

# In Vitro Test Of Antimicrobial Activity Of *Foeniculum Vulgare* Mill. (Fennel) Essential Oil

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**Abstract—** The essential oil of *Foeniculum vulgare* was screened for antimicrobial activity against the Gram positive bacterium *S.aureus*, Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, anaerobic bacterium *Clostridium perfringens*, and three fungal strains; *Aperugillus flavus*, *Aperugillus niger*, and *Microsporium canis*. The Minimum Inhibitory Concentrations (MICs) were determined by broth macro-dilution method. In the preliminary screening using disc diffusion assay against bacteria, the EO exhibited moderate inhibitory activity against the three tested bacteria. MICs and MBCs for bacteria ranged 0.781 to 25 µl /ml. The MIC values indicated that the fennel seed EO was active against all the fungal strains tested in the present study, and exhibited strong antifungal activity.

**Keywords—** *Foeniculum vulgare*, antimicrobial activity, disc diffusion, tube dilution.

## 1. Introduction

The connection between man and his search for drugs in nature dates from the far past, of which there is ample evidence from various sources; written documents, preserved monuments, and even original plant medicines<sup>(1)</sup>. Medicinal and aromatic plants and their derivatives represent an integral part of life in Sudan where flora consists of 3137 species of flowering plants belonging to 170 families and 1280 genera. It is estimated that 15% of these plants are endemic to Sudan. The intersection of diverse cultures and the unique geography holds great potential for Sudanese herbal medicine<sup>(2)</sup>. The percentage of people

dependent on medicinal plants for health care is estimated over 90%. These plants and derived products play an important role in the primary health care in Sudan<sup>(3)</sup>.

*Foeniculum vulgare* is hardy, perennial–umbelliferous herb with yellow flowers and feathery leaves. It grows to a height of up to 2.5 m with hollow stems. The leaves grow up to 40 cm long; they are finely dissected with the ultimate segments filiform (thread like) of about 0.5 mm wide. The flowers are produced in terminal compound umbels. The fruit is a dry seed 4–10 mm long<sup>(4)</sup>. It is widely distributed in most tropical and subtropical countries and have long been used in folk medicines to treat obstruction of the liver, spleen and gall bladder and for digestive complaints such as colic, indigestion, nausea and flatulence<sup>(5)</sup>. In Sudan the plant is used to treat diabetes in traditional medicines<sup>(6)</sup>. The essential oil from plants has usually been isolated by either steam distillation or solvent extraction<sup>(7)</sup>. Trans-anethole, methyl chavicol, fenchone, estragole, d-limonene, neophytadiene, exo-fenchyl acetate and (E)-phytol are the major chemical constituents<sup>(8-11)</sup>. Nineteen compounds were detected in the

essential oil of *F. vulgare* cultivated in Sudan. Monoterpenes comprises the main constituents (98.06%), among which (80.67%) were oxygenated, whereas sesquiterpenes represent only about (0.66%) of the oil. Estragole (68.96%), D-limonene (15.41%) and anethole (8.51%) were the main identified constituents<sup>(4)</sup>. The objective of the present study is to assess the antimicrobial activities of the essential oil of *F. vulgare* against the bacterial and fungal pathogens.

## 2. Materials and methods

### 2.1 Plant material

The plants used in this study was purchased from local markets (Khartoum). They were authenticated in Medicinal and Aromatic plants and Traditional Medicine Research Institute (MAPTMRI). Voucher specimens were deposited at the herbarium of the institute.

### 2.2 Extraction of essential oil

The powdered seeds (500 g) of *F. vulgare* were hydrodistilled in a Clevenger's type apparatus for 6 h and yellow colored oil (yield 4%), with characteristic odor and sharp taste, was obtained. The crude oil was dried over anhydrous sodium sulphate to remove traces of moisture and stored in a refrigerator in the dark at 4°C until use.

### 2.3 Test microorganisms

The EO was tested against the Gram positive bacterium *Staphylococcus aureus* (ATCC 25923), two Gram negative organisms, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and three fungi, *Aperugillus flavus*, *Aperugillus niger*, and *Microsporum canis*.

