# Comparative Analyses of Foeniculum vulgare: Antibacterial Outcomes and Phytochemical Profiling of Essential Oil.

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**ABSTRACT**

Medicinal plants are considered modern resources for producing agents that could act as alternatives to antibiotics in demeanor of antibiotic-resistant bacteria. The aim of the study was to evaluate the chemical composition and antibacterial activities of essential oil of *Foeniculum vulgare* (FV) against *Pseudomonas aeruginosa* and *Bacillus subtilis*. Gas chromatography mass spectrometry was done to specify chemical composion. As a screen test to detect antibacterial properties of the essential oil, agar disk and agar well diffusion methods were employed. Macrobroth tube test was performed to determinate MIC. The results indicated that the most substance found in FV essential oil was Trans-anethole (47.41 %), also the essential oil of FV with 0.007 g/ml concentration has prevented *P. aeruginosa* and with 0.002 g/ml concentration has prevented *B. subtilis* from the growth. Thus, the research represents the antibacterial effects of the medical herb on test *P. aeruginosa* and *B. subtilis*. We believe that the article provide support to the antibacterial properties of the essential oil. The results indicate the fact that the essential oil from the plant can be useful as medicinal or preservatives composition.

**Keywords:** *Foeniculum vulgare*, Essential oil, GC/MS, Antibacterial effects.

## INTRODUCTION

Infections due to bacterial species also stay a serious clinical difficulty. Emerging resistance of bacterial species is seriously reducing the number of efficient antimicrobials. Because of increasing pressure of consumers and legal authorities, the food industry has tended to decrease the use of chemical preservatives in their products to either entirely nil or to adopt more indigenous alternatives for the maintenance or extension of product shelf life[1]. Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times[2-5]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant[6-10]. Essential oils are made from a very intricate mixture of volatile molecules that are produced by the secondary metabolism of aromatic and medicinal plants and can be obtained by various methods, including the use of low or high pressure distillation of various parts of plants or the employment of liquid carbon dioxide or microwaves[11-13]. The span of the essential oils action versus bacteria may achieve values that only prevent the bacterial growth (bacteriostatic) or may be used at either high concentrations or are inherently more aggressive and their

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action results in a reduction in the number of bacterial cells (bactericide)[14-16]. The bacteriostatic action has a reversible character since, after frustration of the agent, the microbial cells will meliorate their reproductive capacity[17,18]. In contrast, the bactericidal effect has a constant effect; as even after the neutralization of the agent, the microbial cells are not capable of growth and reproduction[19,20]. FV commonly known as *Fennel*, is a flowering plant species in the carrot family. It is a hardy, perennial herb with yellow flowers and feathery leaves. It is widely cultivated throughout the temperate and tropical regions of the world for its aromatic fruits, which are used as culinary spices[21]. The FV fruit has a long history of use as both a food and medicine. Traditionally, it is said to act as a carminative (assists with flatulence control) and increase breast milk production. They are also used as a constituent in cosmetic and pharmaceutical products[22,23]. FV is used in traditional medicine for its antiseptic, palliative and anti-inflammatory effects[24]. The most substance found in FV essential oil is Transanethole. Based on knowledge of authors, in comparison to many other pharmaceutical-industrial plants, there is a very little data about chemical composition and antibacterial activity of the essential oil collected from

Kermanshah province, west of Iran. Hence, the aim of the current study was (1): determination of chemical composition of its hydro-distilled essential oil obtained from Kermanshah city, west of Iran by GC–MS, (2): evaluation of antibacterial activity of the essential oil against common pathogens (*P. aeruginosa and B. subtilis*) with broth macro-dilution and agar well and disk diffusion methods.

## MATERIALS AND METHODS

*Plant sample collection*

In the empirical-experimental study, medicine plant collected from Kermanshah. The sample was cleaned from any strange, plants, dust, or any other contaminants.

*Essential oil extraction*

Essential oil from fresh, clean, weighed aerial part FV fruits extracted by hydro-steam distillation using the Clevenger

apparatus were collected and stored in sterile vials. Briefly, 100 to 150 g of plant was introduced in the distillation flask (1L), which was connected to a steam generator via a glass tube and to a condenser to retrieve the oil. This was recovered in a funnel tube. Aromatic molecules of the essential oil were released from the plant material and evaporated into hot steam. The hot steam forced the plant material to release the essential oil without burning the plant material itself. Then, steam containing the essential oil was passed through a cooling system in order to condense the steam. The steam was applied for 3h. After settling the recovered mixture, essential oil was withdrawn. The supernatant essential oil was filtered through anhydrous $Na_2SO_4$ to dry the yielded essential oil. Afterward, the essential oil was collected in tightened vials and stored in a refrigerator. For the antimicrobial activity test, several dilutions of the essential oil were done using dimethyl sulfoxide (DMSO).

