

Antimicrobial Activity of Essential Oils of some Medicinal Plants from Saudi Arabia

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Abstract

Antibacterial and anticandidal activities of essential oils obtained from dill (*Anethum graveolens*) and fennel (*Foeniculum vulgare*) were studied by agar dilution technique. Test organisms were inoculated by radial streaking onto agar plates and incubated for 16–20 hours at 35°C. Antibacterial activity of dill and fennel was evaluated against *Mycobacterium* spp, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. Our results showed that the volatile oils extracted by steam distillation method from roots, stem and leaves of dill and fennel plants did not show antibacterial or anticandidal activities. However, seed extracts from both dill and fennel exhibited varying degrees of growth inhibition of *C. albicans*, *C. tropicalis* and *C. glabrata*. Extracts of dill and fennel seeds prepared by simple solvent extraction method, using acetone, petroleum ether, methanol and chloroform, did not show any antimicrobial activity against common bacterial or fungal pathogens. On the other hand, growth of some *Mycobacterium* species was inhibited by the seed extracts of both fennel and dill. Our results suggest that the anticandidal and antimycobacterial properties of these two herbs may be further investigated to explore the possibility of using them in the treatment of candidal or mycobacterial infections.

Key words: Antimicrobial activity, essential oils, medicinal plants, Saudi Arabia, dill (*Anethum graveolens*), fennel (*Foeniculum vulgare*).

Introduction

Essential oils and extracts from aromatic plants have long been used for a wide variety of medicinal and domestic purposes (Brown, 1995). Antimicrobial properties of essential oils obtained from aerial parts and seeds of aromatic plants such as oregano (*Origanum syriacum* var. *bevanii*), thyme (*Thymbra spicata* subsp. *spicata*), lavender (*Lavandula stoechas* subsp. *stoechas*), rosemary (*Rosmarinus officinalis*), cinnamon (*Cinnamomum zellanicum*), clove (*Syzygium aromaticum*), basil (*Ocimum basilicum*), ginger (*Zingiber officinale*), coriander (*Coriandrum sativum*), cilantro (*C. sativum* L.), eucalyptus (*Eucalyptus dives*), fennel (*Foeniculum vulgare*) and dill (*Anethum graveolens*) are well documented (Soylu, et al., 2006; Lo Cantore, et al., 2004; Kwon, et al., 2002; Elgayyar, et al., 2001; Ruberto, et al., 2000; Jirovetz, et al., 2003; Singh, et al., 2002; Delaquis, et al., 2002; Mazyad, et al., 1999; Chevallier, 1996).

Dill (*Anethum graveolens*), also known as Shapt or dill-weed, is from the family Umbelliferae, and is an annual herb growing to a height of 1.5m. Major compounds found in the essential oil of dill includes furanocoumarin, 5-(4''-hydroxy-3''methyl-2''-butenyloxy)-6,7-furocoumarin, oxypeucedanin, oxypeucedanin hydrate and falcarindiol, all reported to have various degrees of antimycobacterial activity (Stavri & Gibbons, 2005). In addition, D-limonene and D-carvone, found in dill oil extract, have also been shown to possess high antifungal activity against *Aspergillus niger*, *Saccharomyces cerevisiae* and *Candida albicans* (Delaquis, et al., 2002; Jirovetz, et al., 2003). Other contents of dill essential oil includes d-phellandrene, pinene, diterpene, dihydrocarvone, cineole, myrcene, paramyrcene, dillapiole, isomyristicin and myristicin (Jirovetz, et al., 2003; Stavri & Gibbons, 2005). Myristin, apiol and dillapiol present in dill essential oil are effective naturally occurring insecticides. Myristin

in dill is psychoactive and hallucinogenic and the apiol content may be responsible for the diuretic properties (Broiwn, 1995; Chieg, 1984; Stavri, *et al.*, 2005; Lopez, *et al.*, 2005; Delaquis, *et al.*, 2002; Grieve, 1984; Holtom & Hylton, 1979; Mazyad, *et al.*, 1999; Singh, *et al.*, 2002; Laurnet, 1981; Chevallier, 1996).