### 2.4 *In vitro* screening of EO for antibacterial activity

The disc diffusion method was adopted to screen the antimicrobial activity of the against standard control bacteria. The standardized inoculum suspension of each bacterial strain which is equivalent to 0.5 MC farland units was swabbed on the entire surface of Mueller-Hinton agar, then 20 µl of the EO was placed on each sterile 6mm-diameter absorbent filter paper disc and incubated at 37 C° overnight. The inhibition zones were measured and recorded in millimeters (mm). The scale of measurement was the following (disc diameter included): ≥28 mm zone of inhibition (ZI) is strongly inhibitory; < 28 to 16 mm ZI is moderately inhibitory; < 16 to 10 is mildly inhibitory; and <10 mm is non inhibitory. Plates were left at ambient temperature for 15 minutes to allow excess pre-diffusion of extracts prior to incubation at 37°C for 24 hours<sup>(13)</sup>.

### 2.5 Quantitative evaluation of antimicrobial susceptibility

Minimum inhibitory concentrations (MICs) of individual oil and the ratios decided were determined by the tube dilution method as described in the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) against bacteria and fungi. The final twofold dilutions of the EO were prepared volumetrically in Brain Heart Infusion broth medium for bacteria and Sabourud broth for fungi. A single pipette was used for measuring all diluents and then for adding the stock EO to the first tube. A separate pipette was used for each remaining dilution in that set. Because there will be a 1:2 dilution of the

EO when an equal volume of inoculum is added, the EO dilutions were prepared at double the desired final concentration. Within 15 minutes after the inoculum has been standardized, 1 ml of the adjusted inoculum was added to each tube containing 1 ml of EO in the dilution series (and a positive control tube containing only broth), and mix. This results in a 1:2 dilution of each antimicrobial concentration, and a 1:2 dilution of the inoculum.<sup>(14)</sup>The end point (MIC) is the least concentration of antimicrobial agent that completely inhibits the bacterial growth after overnight incubation in 37 C°<sup>(15)</sup>.

### 2.6 Determination of minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) of the EOs on the standard control bacteria was carried out according to National Committee for Clinical Laboratory Standard provision (NCCL). One ml was pipetted from the mixture obtained in the determination of MIC tubes which did not show any growth and streaked on MHA and incubated for 24 h. The least concentration of the EO with no visible growth after incubation was taken as the minimum bactericidal concentration<sup>(16)</sup>.

### 3. Results and discussion

The essential oil of *Foeniculum vulgare* (fennel seeds) was tested for its antimicrobial activity against three bacterial and four fungal strains, Table (1) illustrates the botanical and ethno-pharmacological information about the medicinal plant from which the essential oil was extracted. In the preliminary screening using disc diffusion assay against bacteria, the EO exhibited moderate

inhibitory activity against the three tested bacteria. The susceptibility was estimated quantitatively by means of minimum inhibitory concentrations (MICs) for bacteria and fungi, and minimum bactericidal concentrations (MBCs) for bacterial strains. MICs and MBCs for bacteria ranged 0.781 to 25 µl /ml (Table 2).

These results revealed that the EO of fennel possesses a strong antibacterial activity against the aerobic control bacteria. Our result was opposed to that obtained by Miguel *et al.*, who found that the EO showed a very low antimicrobial activity<sup>(17)</sup>, this might be due to using of commercial oils from different parts of the plant along with using agar diffusion method which gives a general idea but it is not a reliable method to assess the activity of the plant extracts quantitatively, because the most antimicrobial compounds have intermediate polarity or are non-polar. This means that these compounds do not diffuse easily in the aqueous agar matrix. Broth dilution is more suitable to test the antimicrobial activity of the plant extracts than agar diffusion methods<sup>(18)</sup>. Similar to the results of Aprotosoie *et al.*, and Tarek *et al.*, *S.aureus* and *E. coli* were more susceptible than *P.aeruginosa*, the EO exhibited a good activity at low concentration( $\leq 1$  µl /ml) while *P. aeruginosa* was less active even at the highest concentration ( $>16$  µl /ml)<sup>(19, 20)</sup>. MIC value of fennel oil against *E.coli* was 0.0781% in our study, unlike Gulfraz *et al.*<sup>(7)</sup>, who determined MIC value 0.8%. This high concentration may be attributed to determination of MIC against *E.coli* clinical isolate - which is the most prevalent reported microorganism in

