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Full Length Research Paper

Composition and antimicrobial properties of essential oil of *Foeniculum vulgare*

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GC-MS analysis of essential oil obtained from the seed of *Foeniculum vulgare* showed the presence of 31 components containing 95.2% of the total amount and the major component was trans-anethole (70.1%). The analysis of ethanolic and methanolic seed extracts showed the presence of nine components including linoleic acid (56%), palmitic acid (5.6%) and oleic acid (5.2%). The antimicrobial activity of *F. vulgare* oil was assessed by using disk diffusion as well as minimum inhibitory concentration (MIC) method. Fennel oil showed inhibition against *Bacillus cereus*, *Bacillus magaterium*, *Bacillus pumilus*, *Bacillus substilis*, *Eschericha coli*, *Klebsiella pneumonia*, *Micrococcus lutus*, *Pseudomonos pupida*, *Pseudomonos syringae*, and *Candida albicans* as compared to methanolic and ethanolic seed extracts. The lowest MIC values of fennel oil for *Candida albicans* (0.4% v/v), *Pseudomonos putida* (0.6% v/v) and *E. coli* (0.8% v/v) was obtained. It was observed that essential oil and seed extracts of *F. vulgare* exhibit different degree of antimicrobial activities depending on the doses applied. Therefore, fennel oil could be a source of pharmaceutical materials required for the preparation of new therapeutic and antimicrobial agents

Key words: *Foeniculum vulgare*, essential oil, antibacterial agent, GC-MS analysis, analytical techniques.

INTRODUCTION

There is an urgent need to search for new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new infections disease as well as development of resistance to the antibiotics in current clinical use. The first compound with antimicrobial activity was found in the 1930s (Goodman et al., 1991). The development and use of these substance have increased because of the appearance of resistance strains and attention has turned to natural antimicrobial agents in recent years (Daferea et al., 2003) There have been many investigations on the antifungal, antibacterial, and antiviral preparations (Elagayyar et al., 2001) and individual compounds isolated from natural sources. Most of the plants used for medicinal purpose have been identified and their uses are well documented (Vander and Vietinck, 1991).

Essential oils and their components are widely used in medicine as constituents of different medical products, in the food industry as flavouring additives and also in cosmetics as fragrances (Singh et al., 2002). Many essential oils are known to exert antimicrobial activity, but the mechanism of action is often not entirely understood (Karaman et al., 2001). A major problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics, which leads to the insufficiency of antimicrobial treatment. The overuse of antibiotics and consequent antibiotic selection pressure is thought to be the most important factor contributing to the appearance of different kinds of resistant microbes (Valero and Salmeron, 2003).

Foeniculum vulgare Mill, commonly known as fennel, is a small genus of annual, biennial or perennial herbs. It is widely cultivated throughout the temperate and tropical regions of the world for its aromatic fruits, which are used as culinary spices (Akgül and Bayark, 1988). Steam distillation of dried fruit yields, an essential oil referred as 'Fennel oil', used for flavoring purposes in the many parts of the world (Abdallah et al., 1978; Lawrence, 1992).

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Fennel and its preparations are used to cure various disorders, and also act as a carminative, digestive and diuretic agent. Fennel increases elasticity of connective tissues and act as anti-aging agent (Arslan et al., 1989). There are some commercial pharmaceuticals with formula based on fennel essential oil. The antimicrobial properties of the essential oil have been also recognized (Elagayyer et al., 2001; Ruberto et al., 2000). The essential oil from plants has usually been isolated by either steam distillation or solvent extraction (Sajid et al., 2008). The chemical constituents and some antimicrobial properties of *F. vulgare* were studied previously (Cavalerio et al., 1993). The objective of present study was to assess level of essential oil and its fractionation by GC-MS as well as evaluation of antimicrobial activities of ethanol and methanol seed extracts of *F. vulgare.*

MATERIALS AND METHODS

Collection and preparation of samples

Ripe seed samples of *F. vulgare* were purchased from local market of Rawalpindi. Seed of *F. vulgare* were subjected to shadow drying followed by oven drying at 80°C for over night. The dried seed samples were converted into powder form and stored for further process. All chemicals used in this experiment were purchased from local dealers of Sigma (USA) and Merck (Germany).

Isolation of oil

The powdered seed (100 g) of *F. vulgare* were hydrodistilled in a Clevenger's type apparatus for 6 h and yellow colored oil (yield 3.8 %), 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 sterilized vial in a refrigerator in the dark at 4°C until use. The chemical analysis of fennel oil and extract was undertaken by Gas Chromatography (GC) and Gas Chromatography–Mass Spectroscopy (GC–MS) techniques as reported by Adams (1995) and Betts (1992).