Fennel (*Funicular vulgare*) also belongs to the same botanical family, Umbelliferae. Other names include Shamer, Finocchio, carosella and Florence fennel. Fennel is a sweet, aromatic, diuretic herb that relieves digestive problems, increases lactation, relaxes spasms and reduces inflammation with expectorant, carminative and aromatic properties (Ostad, *et al.*, 2001; Ozbek, *et al.*, 2003; Ensminger, 1986). Extracts from fennel plant has been used as an antispasmodic, diuretic, analgesic and antipyretic and has antimicrobial properties; it can also be used for skin disorders, conjunctivitis and blepharitis of the eye (Ruberto, *et al.*, 2000; Wood, 1988; Ozbek *et al.*, 2003; Ostad, *et al.*, 2001; Fortin, ...; Ensminger, *et al.*, 1986).

Previous reports have shown that fennel essential oil contains anethole, estragole and fenchone plus an additional 18 compounds which include alpha-pinene, camphene, B-pinene, alpha-phellandrene, myrcene, limonene, B-phellandrene, gamma-terpinene, cis-cimene, terpinolene, carvacrol, camphor, borneol, cineol and p-cymene (Karlsen, *et al.*, 1969; Kwon, *et al.*, 2002; Rubert, *et al.*, 2000; Mimica-Dukic, *et al.*, 2003). These compounds, especially carvacrol, borneol, camphor and anethole, have been reported to inhibit the growth of *Phytophthora infestans*. Sporangial production was inhibited by the essential oil of fennel (Soylu, *et al.*, 2006).

Despite the enormous number of studies done on the antimicrobial properties of aromatic plants, very little work has been published on the antimicrobial properties of *Foeniculum vulgare* (fennel) *Anethum graveolens* (dill) utilizing microbial and fungal isolates. The present study was conducted to extract essential oils from fennel and dill plants using hot water steam distillation and organic solvent extraction techniques, and evaluate *in vitro* the antimicrobial activity of extracted oils on the growth of common pathogens such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and some *Mycobacterium* species. It is hoped that the present report will contribute

to the existing state of knowledge about the antimicrobial properties of aromatic plants.

Materials and Methods

Aromatic Plants

Mature and fresh dill and fennel plants were purchased from vegetable supermarket in Riyadh city (Saudi Arabia) while seeds were purchased from traditional and folk medicine stores. Plants and seeds were both identified by a plant taxonomist at the Botany Department, College of Science, King Saud University, Riyadh (Saudi Arabia). Plants and seeds were brought to the laboratory and processed immediately for the extraction.

Extraction of Essential Oils

Essential oils can be extracted using a variety of methods, although some are not commonly used today. Currently, the most popular method for extraction is steam distillation in which water is heated to produce steam that carries the most volatile chemicals of the aromatic material with it. The steam is then chilled (in a condenser) and the resulting distillate is collected. The Essential Oil normally float on top of the Hydrosol (the distilled water component) and may be separated off.

We employed hot water steam distillation method to extract Dill and Fennel essential oils. Briefly, 250 grams of fresh and cleaned roots, leaves and stem of the plants were placed into a pot, 500 ml of sterilized distilled water was added and the contents were boiled for 30 minutes. Dill and fennel seeds were processed by following the same procedure (250 grams of seeds / 500 ml distilled water) was performed. This mixture was allowed to cool and then squeezed to obtain the extract. Finally, this extract was subjected to conventional steam distillation using the Clevenger apparatus for a period of 3-5 hours. The oil obtained was kept refrigerated and protected from direct light.

