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Evaluation of the Antibacterial Activity of Different Parts of *Foeniculum vulgare* (fennel) Extracts

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ABSTRACT

Background & Aim: *Foeniculum vulgare* Mill. (Apiaceae) is a medicinal and aromatic plant. Various pharmacological experiments in vitro and in vivo models have convincingly showed *F. vulgare* ability in antifungal, antibacterial, antioxidant, antithrombotic and hepatoprotective activities.
Experimental: The purpose of this study was to evaluate the antibacterial activity of extracts of *Foeniculum vulgare* on the number of pathogen bacteria. For this purpose, ethanol, methanol and acetone extracts (through maceration method) of different parts of the *Foeniculum vulgare* (flowers, leaves and fruit) were prepared and antibacterial activity was tested against some gram positive (*Staphylococcus aureus, Bacillus cereus*) and gram negative (*Pseudomonas aeruginosa, Escherichia coli*) bacterial species through standard disc diffusion method.
Results: The findings showed that the most sensitive bacteria to the extracts was *Staphylococcus aureus* and the most resistant bacteria was *Pseudomonas aeruginosa*. According to the gained results in the case of fennel flower and fruits extract, methanol and acetone extract showed the highest and lowest antibacterial activity, respectively, while in the case of fennel leaves ethanolic extract exhibited the most inhibitory effect. In general, the results of this study indicated that the extract of fennel flowers had more antibacterial properties in comparison with other parts of plant.
Recommended applications/ industries: The antibacterial activity of fennel is due to the presence of terpenoids and phenolic compounds. These compounds are potentially effective on membrane permeability of bacteria and thereby cause the cell death. Therefore, fennel extract can be used to control bacteria resistant to several antibiotics.

1.Introduction

The increasing resistance of many clinical strains of bacteria to antibiotics has led to look for safe and effective agents for the treatment of chronic bacterial infections (Javadian *et al.*, 2014). Thus much attention is devoted to the production of new antibacterial agents that are used to control pathogens (Brković *et al.*, 2006). Medicinal plants are important sources of potentially useful new compounds for the development of antimicrobial agents. The first step towards this goal is screening the plants which are very used in medicine (Manonmani and Mohideen, 2011). Many of the herbs used in traditional medicine are much cheaper than

modern drugs and easily available in rural areas. Plants generally produce secondary metabolites that are used as an important source of microbicides, pesticides and many pharmaceutical drugs. Herbal products are still the main source of pharmacological agents used in traditional medicine and they have attracted great interest (Shahid *et al.*, 2013). *Foeniculum vulgare* Mill. is a biennial medicinal plant belonging to the family Apiaceae (Umbelliferae) (Shahat *et al.*, 2011). It is a hardy, perennial umbelliferous herb with yellow flowers and feathery leaves (Javadian *et al.*, 2014). Essential oil of fennel is used as flavoring agents in food products such as beverages, bread, pickles, pastries, and cheese. It is also used as a component of cosmetic and medicenal products (Shahat *et al.*, 2011).

F. vulgare has been reported to contain 6.3% of moisture, 9.5% protein, 0% fat, 13.4% minerals, 18.5% fibre and 42.3% carbohydrates (Brković *et al.*, 2006). The minerals and vitamins present in *F. vulgare* are calcium, potassium, sodium, iron, phosphorus, thiamine, riboflavin, niacin and vitamin C. It has antioxidant, antitumor, chemopreventive, cytoprotective, hepatoprotective, hypoglycemic oestrogenic, anti-inflammatory, antimicrobial, bronchodilatory, estrogenic, diuretic, lithontripic, galactogogue, emmenagogue, antithrombotic, hypotensive, gastroprotective, memory enhancing activities. Recently, antimicrobial activities of fennel seed extracts and essential oils have been studied and the value of this commonly used kitchen spice has been discovered (Javed *et al.*, 2012). The purpose of this study was to evaluate the antibacterial activity of different parts of the *Foeniculum vulgare* (flowers, leaves and fruit) extracts on the number of pathogen bacteria.

2. Materials and Methods

2.1. Preparation of plant Samples

Plant samples were collected from University of Agriculture and Natural Resources of Ramin of Khuzestan and identified based on classification criteria in the Department of Biology, University of Shahid Chamran. Then different parts of plant (flowers, leaves and fruits) were placed in an oven for 75 hours at 25 ° C to dry completely. After that, plant samples were powdered by using electric mill. This obtained powder was used for extraction of bioactive compounds by maceration method with different solvents.

2.2. Maceration method

The finely powered samples were weighed and 5g of samples were dissolved in various organic solvents including 80% ethanol, methanol and acetone. Following 48 hours incubation at room temperature and gentle mixing, the samples were filtered using Whatman filter paper to separate the filtrate for antimicrobial assays (Seenivasan *et al.*, 2010).