resistant data in many countries- instead of using standard control strain of *E.coli* <sup>(21)</sup>. On other hand, the MIC of the current study was more than that recorded by Bisht *et al.*, who determined MIC 0.062% for *E.coli* <sup>(22)</sup>. The major components in fennel oil that possess antimicrobial properties are trans-anethol and fenchone <sup>(23)</sup>. The antimicrobial properties of essential oil of fennel and its major constituents, anethole, have been shown to be able to suppress several human and plant pathogenic fungi <sup>(24)</sup>. The MIC values indicated that the fennel seed EO was active against all the fungal strains tested in the present study, and exhibited strong antifungal activity. For *Microsporum canis*, the MIC was < 0.625%. For *Aperugillus flavus* and *Aperugillus niger* strains, the MIC value was at the same level (Table 2). Zeng *et al.*, investigated the antifungal

effects of fennel seed EO from varied aspects, such as MIC and minimum fungicidal concentration, mycelia growth, spore germination and biomass. The results indicated that the EO had potent antifungal activities on *Trichophyton rubrum*, *Trichophyton tonsurans*, *Microsporum gypseum* and *Trichophyton mentagrophytes*, which is better than the commonly used antifungal agents fluconazole and amphotericin B <sup>(25)</sup>. The volatile oil showed complete zone inhibition against *Aspergillus niger*, *Aspergillus flavus*, *Fusarium graminearum* and *Fusarium moniliforme* at 6 IL dose. It was found to be effective for *A. niger* even at 4 IL dose. Moreover, using food poison technique, the volatile oil and extract both showed good to moderate zone of inhibition <sup>(26)</sup>.

**Table (1): Preliminary screening for antibacterial activity of fennel seed EOs by disc diffusion assay**

Family/Botanical name/Synonyms/ Vernacular name	Yield %	Conc%	Antibacterial activity (zone of inhibition in mm)		
			<i>S.aureus</i>	<i>E.coli</i>	<i>P.aeruginosa</i>
<b>Apiaceae</b> <i>Foeniculum vulgare</i> Mill. <b>Synonym:</b> <i>Foeniculum capillaceum</i> Gilib., <i>Anethum foeniculum</i> L. <i>Foeniculum officinale</i> All. <b>Vern.</b> chemar	4%	20%	19	20	15
		10%	13	17	14

**Table (2): Minimum inhibitory concentrations (MICs) of fennel seed essential oil**

<i>S.aureus</i>		<i>E.coli</i>		<i>P.aeruginosa</i>		<i>A.flavus</i>	<i>A.niger</i>	<i>M.canis</i>
MICs	MBCs	MICs	MBCs	MICs	MBCs	MIC	MIC	MIC
µl/ml		µl/ml		µl/ml		µl/ml	µl/ml	µl/ml
0.781	0.781	0.781	0.781	25	25	0.125	0.125	<0.0625

## References

1- Petrovska BB. Historical review of medicinal plants usage. Pharmacogn Rev. 2012; 6(11): 1–5.

2- Khalid H, Abdalla WE, Abdelgadir H, Opatz T, Efferth T .Gems from traditional north-African medicine: medicinal and aromatic

- plants from Sudan. *Nat Prod Bioprospect.* 2012; 2(3): 92–103.
- 3- Ibrahim FY, El-Khateeb AY. Effect of herbal beverages of *Foeniculum vulgare* and *Cymbopogon proximus* on inhibition of calcium oxalate renal crystals formation in rats. *Annals of Agricultural Sciences.* 2013; 58(2):221-229.
- 4- Rather M, Dar AB, *et al.* *Foeniculum vulgare*: A comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. *Arabian Journal of Chemistry.* 2012 Article in press.
- 5- Garga C, Khan S A, Ansari S H, Suman A, Garg M. Chemical composition, therapeutic potential and perspectives of *Foeniculum vulgare*. *Phcog Rev* 2009; 3:346-52.
- 6- Monalisa MN, Rahmatullah M. Antihyperglycemic, analgesic activity, and acute toxicity studies with methanol extract of *Foeniculum vulgare* seeds. *World Journal of Pharmacy and Pharmaceutical Sciences.* 2015; 4(9): 198-206.
- 7- Gulfraz M, Mehmood S, Minhas N, Jabeen N, Kausar R, Kokab, *et al.* Composition and antimicrobial properties of essential oil of *Foeniculum vulgare*. *African Journal of Biotechnology.* 2008; 7(24):4364-68.
- 8- García-Jiménez N, Péerez-Alonso MJ, Velasco-Negueruela A. Chemical composition of fennel oil, *Foeniculum vulgare* Miller, from Spain. *Journal of Essential Oil Research.* 2000; 12(2): 159-162.
- 9- Zhaoa NN, Zhoub L, Liua ZL, Duc SS, Dengd ZW. Evaluation of the toxicity of the essential oils of some common Chinese spices against bostrychophila. *Food Control.* 2012; 26(2):486–490.
- 10- Senatore F, Oliviero F, Scandolera E, Taglialatela-Scafati O, Roscigno G, Zaccardelli M, *et al.* Chemical composition, antimicrobial and antioxidant activities of anethole-rich oil from leaves of selected varieties of fennel [*Foeniculum vulgare* Mill. ssp. *vulgare* var. *azoricum* (Mill.) Thell]. *Fitoterapia.* 2013; 90:214–219.
- 11- Diao W, Hu Q, Zhang H, Xu J. Chemical composition, antibacterial activity and mechanism of action of essential oil from seeds of fennel (*Foeniculum vulgare* Mill.). *Food Control.* 2014; 35(1):109–116.
- A. 12- Hassan OM, Elhassan IA. *Characterization of essential oils from fruits of umbelliferous crop cultivated in Sudan II. Coriandrum sativum L. (Coriander) and Foeniculum vulgare Mill (Fennel).* 2017; 6(1): 113-116.
- 13- Elgayyar M, Draughon FA, Golden DA, Mount JR. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *Journal of Food and Protection.* 2001; 64(7):1019-1024.
- 14- Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard\_Seventh Edition.* CLSI document M7-A7 (ISBN 1-56238-587-9). Clinical and Laboratory

- Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2006.
- 15- Cheesbrough M. Medical Laboratory Manual for Tropical Countries. Volume II. Low-priced edition. Butterworth & Co Ltd (*pub*), Cambridge, 2008, p.197-198, 243-244.
- 16- Doughari JH, El-mahmood AM, Tyoyina I. Antimicrobial activity of leaf extracts of *Senna obtusifolia* (L). African Journal of Pharmacy and Pharmacology. 2008; 2(1):007-013.
- 17- Miguel MG, Cruz C, Faleiro L, Simões MT, Figueiredo AC, Barroso JG, *et al.* *Foeniculum vulgare* essential oils: chemical composition, antioxidant and antimicrobial activities. Natural product communications. 2010; 5(2):319-28.
- 18- Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica. 1998; 64:711–713.
- 19- Aprotosoiaie AC, Hăncianu M, Poiată A, Tuchiluş C, Spac A, Cioană O, et al. *In vitro* antimicrobial activity and chemical composition of the essential oil of *Foeniculum vulgare* Mill. Revista medico-chirurgicală a Societății de Medici și Naturaliști din Iași. 2008; 112(3):832-6.
- 20- Tarek N, Hassan HM, El-Gendy AO. Comparative chemical and antimicrobial study of nine essential oils obtained from medicinal plants growing in Egypt. Beni-Suef University Journal of Basic and Applied Sciences. 2014; 3(2):149-156.
- 21- Aly M, Balkhy HH. The prevalence of antimicrobial resistance in clinical isolates from gulf corporation council countries. Antimicrobial Resistance and Infection Control. 2012; 1:26.
- 22- Bisht DS, Menon KR & Singhal MK. Comparative antimicrobial activity of essential oils of *Cuminum cyminum* L. and *Foeniculum vulgare* Mill. seeds against *Salmonella typhimurium* and *Escherichia coli*. Journal of Essential Oil Bearing Plants. 2014; 7(4): 617-622.
- 23- Kazemi M, Mousavi E, Kharestani H. Chemical composition and antimicrobial activities of essential oils of *Varthemia persica*, *Foeniculum vulgare* and *Ferula lycia*. Current Research in Bacteriology. 2012; 5:42-52.
- 24- Soylu S, Yigitbas H, Soylu EM, Kurt S. Antifungal effects of essential oils from oregano and fennel on *Sclerotinia sclerotiorum*. Journal of Applied Microbiology. 2007; 103:1021-1030.
- 25- Zeng H, Chen X, Liang J. *In vitro* antifungal activity and mechanism of essential oil from fennel (*Foeniculum vulgare* L.) on dermatophyte species. Journal of Medical Microbiology. 2015; 64: 93–103
- 26- Singh G, Maurya S, de Lampasona MP, Catalan C. Chemical constituents, antifungal and antioxidative potential of *Foeniculum vulgare* volatile oil and its acetone extract. Food Control. 2006; 17:745–752.