Extraction of essential oils from these two herbs was also done by simple solvent extraction method using four solvents namely, acetone, petroleum ether, methanol and chloroform. The seeds (250 grams) of both dill and fennel plants were macerated in a mortar and pestle and then saturated with respective solvents in separate flasks with frequent shaking or rotation. The solvent dissolved all extractable matter from the seeds including non-aromatic material and highly volatile aromatic molecules. The

solution, containing both solvent and dissolvable seed material, was filtered and the filtrate was allowed for 24 hours evaporation at room temperature (22 - 23°C) for the total evaporation of alcoholic solvent to separate the oil. The extracted volatile oils were stored refrigerated until further use.

Microorganisms

Antimicrobial activity tests were carried out against *Staphylococcus aureus* (ATCC 25923), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 10231), *Mycobacterium smegmatis* (ATCC 35797), *Mycobacterium chelonae* (ATCC 35751) and *Mycobacterium xenopi* (ATCC 14470). In addition, *C. albicans*, isolated from the vaginal swab of a vaginal infection patient at King Khalid University Hospital (KKUH), Riyadh, *C. tropicalis* and *C. glabrata* (isolated from the urine of a male urinary tract infection patient, KKUH) were also tested. Organisms were maintained on blood agar.

Antimicrobial assay

The antimicrobial screening protocol was essentially the agar dilution method described by Mitscher *et al.* (Mitscher *et al.*, 1972) (24) for the evaluation of antimicrobial activity in the extracts of higher plants. Extracted oils from both dill and fennel were evaluated at a concentration of 1000 µl/ml in dimethyl sulfoxide (DMSO) by dilution with 10 ml of molten blood agar at 45-50°C. The agar and extracted oil were mixed thoroughly and the mixture was poured into a Petri dish on a levelled surface to obtain an even agar depth of 3-4 mm. Inocula for the screening assay were prepared

by growing overnight cultures of bacteria in Mueller-Hinton broth. The cultures were diluted to 1:1000 in broth to give 10⁴ colony forming units (CFU) per µl of the inoculum. Plates for the determination of anticandidal activity of dill/fennel oil were prepared by dispensing 15 ml of sterile Sabouraud dextrose agar (SDA) into 100 x 15 mm. sterile Petri dishes. The inocula were prepared by addition of 1 ml of overnight *Candida* cultures to 9 ml of Mueller-Hinton broth to yield 10⁴ colony forming units (CFU) per µl of the inoculum.

Sterile cotton-tipped applicators were used to streak the entire surface of agar plates. Cylindrical plugs were removed from the solidified agar plates, using sterile cork borer, to produce wells having a diameter of approximately 11 mm. Then 100 µl of the volatile oil and/or extract was added to each well. Dimethyl sulfoxide (DMSO) was used as a negative control in all the experiments. Plates are then incubated at 30–32 °C for 24 hours. Antimicrobial activity was recorded as the width (in millimetres) of the clear zone of inhibition surrounding the agar well. The results were reported as positive (+) if there is inhibition of growth and negative (-) if there is no inhibition of growth. Triplicate sets of plates were prepared on each occasion and experiments were repeated three times. The mean of three readings was calculated and used in the analysis.

Results

The results shows a wide variety Very little quantity of oil (250–300 µl/250 grams) could be extracted from the roots, stem and leaves from both dill and fennel by water steam distillation method. Table 1 shows the *in vitro* effect of these essential oils on the growth of *S.aureus*, *B. subtilis*, *E. coli*, *Ps. aeruginosa* and *C.*

Table 1. Determination of anti-bacterial and anti-candidal activities of Dill and Fennel volatile oil extracted by water steam distillation method.

