococcus pneumoniae*	20.2 ± 0.18	--	17.0 ± 0.41
8	*Aspergillus niger*	22.7 ± 0.40	--	16.7 ± 0.23
9	*Candida albicans*	20.5 ± 0.15	--	17.8 ± 0.24
10	*Rhizopus stolonifer*	23.0 ± 0.49	--	17.4 ± 0.30

Values are expressed as mean ± SD (n = 3) and values followed by same letter are not significantly different at the $P < 0.05$; Determined by Duncan's Multiple Range test. Positive control = Ampicillin/Griseofulvin, negative control = DMSO.

Table 3. Antimicrobial activities of essential oil from methanolic extract of *Foeniculum vulgare* on different microbes and their corresponding MIC.

S. No.	Name of the organism	*MIC* (µg/ml)	
		Essential oil	Positive control
1	*Escherichia coli*	6.0	56
2	*Bacillus cereus*	12	28
3	*Lactobacillus acidophilus*	24	28
4	*Micrococcus luteus*	48	56
5	*Staphylococcus aureus*	48	56
6	*Klebsiella pneumoniae*	48	56
7	*Streptococcus pneumoniae*	24	28
8	*Aspergillus niger*	24	28
9	*Candida albicans*	6.0	7.0
10	*Rhizopus stolonifer*	12.0	14.0

Positive control is Ampicillin/Griseofulvin.

4. Discussion

In the present investigation, *Foeniculum vulgare* essential oil GC-MS analysis showed presence of 36 compounds representing more than 99.98% of the essential oil [Table 1]. The inhibition zones of the essential oils on tested organism show a significant correlation with MIC values ($P < 0.05$). Based on growth inhibition zone diameters obtained in tests, results were divided into three categories *i.e.* resistant (>7 mm), intermediate (>12 mm), and susceptible (>18 mm). Maximum growth inhibition diameter was obtained 26.5 ± 0.14 mm against *Micrococcus luteus* followed by 25.9 ± 0.14 mm against *Lactobacillus acidophilus*. *Foeniculum vulgare* oil has shown significantly higher growth inhibition zone diameters in *Aspergillus niger* 22.7 ± 0.40 mm, *Candida albicans* 20.5 ± 0.15 mm and 23.0 ± 0.49 mm in *Rhizopus stolonifer* than the broad spectrum antifungal drug griseofulvin 17.4 ± 0.30 mm [Table 2]. Similar growth-inhibition zone diameters were reported in *P. aeruginosa* 33.3 mm, *B. subtilis* 29.9 mm *P. vulgaris* 29.4 mm, *K. pneumoniae* 20.8 mm and *S. aureus* each [16], *Clostri-*