*Gas chromatography mass spectrometry (GC/MS)* FV essential oil was analysed using GC/MS (Shimadzu capillary GC-quadrupole MS system QP 5000) with two fused silica capillary column DB-5 (30 µm, 0.25 mm i.d, film thickness 0.25 µm) and a flame ionization detector (FID) which was operated in EI mode at 70 eV. Injector and detector temperatures were set at 220°C and 250°C, respectively. One microliter of each solution in hexane was injected and analyzed with the column held initially at 60°C for 2 min and then increased by 3°C/min up to 300°C. Helium was employed as carrier gas (1 ml/min). The relative amount of individual components of the total essential oil is expressed as percentage peak area relative to total peak area. Qualitative identification of the different constituents was performed by comparison of their relative retention times and mass spectra with those of authentic reference compounds and mass spectra.

*Source of microorganisms*

Tow bacterial species namely *P. aeruginosa* (PTCC No. 1707) and *B. subtilis* (ATCC No. 21332) were procured from Iranian Research Organization for Science and Technology as lyophilized. Each bacterial strain was activated on Tryptic Soy broth, constant at 37°C for 18 h. Then 60 µl of the broth was transferred to Nutrient agar and incubated at 37°C for another 24 h; cell concentration was then adjusted to obtain final concentration of $10^8$ cfu/ml using Muller Hinton broth.

*Culture media*

Mueller-Hinton Agar (Müller-Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing) was prepared according to the manufacturer's instruction (Oxoid, UK), autoclaved and dispensed at 20 ml per plate in 12 x 12cm Petri dishes. Set plates were incubated overnight to ensure sterility before use. *Evaluation of antimicrobial activities*

Agar disk and agar well diffusion were used as screen tests to evaluate antibacterial property of essential oil of FV based on standard protocol. The solution of the essential oil was yielded in 1g/ml from which six fold serial dilutions (v/v) were prepared. 60 µl of each dilution was poured on each disk and well in order. After a period of 24 hours incubation, the diameters of growth inhibition zones around the disks and wells were measured. DMSO was used as negative control whereas kanamycin and cephalexin were used as positive controls in case of *E. coli* and *S. aureus*, respectively. Minimum inhibitory concentration (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. Minimum bactericidal concentration (MBC) means the lowest concentration of the agent which causes death to test bacteria. The last can be revealed by pouring 60 µl of MIC tube and six dilutions before contents on agar plate. In the case, after incubation period, the lowest concentration which makes no growth indicates MBC. For determination of MIC value, macrobroth dilution method was applied. Interpretation of the results was done due to national accepted letter[25].

*Statistical Analysis*

Antibacterial effect was determined by One way variance analysis (ANOVA), using the SPSS 18 software package. Data were considered statistically significant at $p \leq 0.05$.

## RESULTS

*Chemical composition*

16 compounds such as Trans-anethole (47.41 %), Limonene (32.21 %), β-Ocimene Z (2.41 %), Cisanethole (2.22 %), Fenchone (2.37 %), α-Fenchyl acetate (1.65 %), α-Phellandrene (1.22 %), β-Fenchyl acetate (1.12 %), β-Myrcene (0.73 %), α-Pinene (0.71 %), Germacrene-D (0.37 %), β-Ocimene E (0.23 %), βFarnesene (0.21 %), α-Copaene(0.14 %), Camphene (0.12 %), β-Pinene (0.09 %) representing 93/21% of the total essential oil composition of FV were identified using mass gas-chromatograph. The most substance found in FV essential oil was Trans-anethole. In contrast, *β-Pinene* was the least constituents discovered in the plant.

*Agar disk diffusion test*

In case of FV, the widest zone was formed due to 0.031 g/ml of the essential oil in *B. subtilis* culture, and it was no halo in 0.002 g/ml and less for both of bacteria. No

Table 1: The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of FV essential oil.

| Dilution(g/ml) | Inhibition zone in disk diffusion (mm) | |
|---|---|---|
| Microorganism | *P.* | *B. subtilis aeruginosa* |
| Positive control | 22 | 22 |
| 1/32 (0.031) | 11 | 14 |
| 1/64 (0.015) | 10 | 9 |
| 1/128 (0.007) | 9 | 8 |
| 1/256 (0.003) | 8 | 8 |
| 1/512 (0.002) | 0 | 0 |
| 1/1024 (0.001) | 0 | 0 |
| Negative control | 0 | 0 |

Table 2: The diameters of growth inhibition zones in agar well diffusion test in different dilutions of FV essential oil.

| Dilution(g/ml) | Inhibition zone in (mm) | |
|---|---|---|
| Microorganism | *P. aeruginosa* | *B. subtilis* |
| 1/32 (0.031) | 10 | 9 |
| 1/64 (0.015) | 9 | 8 |
| 1/128 (0.007) | 8 | 8 |
| 1/256 (0.003) | 8 | 0 |
| 1/512 (0.002) | 0 | 0 |
| 1/1024 (0.001) | 0 | 0 |
| Negative control | 0 | 0 |

inhibition zone was observed due to DMSO. Growth inhibition zones due to different dilutions are listed in table 1. *Agar well diffusion test*

In regard to FV essential oil, the widest zone was seen in 0.031 g/ml, due to *P. aeruginosa* (10 mm). It was no growth inhibition in 0.002 g/ml and less for all bacteria. No inhibition zone was observed due to DMSO. The data are discoverable in table 2.