Tested Organism	Dill				Fennel			
	Root	Stem	Leaves	Seeds	Root	Stem	Leaves	Seeds
<i>S. aureus</i> ATCC 25923	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>B. subtilis</i>	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>E. coli</i> ATCC 25922	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>P. aeruginosa</i> ATCC 27853	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
<i>C. albicans</i> ATCC 10231	(-)	(-)	(-)	(+)	(-)	(-)	(-)	(+)

(-) = no inhibition of microbial growth

(+) = inhibition of microbial growth

albicans. According to our results, volatile oil extracted from the roots, stem and leaves of both Fennel and Dill did not show any inhibition of the growth of the above mentioned test organisms. On the other hand, slightly better quantity of oil could be extracted from the seeds of both plants (1.25 – 1.50 ml / 500 grams) using steam distillation method. Extracted seed oil was tested on to inoculated discs with the test organisms. Table 1 show that no inhibition of the growth was seen in *S.aureus*, *B. subtilis*, *E. coli* and *Ps. aeruginosa* except *C. albicans* where considerable inhibition of growth was observed.

In order to identify some of the other common *Candida* species that might be sensitive to Dill and Fennel oils, further testing was performed employing the similar experimental conditions. Table 2 shows the anticandidal activity of Dill and Fennel seed oils extracted by steam distillation method. Of the four *Candida* isolates used in this experiment, *C. tropicalis* and *C. glabrata* were isolated from the urine specimen of a patient having lower urinary tract infection while *C. albicans* was isolated from the vaginal swab of a female patient having vaginal infection. Varying zones of inhibition were observed; a higher antifungal activity was observed in Dill volatile oil against all tested *Candida* species, ranging from 20-40 mm zone of inhibition. Similar results were obtained with Fennel oil showing a zone of inhibition of 18 – 20 mm against all the *Candida* species except *C. glabrata* where no inhibition of growth was observed (Table 2).

Antimicrobial activity of Dill and Fennel seed extracts obtained by simple solvent extraction method was also evaluated against same set of pathogens. Table 3 shows the antibacterial and anticandidal activities of Dill and Fennel extracts using acetone, petroleum ether, methanol and chloroform. According to our results, no antibacterial or anticandidal activity was seen in any of the four extracts against *S. aureus*, *B. subtilis*, *E. coli*, *Ps. aeruginosa* and *C. albicans*.

However, when these solvent extracts were tested against some *Mycobacterium* species, considerable amount of antibacterial activity was observed against *M. chelonae*, *M. xenopi* and *M. smegmatis* in all of the four Dill extracts. Similarly, methanol and petroleum ether extracts of Fennel also inhibited the growth of *M. chelonae*, *M. xenopi* and *M. smegmatis*. However, acetone and chloroform extracts from fennel did not show any type of antimycobacterial activity. In

Table 2. Anticandidal activity of Dill and Fennel volatile oil extracted from seeds by water steam distillation.

<i>Candida</i> species	Source of Tested Isolates	Zone of inhibition (millimetres)	
		Dill volatile oil	Fennel volatile oil
<i>C. albicans</i>	ATCC 10231	40 mm	20 mm
<i>C. albicans</i>	Vaginal Swab	35 mm	20 mm
<i>C. tropicalis</i>	Urine	40 mm	18 mm
<i>C. glabrata</i>	Urine	20 mm	0.0 mm

addition, essential oils obtained from Dill and Fennel through steam distillation were also tested against these *Mycobacterium* species. Our results indicated that neither of the two volatile oils exhibited any type of activity against *Mycobacterium* species (Table 4).

Discussion

Essential oils are natural plant products which accumulate in specialized structures such as oil cells, glandular trichomes, and oil or resin ducts. The formation and accumulation of essential oils in plants have been thoroughly reviewed by Croteau (1986), Guenther (1972) and Runeckles and Mabry (1973). Chemically, the essential oils are primarily composed of mono- and sesquiterpenes and aromatic polypropanoids synthesized via the mevalonic acid pathway for terpenes and the shikimic acid pathway for aromatic polypropanoids (Croteau,1986; Guenther,1972; Runeckles & Mabry, 1973). The essential oils from aromatic plants are for the most part volatile and thus, lend themselves to several methods of extraction such as hydrodistillation, water and steam distillation, direct steam distillation, and solvent extraction (ASTA 1968; Guenther 1972; Heath 1981). The specific extraction method employed depends upon the plant material to be distilled and the desired end-product. The essential oils which impart the distinctive aromas are complex mixtures of organic constituents, some of which being less stable, may undergo chemical alterations when subjected to high temperatures. In this case, organic solvent extraction is required to ensure no decomposition or changes have occurred which would alter the aroma and fragrance of the end-product.