2.3. Antibacterial assay

In order to discover antibacterial activity of different extracts Kirby-Bauer disc diffusion method was used. Briefly, sterile blank discs were saturated with different concentrations of extracts (25, 50, 100, 200 mg/ml). So each disc approximately absorbed 40 µl of diluted extract. The test bacteria including *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633) as gram positive species and *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC9027) as gram negative species were inoculated into Muller Hinton broth (Merck, Germany) and incubated at 37°C till providing 0.5 McFarland turbidity. Then a lawn culture was prepared from each species on Muller Hinton agar (Merck, Germany) and the prepared discs were placed on these cultures. Simultaneously standard antibiotic discs were also used. After 15 min at room temperature, the plates were incubated at 37°C for 24h and the inhibition zone diameter (mm) was measured and recorded. All tests were done as triplicates (Seyyednejad *et al.*, 2014).

3. Results and discussion

According to present results, all extracts obtained from different parts of the fennel plant using various solvents showed different anti-bacterial properties. The result of the antibacterial activity of fennel flowers is presented in Table 1.

The results showed that the highest concentration of acetone extract (200 mg/ml) had maximum effect on *Bacillus subtilis* (Gram-positive) with a diameter of inhibition zone (12 mm), but no antibacterial activity was observed for this extract against other Gram-negative bacteria tested in this study. The highest concentration of ethanol extract of fennel flowers showed maximum effect on the four tested bacteria (with a maximum diameter inhibition 11.3 mm) and the

lowest concentration of ethanol extract (25mg/ml) had no antibacterial activity. Methanol extract of fennel flowers showed significant effect on the Gram-positive bacteria. The highest concentration of methanol extract (200 mg/ml)of fennel flowers had maximum effect on *Staphylococcusaureus* (Gram-positive) with a diameter of inhibition zone of 15 mm and the lowest concentration of methanol extract was more effective on this bacteria too (with a diameter of inhibition zone of 10.3 mm). In general in this study, methanol and acetoneextract of fennel flowers showed the highest and lowest antibacterial activities, respectively.

Table 1. Inhibition zone diameter (mm) of fennel flower extract.

Solvent	Extract concentration (mg/ml)	*S. aureus*	*B. subtilis*	*E.coli*	*P. aeruginosa*
Acetone	25	-	-	-	-
	50	-	-	-	-
	100	8.0±0.8	10.3±1.0	-	-
	200	8.7±1.3	12.0±0.5	-	-
Ethanol	25	-	-	-	-
	50	-	6.0±0.3	-	-
	100	9.0±0.5	5.3±1.0	8.3±1.0	8.3±1.5
	200	11.3±1.5	10.3±0.8	10.0±1.5	9.7±0.5
Methanol	25	10.3±0.8	12.3±0.5	-	-
	50	12.0±1.0	10.3±0.3	-	-
	100	13.3±1.5	10.7±1.0	-	-
	200	15.0±0.5	9.0±0.3	8.0±0.5	9.3±0.8

The result of the antibacterial activity of the extracts of fennel leaves is presented in Table 2. According to the results, the highest concentration of acetone extract of fennel leaf had maximum effect on *Bacillus subtilis* (Gram-positive) with a diameter of 10 mm and other tested concentrations did not show any antibacterial activity. The lowest concentration of ethanol extract of fennel leaves had the maximum effect on Gram-positive bacteria (*Bacillus subtilis)* with a diameter of 12 mm and the highest concentrations of this extract had maximum effect on both tested Gram-positive bacteria. The high concentration of methanol extract (100 and 200 mg/ml) of fennel leaves had the maximum effect on *Staphylococcus aureus* (with a diameter of 6.7 and 10.3 mm, respectively) while other it did not show any antibacterial activity on *B. subtilis*and two Gram-negative bacteria. Generally, ethanol extract of fennel leaves had the most antibacterial properties.

Table 2. Inhibition zone diameter (mm) of fennel leaf extract.

Solvent	Extract concentration (mg/ml)	*S. aureus*	*B. subtilis*	*E.coli*	*P. aeruginosa*
Acetone	25	-	-	-	-
	50	-	-	-	-
	100	-	-	-	-
	200	6.7±0.8	10.3±0.3	8.3±0.5	7.7±0.3
Ethanol	25	-	-	-	-
	50	7.7±1.0	7.3±1.3	-	-
	100	9.3±1.5	10.0±1.5	-	-
	200	10.0±0.3	12.0±1.0	8.7±0.3	8.3±0.5
Methanol	25	-	-	-	-
	50	-	-	-	-
	100	6.7±0.3	-	-	-
	200	10.3±0.5	-	-	-

According to Table 3, the highest concentration of acetone extract of fennel fruit had maximum effect on the *E. coli* and *Staphylococcus aureus* (with a diameter of 10 mm) and the lowest concentration of this extract did not show any antibacterial effects. The highest concentration of ethanol extract of fennel fruits only affected *Bacillus subtilis* (with a diameter of 10 mm) and other tested concentrations didn't show any antibacterial activity. Also, antibacterial activity was not observed for the methanolic extract. According to the gained results methanol and acetone extracts of fennel fruits showed maximum and minimum antibacterial activities, respectively.