dium ferfringens, *E. coli* and *Lactobacillus acidophilous* [17], *Bacillus species* [18], *Staphylococcus aureus* [19] [20], and *Salmonella enteritidis* in presence of different essential oils [21]. Coriander oil (*Foeniculum vulgare*) has shown 6.0 µg/ml MIC valueagainst *E. coli*, 12.0 µg/ml against, *Bacillus cereus*, 24.0 µg/ml *Lactobacillus acidophilus* [**Table 3**]. Similarly, it has shown MIC value in a range of 6 - 24 µg against *Aspergillus niger*, *Candida albicans*, *Rhizopus stolonifer* [**Table 3**]. Similar MIC 6 µg/ml was reported in allicin and diallyl sulfur compounds against *Helicobacter pylori* [22]. Similarly luteolin [23], thymus [24], phenolics [17] (2006) and Cavacrol [3], di-terpenoids [25] isolated from various essential oils have also shown strongeranti-microbial activity against few bacteria mainly against oral pathogens [26] and *Escherichia coli* O157:H73. Similarly, juniper oil extracted from *Juniperus communis* (L) has shown strong bactericidal activity against both Gram-positive and Gram-negative bacteria with MIC values between 8% and 70% v/v [27]. Same oil has also shown stronger fungicidal activity against *Candida* sp. (MIC from 0.78% to 2% v/v). *Sardinian juniperus* essential oil was found active against foodborne pathogens and spoilage microorganism [7]. Similar, antimicrobial activity was exhibited by different *Mentha* species *i.e. Mentha longifolia* L., *Mentha aquatica* and *Mentha piperita* L. against *E. coli* with very low MIC value (4 µL/ml) [4] and *Hypericum* species such as *Hypericum scabrum*, *Hypericum scabroides* and *Hypericum triquetrifolium* essential oils [28]. Moreover, di-terpenoids isolated from *Sagittaria pygmaea* has shown antibacterial activity against *Streptococcus mutans* (ATCC 25175) with MIC value of 15.6 µg/ml [25]. Essential oils from *Coriandrumsativum* (L) [29] *Cinnamomum osmophloeum* [30]. Besides this, *Dracocephalum foetidum* essential oil also exhibited strong antibacterial activity against methicilin-resistant *Staphylococcus aureus* (MRSA) [31]. For comparison of antimicrobial activity of essential oils certain broad spectrum antibiotics were also tested against same bacterial strains, which have shown marginal activity orintermediate effect. In various bioassays, *Foeniculum vulgare* essential oil has shown very high anti-bacterial and anti-fungal activities *in vitro.* Similar, antimicrobial activity of essential oils of *Laserpitium latifolium* and *L. ochridanum* against one Gram-positive and three Gram-negative bacteria and two *Candida albicans* strains. Essential oil showed a high antimicrobial potential against *Staphylococcus aureus*, *S. epidermidis*, *Micrococcus luteus*, or *Candida albicans* (minimal inhibitory concentrations of 13.0 - 73.0 µg/ml [32]. Essential oils (E.O.s) from *Zataria multiflora* Boiss. (zataria) and *Origanum vulgare* (oregano) showed extensive antimicrobial activity in a wide range of food spoilage or pathogenic fungi, yeast and bacteria, and on hepatitis A virus [33], Contrary to this, essential oils showed weak inhibitory activity against the Gram-positive pathogens. Essential oils of *Pereskia aculeata* Mill. and *P. grandifolia* Souza *et al*., 2014) [34], Similarly Piper species: *Piper abbreviatum*, *P. erecticaule* and *P. lanatum* displayed weak activity towards Gram-positive bacteria with MIC values in the range 250 - 500 µg/ml. *P. erecticaule* oil showed the best activity on *Aspergillus niger* (MIC 31.3 µg/ml), followed by *P. lanatum* oil (MIC 62.5 µg/ml) [35]. *Juniperus excelsa* Bieb. leaf essential have showed high activity towards: *Staphylococcus aureus*, *Streptococcus pyogenes* and *Haemophilus influenzae* (MIC = 125 µl/ml). The pinene-type of essential oil showed moderate activity against *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus pyogenes*, *Corynebacterium* spp. and *Campylobacter jejuni* (MIC > 50%) [36]. Essential oils isolated from the leaves of *Cosmos bipinnatus* showed effects against both Gram-negative and Gram-positive bacteria isolates. The MIC of Gram-positive strains ranged between 0.16 and 0.31 mg/ml while those of Gram-negative bacteria ranged between 0.31 and 0.63 mg/ml. The Gram-positive bacteria were more susceptible to the essential oil than the Gram-negative bacteria [37].

Due to presence of volatile components, *i.e.* phenolic compounds in higher concentration [17] and its diffusion at room temperature fennel essential oil displayed high susceptibility against both Gram-negative and Gram-positive bacteria. In addition, it may also increase the plasma membrane permeability that results in higher leakage of fluid material from bacterial cells [32] and inhibit microbial respiration [38]. Therefore, major antimicrobial activity seems to be post diffusion action of essential oils on growth and metabolism of both the bacterial and fungal strains [39] [40]. No doubt, Saunf (*Foeniculum vulgare*) essential oil contains so many promising molecules, which can be used for therapeutic purposes mainly pharmacological potential [41]. Like other plant, natural products essential oils possess broad-spectrum antimicrobial activity against pathogenic microbial strains [42]. As high antimicrobial susceptibility obtained in tests incomparison to drugs, olive essential oil and its components can be used for formulation of highly active non-antibiotic drug that may be less toxic and show lesser side effects. The antibacterial activity can be attributed to effects of the combination of several components of the oil. The results indicate that the *Foeniculum vulgare* might be exploited as natural antibacterial agent and have application in the treatment of several infectious diseases caused by these bacteria.

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