In the present study, volatile oils from both dill and fennel plant leaves, stems, roots and seeds, extracted by hot water steam distillation as well as organic solvent

Table 3. Antimicrobial activity of Dill and Fennel extracts by simple solvent extraction method.

Plants	Extracts	<i>S. aureus</i> ATCC 25923	<i>B. subtilis</i> ATCC 6633	<i>E. coli</i> ATCC 25922	<i>Ps. aeruginosa</i> ATCC 27853	<i>C. albicans</i> ATCC 10231
Dill	Acetone	(-)	(-)	(-)	(-)	(-)
	Petroleum ether	(-)	(-)	(-)	(-)	(-)
	Methanol	(-)	(-)	(-)	(-)	(-)
	Chloroform	(-)	(-)	(-)	(-)	(-)
Fennel	Acetone	(-)	(-)	(-)	(-)	(-)
	Petroleum ether	(-)	(-)	(-)	(-)	(-)
	Methanol	(-)	(-)	(-)	(-)	(-)
	Chloroform	(-)	(-)	(-)	(-)	(-)

(-) = no inhibition of microbial growth

(+) = inhibition of microbial growth

Table 4. Antimicrobial activity of Dill and Fennel extracts by simple solvent extraction method against some Mycobacterium species.

Plants	Type of extracts	<i>M. smegmatis</i> (ATCC 35797)	<i>M. chelonae</i> (ATCC 35751)	<i>M. xenopi</i> (ATCC 14470)
Dill	Acetone	(+)	(+)	(+)
	Petroleum ether	(+)	(+)	(+)
	Methanol	(+)	(+)	(+)
	Chloroform	(+)	(+)	(+)
	Volatile oil	(-)	(-)	(-)
Fennel	Acetone	(-)	(-)	(-)
	Petroleum ether	(+)	(+)	(+)
	Methanol	(+)	(+)	(+)
	Chloroform	(-)	(-)	(-)
	Volatile oil	(-)	(-)	(-)

(+) = inhibition of Mycobacterium growth

(-) = No inhibition of Mycobacterium growth

extraction methods, showed no inhibitory activity to our selected Gram positive and Gram negative bacteria (Tables 1,3). This is in contrast to several other studies that have reported antibacterial activity of these essential oils. (Soylu, *et al.*, 2006; Lo Cantore, *et al.*, 2004; Kwon, *et al.*, 2002; Elgayyar, *et al.*, 2001; Ruberto, *et al.*, 2000; Fyfe, *et al.*, 1997; Stavri & Gibbons, 2005; Lopez, *et al.*, 2005; Jirovetz, *et al.*, 2003; Mazyad, *et al.*, 1999; Singh, *et al.*, 2002; Delaquis, *et al.*, 2002). This may be either due to the high volatility of the oil, leading to the escape

or evaporation of its major antibacterial constituents during boiling or the insufficient release of the oil during extraction. Lemberkovic, *et al.* (2003) has recently shown that the composition of essential oils in aromatic plants is greatly affected by the method of extraction, mainly the distribution of monoterpenes, monoterpene-esters, mono- and sesquiterpene compounds and azulene sesquiterpene. Furthermore, Hili *et al.*, (1997) has reported that the use of dispersing solvent (DMSO) reduced the antimicrobial activity of cinnamon oil to 50-folds when no dispersant solvent was used. In our study, we also employed DMSO as the diluent for both dill and fennel oils/extracts prior to the preparation of agar plates for antimicrobial assay.