Table 3. Inhibition zone diameter (mm) of fennel fruit extract.

Solvent	Extract concentration (mg/ml)	*S. aureus*	*B. subtilis*	*E.coli*	*P. aeruginosa*
Acetone	25	-	-	-	-
	50	8.0±0.2	-	-	-
	100	9.0±0.5	8.0±0.5	8.0±0.2	-
	200	10.0±0.50	8.0±0.2	10.0±0.5	-
Ethanol	25	-	-	-	-
	50	-	-	-	-
	100	-	-	-	-
	200	-	10.0±0.75	-	-
Methanol	25	-	-	-	-
	50	-	-	-	-
	100	-	-	-	-
	200	-	-	-	-

The results of this study indicated that the extract of fennel flowers in comparison with other components of fennel had more anti-bacterial properties. The findings showed that the most effective solvent was methanol and *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the most sensitive and resistant bacteria bacteria to the extracts, respectively.

Recently, many researchers have turned to the extraction of bioactive compounds from natural sources. Global attention is on the formulation of natural products based on the absence of toxicity, their complete biodegradability, their availability from renewable sources, and in many cases their low cost compared to similar chemical compound (Akeel *et al.*, 2014).

Fennel is an example of natural resources that can extract its bioactive compounds to use for antibacterial purposes. According to present results *Staphylococcus aureus* and *Pseudomonas aeruginosa* showed the most and lowest inhibition zone. This is in coincidence with those of earlier studies by Akeel *et al.* (2014). According to Manzoor *et al.* (2012) antibacterial activity of fennel is dedicated to terpenoids and phenolic compounds. They stated that these purified compounds exhibited high antibacterial activity. Phenolic compounds are important plant active compounds, which have antimicrobial activity and can act as a defense mechanism against pathogenic microorganisms. Due to the hydrophobic properties of phenolic compounds, these compounds are potentially affects the membrane permeability of bacteria and thereby it disrupt the bacterial cell membrane structure and cause to more permeability that it would leak out ions and other cell contents (Manzoor *et al.*, 2012). Although the withdrawal of limited quantities of these substances is tolerable for bacteria but it affects the viability and leaving a large amount of cell contents or exit of ions and vital molecules caused the death of cell (Tringali, 2004).

Kooti *et al.* (2015) reported that *Foeniculum vulgare* extract had a significant antibacterial effect against a lot of bacteria except Pseudomonas aeruginosa. The potential mechanisms of anti-microbial activity of *Foeniculum vulgare* is due to the presence of active compounds with antimicrobial activity such as oleic acid and coumarin in aqueous and alcoholic extract.

Dua *et al.* (2013) noted that fennel extract caused damage to the bacterial cell membranes of gram positive bacteria leading to the leakage of the biomolecules from cells, but *E.coli* and *P.aeruginosa* were resistant. Gallic acid, among the phenolics and quercetin among the flavanols had the most antimicrobial activity in the methanolic extract of fennel. Presence of high amount of gallic acid,

quercetin and kaempferol along with other polyphenols in the extract indicates high efficacy of fennel as a reducing agent and its possible role as antimicrobial agent (Dua *et al*., 2013).

Patra *et al.* (2002) reported that antheole and its isomers are responsible for the antibacterial activity of fennel and it can be used as additives in pharmaceutical industries as well as in stabilizing foods against oxidation.

In general, we indicated that the extracts of different parts of fennel inhibited the growth of gram-positive bacteria more than gram-negative ones. Gram-positive bacteria have peptidoglycan compounds in outer membrane while Gram-negative bacteria have only a thin layer of peptidoglycan and a large part of the wall is lipoprotein and lipopolysaccharide and for this reason they are more resistant to the antagonistic effects of fennel extracts (Gulfraz *et al.*, 2008).

According to the data we obtained fennel fruit does not have appreciable antibiotic properties unlike other researchers' findings such as Gulfraz (2008).

The results showed that fennel extract has antibacterial effect on all bacteria strains (Except in the fruit part that have little impact) so its' extract can be used to control multiple-antibiotic resistant bacteria.

4. Conclusion

Foeniculum vulgare has emerged as a good source of traditional medicine and it provides a noteworthy basis in pharmaceutical biology for the development/ formulation of new drugs and future clinical uses. The results of this study indicate that methanol extract of fennel flowers in comparison with other solvents and other parts of fennel have more anti-bacterial properties and it can be used as a new source of antibiotics against pathogens.

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