GC-MS conditions for analysis of essential oils

Fractionation of fennel oil was carried out in Agilent (G 6890 N) gas chromatography net work system, under following conditions: column, HP innowax capillary; 60.0 m x 0.25 x 0.25 um; oven temperature programme, the column held initially at 60°C with 10°C /min heating ramp for 1 min and increased to 200°C with 10°C/min heating ramp for 1 min and increased to 200°C with 5°C /min heating ramp for 10 min. Then the final temperature was increased to 220°C with 5°C/min heating ramp for 20 min; injector temperature, 250°C; detector (FID) temperature, 275°C carrier gas (helium); inlet pressure, 40.60 psi; linear gas velocity, 39 cm/s; column flow rate, 1.5 ml/min split ratio, 40:1; injected volume, 1 µL. MS conditions ionization energy: 70 eV, ion source temperature: 280°C interface temperature: 250°C; mass range: 35 - 450 atomic mass units as reported by Adams (1995).

Identification of compounds

The percentages of oil components were obtained after triplicate analysis from electronic integration measurements using flame ionization detection (FID). The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes C_8 – C_{16}. Chemical constituents were identified by comparing their mass spectra with Wiley and Nast library

Antimicrobial analysis

The test microorganisms used in this experiment were obtained from the culture collections section of Pakistan Institute of Medical Research Islamabad. Those were further re-cultured for this experiment. The antimicrobial activities of the seed extracts of *F. vulgare* and fennel oil were investigated by disc-diffusion method on Muller-Hinton broth. It was performed using a 24 h old bacterial culture at 37°C, reseeded on Nutrient Broth. Fungi were reseeded on potato-glucose agar and incubated for 72 h at 20°C under alternating day-night light conditions. The cultures were adjusted to 5.6 x 106 CFU/ml with sterile water. 1 ml of the suspension was added over the plates containing Mueller Hinton broth to get a uniform microbial growth on broth control and test plates. The seed extracts were dissolved in ethanol and methanol (30 mg/mL) separately and sterilized. Under aseptic conditions, empty sterilized disc (Whatman no.5, 6 mm diameter) were impregnated with 20 µg of oil and seed extracts and placed on the agar surface. The plates were left for 30 min at room temperature to allow the diffusion of the oil or seed extracts, and they were incubated at 37°C. After the incubation period (24 h), the zone inhibition was measured in millimetre. Negative controls were prepared using the same solvents. Sulbactum, Cefoperazona, Ofloxan and Netilmicin were used as standard antibiotics for comparison. Minimum inhibitory concentrations (MICs) were determined after 24 h for bacteria and after 48 h for *C. albicans*. The MIC values are defined as the lowest concentration of any sample required for inhibiting the visible growth of organism on the agar plate. The organisms were maintained on blood agar (BA). Over night cultures were prepared by inoculating approximately 2 ml Muller Hinton broth (MHB) with 2 - 3 colonies of each organism taken from BA. Broths were incubated overnight at 35°C with shaking. Inocula were prepared by diluting overnight cultures in saline to approximately 10^8 cfu/mL from bacteria and 10^7 cfu / mL *C. albicans*. These suspensions were further diluted with saline as required.

Statistical analysis

The results were reported in the form of mean, standard deviation and percentage value of three measurements. The statistical analysis was performed by using one way ANOVA.

RESULTS AND DISCUSSION

Essential oil

Oil obtained from *F. vulgare* was further analyzed by GC-MS. A total of 33 compounds were found in essential oil of fennel by GC-MS equal to total 95.2%. Whereas trans-anthole was major components of oil (70.1%) followed by fenchone (6.9%) and methyl chavicol (4.8%) but the levels of other compounds were low (Table 1). The analysis of ethanolic and methanolic extracts of fennel fruit indicates linoleic acid (56.0%) followed by oleic acid (5.2%), whereas other compounds were present with lower concentrations (Table 2).

Table 1. Chemical composition of oil from *F. vulgar*.

Compounds	% FID	∂KI
Ethanol	Tr	-
Acetic acid ethyl ester	Tr	807
3 methylbutanol	0.2	832
2-methylbutanol	Tr	831
α-Thujene	Tr	831
α pinene	0.3	943
Camphene	Tr	854
Subinene	Tr	976
β-Pinene	0.3	981
Mycerene	0.2	994
δ-3-Carene	0.1	1014
α-Terpinene	Tr	1021
P-Cymene	2.0	1030
1,8-Cineole	3.2	1036
Trans-β-Ocimene	0.1	1052
γ-Terpinene	2.3	1065
Fenchone	6.9	1089
Linalool	1.3	1098
Camphor	0.4	1145
Beta-Terpineol	Tr	1144
Terpinen-4-0l	0.3	1176
α -Terpinol	0.4	1187
Methyl chavicol	4.8	1196
Fenchyl acetate	0.3	1223
Cuminal	0.5	1247
Cis-Anethole	0.4	1253
P-Anisaldehyde	0.4	1259
Trans-Anethole	70.1	1288
Thymol	0.2	1292
α-Copaene	0.2	1380
β-Caryophyllene	0.3	1421
Total	95.2%	-

Percentages (%) are mean of three replications obtained from electronic measurements using flame ionization detection (FID).
Tr = Trace.