Our results showed that essential oils from dill and fennel seeds had considerable antimycobacterial and anticandidal properties. In a study by Lo Cantore *et al.*, however, essential oil extracted from fennel showed a lesser antibacterial effect compared to coriander oil in inhibition of *Escherichia coli* and *Bacillus megaterium* (Lo Cantore, *et al.*, 2004). The antimycobacterial property observed in the oil and extract is probably due to the presence of furanocoumarin in dill (Stavri & Gibbons, 2005). Further studies are, however, needed to reconfirm the antimycobacterial properties of fennel extract and identify the active component through advanced techniques, such as GC-MS.

Only a few studies have reported the antifungal properties of essential oil of dill and fennel (Soylu, *et al.*, 2006; Elgayyar, *et al.*, 2001; Mimica-Dukic, *et al.*, 2003). Our results confirm earlier reports that both

essential oils and solvent extracts from these plants have proven antifungal effects. These extracts were consistently found to be effective on fungal growth by inhibition of sporangial production. In a separate study done by Kwon YS, dillapional, scopoletin, dillapiol, bergapten, imperatorin and psoralen found in fennel stem extracts have antimicrobial activity against *Bacillus subtilis*, *Aspergillus niger*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Cladosporium cladosporioides* (Kwon, *et al.*, 2002; Elgayyar, *et al.*, 2001).

In conclusion, essential oils from both fennel and dill have shown considerable antifungal activity against commonly encountered *Candida* species in our study. The lack of antibacterial activity in both fennel and dill extracts, obtained either by hot-water steam distillation or simple extraction method, may be due to the absence or denaturation of some of the active components of the essential oils which are responsible for bacteriostatic or bactericidal activities. The analysis of the chemical composition of our extracts by GC-MS might have identified these constituents in our preparations. On the other hand, the detection of antimycobacterial activity in our extracts establish the need for further studies. The antifungal characteristics of these two herbs can be further investigated to be used in the treatment of fungal infections. These essential oils can be highly inhibitory to selected pathogenic and spoilage microorganisms, and may provide better alternatives and/or supplements to the conventional antimycobacterial or antifungal additives in foods.

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النشاط المضاد للميكروبات في الزيوت الطيارة لبعض النباتات الطبية في المملكة العربية السعودية

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الملخص

تم دراسة النشاط المضاد للبكتيريا والمضاد للكانديدا للزيوت الطيارة المتحصل عليها من الشبت (*Anethum graveolens*) والشمر (*Feoniculum vulgare*) بطريقة التخفيف على الآجار، حيث اختبرت الكائنات الحية بتخطيطها على الأطباق وتحسينها لمدة ١٦-٢٠ ساعة عند درجة حرارة ٣٥ م°. تم دراسة تأثير النشاط المضاد للبكتيريا لكل من الشبت والشمر على الأحياء الدقيقة التالية:

Mycobacterium spp. *Staphylococcus aureus* *Bacillus subtilis* *Escherichia coli*

Pseudomonas aeruginosa و *Candida albicans*

أوضحت النتائج أن مستخلص الزيوت الطيارة بالتقطير البخاري من جذور، سيقان، وأوراق نباتين الشبت والشمر لم تظهر أي نشاط مضاد للبكتيريا أو الكانديدا، في حين أن مستخلص الزيوت الطيارة للبدور أظهر تثبطا بدرجات متفاوتة على كل من *C. albicans* *C. tropicalis* and *C. glabrata* كذلك نجد أن مستخلص البذور للنباتين الشبت والشمر في المذيبات العضوية (اسيتون، بتروليم أثير، ميثانول، والكلوروفورم) لم تظهر أي نشاط مضاد للبكتيريا أو الفطريات الممرضة، في حين أنه ثبت نمو أنواع من *Mycobacterium*. من هذه النتائج يمكننا مواصلة الدراسة للتحقق من امكانية استخدام هذه الأعشاب في علاج الاصابات بالكانديدا و الميكوبكتيريا.