*MIC* and *MBC ascertaining*

The values of MIC were acquired in 0.007 g/ml for *P. aeruginosa* and 0.002 g/ml for *B. subtilis*. The values of MBC are 0.015 g/ml for *P. aeruginosa* and *B. subtilis* (table 3).

## DISCUSSION

The use of plant compounds to remedy infections is an old practice in a large part of the world, especially in developing countries where there is dependence on traditional medicine for a versatility of diseases[26,27]. Interest in plants with antimicrobial properties has revived as a result of new obstacles associated with the use of antibiotics[28,29]. FV is a small genus of annual, biennial or perennial herbs distributed in central Europe and Mediterranean region[22,30]. Mature FV fruit and its essential oil are used as flavoring agents in food products such as liqueurs, bread, pickles, pastries, and cheese. They are also used as a constituent in cosmetic and pharmaceutical products[22,23]. FV is one of the medicinal plants which have been used for different purposes in traditional medicine of Iran, such as antimicrobial, antifungal, and antibacterial.

Table 3: MIC and MBC of essential oil of FV.

| Microorganism | *P. aeruginosa* | *B. subtilis* |
|---|---|---|
| MIC | 1/128 (0.007) | 1/512 (0.002) |
| MBC | 1/64 (0.015) | 1/64 (0.015) |

*Yield and analysis of FV*

Concerning the method of essential oil and preventing from using high temperature to decrease the rate of destruction of effective herbal compound. The most substance found in FV essential oil was Trans-anethole with 47.41 %. Trans-anethole is an alkyl alkylphenolether. Both the cis and trans isomers of transanethole occur in nature with the trans isomer always being the more abundant. Natural anethole occurs in FV essential oil. It has been shown to block grow of bacteria, inflammation and carcinogenesis. There is a partial difference between these results and the similar studies. The composition of medicinal plant can highly be affected by their secretary tissue condition and developmental stage[31]. The previous findings showed that terpenes, phenols, aldehydes and ketones are the major components of essential oils[32], and it is generally believed that essential oils principally performed against the cell cytoplasmic membrane of microorganisms. The hydrophobicity is an important characteristic of essential oils and their components which enables them to accumulate in cell membranes, disturbing the structures and causing an increase of permeability[33]. Some chemical constituents from FV have been identified as active antimicrobial principles such as a phenyl propanoid derivative – Dillapional was found to be the active antimicrobial principle of the FV stem. Another molecule – Scopoletin which is a coumarin derivative has been isolated from FV and reported to possess marginal antimicrobial effect[34].

*Antibacterial activity*

As the table showed, FV essential oil have prevented the growth of *P. aeruginosa* and *B. subtilis*. Also, by increasing the concentration of FV essential oil, the inhibition zone in many of samples increased. The results determined that in tested bacteria, there was a significant difference in terms of sensitivity to the essential oil. The FV essential oil have maximum activity against *B. subtilis* (14 mm), which is comparable with a zone of inhibition exhibited by cephalexin (22 mm). Also, the results indicated that essential oil of FV with 0.007 g/ml concentration has prevented *P. aeruginosa* and with 0.002 g/ml concentration has prevented *B. subtilis*, from the growth. In the study, the level of MBC was observed 0.015 g/ml for FV. Thus, the research represents the antibacterial effects of the medical herb on *P. aeruginosa* and *B. subtilis*. There have been several reports on FV essential oils, including reports on the relative concentration of FV antibacterial activity[35-37]. A number of authors have mentioned the antimicrobial activity of essential oils of the plant, however, the mechanism of action has not been studied in great detail[38,39]. The essential oil extracted from the fruits of FV exhibited antibacterial effect against foodborne pathogens such as *Bacillus megaterium* and *Listeria monocytogenes*[35,36,39]. The seed essential oil of FV has also been reported to possess antibacterial activity against some human pathogenic bacteria. Ethanol and water extracts of FV have shown activity against Campylobacter jejuni and Helicobacter pylori[40].

The results indicated essential oil of FV possess antibacterial effect, and the antibacterial activity of the essential oil was due to the presence of various active compounds. Hence, the phytochemical compounds responsible for the antibacterial effects of bacteria can be subjected to isolation of the therapeutic antimicrobials. Our results defend the use of the plant in traditional medicine and offer that FV possess compounds with good antibacterial properties. It can be used as antibacterial

supplements in the developing countries towards the development of new remedial agent.

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