Antimicrobial activity

According to the results given in Tables 3 and 4, essential oil of *F. vulage* had significant antimicrobial activities against some of microorganisms as compared to the methanolic and ethanolic seed extracts. Therefore in general, the essential oil showed better activity than the extracts. The diameters of growth inhibition zone ranged from 14 to 31 mm (including the diameter of the disc—6 mm) with the highest inhibition zone values observed against *B. magaterium* (31 mm) and *B. substilis* (29 mm). In all the extracts showed less activity than essential oil. However, some organisms showed resistance towards both oil and seed extracts. The essential oil showed greater or similar activity on Gram-positive and Gram-negative bacteria and *C. albicans* (Table 3) strains at a concentration of 100 µg/disk as compared to ofloxacin, (6 ug/disc) and miconazole nitrate (50 µg/disc) used as positive controls. The minimum inhibitory concentration (MIC) values of 0.4% (v/v) were observed against *C. albicans*, 0.6% for *P. putida* and 0.8% for *E. coli* (Table 4). Due to increase in incidence of new and re-emerging infectious disease and development of resistance towards antibiotics (Elagayyer et al., 2001), the oil and seed extracts of *F. vulgare* must be further explored in development of new and useful drugs for treating various infections of human and animals.

Higher concentration of *trans*-anethole (70.1%) was found in fennel oil which contains both antioxidants and antimicrobial activities (Muckenstrum et al., 1997). While,

Table 2. Chemical composition of ethanolic and methanolic extracts of *F. vulgare*.

Compound	% FID	
	Methanolic extract	**Ethanolic extract**
4 hydroxy-4-methyl-2 pentanone	0.2	0.1
Undecane	0.2	0.2
Cis-Anethole	0.5	0.4
trance anethole	0.6	0.5
Palmitic acid	5.6	5.4
Methyl oleat	0.3	0.2
Linoleic acid	56.0	53.0
Oleic acid	5.2	5.1
Stigmast-5-en-3-ol	0.6	0.5
Total	69.2	67.0

Percentages are mean of three replications obtained from electronic measurements using flame ionization detection (FID).

Table 3. Antimicrobial activity of seed extracts and oil of *F. vulgar* tested against bacterial and yeast strains by using disk diffusion method. Zone of inhibition in diameter (mm) around test disk.

Microorganism	Oil	Methanolic fruit extracts	Ethanolic fruit extracts
B. cereus	26 ± 0.4	18 ± 0.1	16± 0.5
B. magaterium	31 ± 1.1	25 ± 0.6	22 ±0.8
B. pumilus	28 ± 0.5	21 ± 0.3	19± 0.5
B. substilis	29 ± 0.45	19 ± 0.5	17± 0.3
B. gladioli	-	-	-
E. coli	16 ± 0.4	14 ± 0.5	12± 0.2
K. pneumonia	14 ± 0.5	11 ± 0.6	10 ± 0.5
M. lutus	14 ± 0.4	12 ± 05	11 ± 04
P. putida	17 ± 0.2	13 ± 0.4	11 ± 0.3
P. syringae	28 ± 0.5	24 ± 0.6	21 ± 0.5
S. aureus	-	-	-
S. epidermis	-	-	-
S. pneumonia	-	-	-
S. pyogenes	-	-	-
C. albicans	13 ± 0.3	10 ± 0.2	10 ± 0.7

- = Not tested

Table 4. The MIC (%, v/v) values of seed extracts *F. vulgar* tested against microorganism in microdilution assays.

Microorganism	Oil	Methanolic fruit extract
B. cereus	1.6	3.1
B. magaterium	1.4	3.7
E. coli	0.8	2.6
K. peneumonia	2.6	2.5
M. lutus	2.1	3.1
S. epidermis	1.9	2.1
P. syingae	2.6	1.3
P. putida	0.6	1.4
C. albicans	0.4	1.6

Patra et al. (2002) reported that antheole and its isomers are responsible for antimicrobial activities of fennel oil. Essential oils and their components are widely used in medicine, in the food industry and in cosmetics. There is an urgent need for familiarity with these products and their components because they are available over-the-counter from herbal suppliers and natural food stores and in many cases are not harmless. Due to antimicrobial activity, seed extracts of *F. vulgare* can be used as supplement in pharmaceutical industries. It can also be used in stabilizing food against oxidative deterioration. It was reported by Nenad et al. (2007) that the main advantage of natural agents is that they do not enhance the antibiotic resistance, a phenomenon commonly encountered with the long-term use of synthetic antibiotics. There are reports of the active principles of essential oils from various plants with antibacterial or antifungal activity. The antimicrobial activity of essential oils is assigned to a number of small terpenoids and phenolic compounds, which also in pure form demonstrate high antibacterial activity (Corner, 1993). The essential oils and their components are known to be active against a wide variety of microorganisms, including Gram-negative and Gram-positive bacteria. Gram-negative bacteria were shown to be generally more resistant than Gram-positive ones to the antagonistic effects of essential oils because of the lipopolysaccharide present in the outer membrane, but this was not always true. It is concluded that *F. vulgare* seed extracts and oil are rich in *trans*-anethole and other compounds and are effective against *C. albican, E. coli and P. putida* and other similar organisms. Therefore *F. vulgare* seed extracts and oil are valuable not only for increasing shelf life of foodstuffs but it could be a future target for replacing synthetic antibacterial agents.

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