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Short Communication

Antimicrobial Screening of Fennel at the Seedling Stage

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Abstract

A wide range of medicinal plant parts that possess varied medicinal properties are used for extracts as raw drugs. In this study, the ethanolic extract obtained from aerial parts of fennel (*Foeniculum vulgare* Mill.) seedlings was evaluated *in vitro* to examine their antimicrobial activity against four Gram-negative and three Gram-positive bacteria and two fungi. Aerial parts of one-month-old seedlings were air dried and powdered. Each powdered sample (20 g) was extracted with 200 ml ethanol (96%) using a shaking water bath for 24 h at room temperature. The solvent was removed under vacuum at 40°C using a rotary vacuum evaporator. The antimicrobial effect of the aerial part of fennel seedlings was tested by the disc diffusion method. The experiment was a randomized complete block with three replicates for each sample. Fennel seedling extract had no inhibitory effect against Gram-positive bacteria and fungi, but had a weak effect against Gram-negative bacteria.

Key words*:* Antimicrobial activity, Bacteria, Fennel, *Foeniculum vulgare*, Fungi

Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably from plant origin [1, 2]. According to recent estimates about 25% of the drugs used presently come from higher plants [3]. Natural products are of great interest in the process of drug discovery, due to their large diversity in nature, permitting the identification of lead molecules of greater interest for the development of new therapeutic agents [4]. A wide range of medicinal plant parts that possess varied medicinal properties is used for extracts as raw drugs. The different parts used include roots, stems, flowers, fruit, twigs and modified plant organs [5]. Some biologically active compounds isolated from spices and herbs have been used to inhibit the growth of pathogenic microorganisms because of the resistance that those microorganisms have built up against antibiotics [6]. The emergence and spread of antimicrobial resistance is an array of problems caused by various interconnected factors, many of which are related to misuse and overuse of antibiotics [2]. Fennel (*Foeniculum vulgare* Mill.) is used as a spice and also as an important ingredient in various folklore medicines throughout the world. Moreover, this plant has been extensively investigated for its several medicinal and therapeutic activities and has been reported to possess carminative, flavoring, antioxidant, antibacterial, antifungal and mosquito-repellent properties [4, 7, 8, 9]. The aim of this study was to evaluate the antimicrobial potential of fennel seedlings.

Materials and Methods

Preparation of plant samples

Fennel seeds (provided from Zeitoon Talaee Kavir Co., Esfahan, Iran) were surface sterilized with a solution of water and bleach (95:5) for 10 min. Seeds were placed on sterilized filter paper in 6-cm open Petri dishes to germinate. Three-day-old seedlings were sown in plastic pots filled with perlite. Each pot, into which 15 seedlings were sown, was 14 in diameter, 8 cm in height, and contained 150 g perlite. Hoagland's solution [10] was used to irrigate the pots. Since the pots did not have drainage, the nutrient solution was applied only on the first day. The pots were weighed after the first irrigation, and same mass was maintained by

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irrigating with distilled water daily for one month. Hoagland's solution was applied anew every 2 weeks. Samples were maintained in a growth chamber with a 16-h photoperiod (25 °C during the day and 15 °C at night) at a light intensity of 8000 lux.

Extraction from shoots

Aerial parts of one-month-old seedlings were air dried and powdered. Each powdered sample (20 g) was extracted with 200 ml ethanol (96%) using a shaking water bath for 24 h at room temperature. The extracts were separated from solids by filtering through Whatman No. 1 filter paper. The remaining residue was re-extracted twice and the extracts were pooled. The solvent was removed under vacuum at 40°C using a rotary vacuum evaporator.

Antimicrobial testing

The antimicrobial effect of the aerial part of fennel seedlings was tested by the disc diffusion method [11]. The discs (6.4 mm in diameter) were impregnated with 10 µl of 200 mg/ml extract. Each disc had 2 mg of extract. Ten µg of Gentamicin, Ampicillin and Ketoconazole (Padtan Teb, Iran) were used as positive controls for Gram-negative bacteria, Gram-positive bacteria and fungi, respectively. The microorganisms *Staphylococcus aureus* (PTCC 1112), *Escherichia coli* (PTCC 1330), *Salmonella typhi* (PTCC 1609), *Pseudomonas aeruginosa* (PTCC 1074), *Bacillus subtilis* (PTCC 1023), *Klebsiella pneumonia* (PTCC 1053), *Aspergillus niger* (PTCC 5010), *Candida albicans* (PTCC 5027) and *Staphylococcus epidermidis* (PTCC 1114) were used in this experiment. All microorganisms were obtained from the Persian Type Culture Collection (PTCC), Tehran, Iran. Microorganisms were cultured for 16-24 h at 37 °C and prepared to turbidity equivalent to McFarland Standard No. 0.5 [12]. The suspensions (100 µl including 10^8 cfu/ml bacteria and or 10^6 cfu/ml fungi) were then spread on a plate of nutrient agar (HiMedia, India) for the antibacterial assay or on Sabouraud dextrose agar (CONDA, Spain) for the antifungal assay. The bacterial samples were incubated for 24 h at 37 °C and fungal Petri dishes for 24-48 h at 20 °C. The extract and controls were tested in duplicate and the experiments were repeated four times. Antimicrobial activity was evaluated by measuring the inhibition zone in mm.

Statistical analysis and computations

All experiments were organized in a randomized complete block with three replicates each. Raw data were imported to Microsoft Excel for calculations and graphical representation. SPSS version 17.0 was used for analysis of variance and comparison of means by Duncan's multiple range test at $\alpha \leq 0.05$.

Results and Discussion

The ethanolic extract of fennel seedlings had no significant effect against the tested Gram-positive bacterial and fungal samples (Fig. 1), and a low effect against experimented Gram-negative bacteria; the greatest effect was against *Klebsiella pneumonia* and *Escherichia coli*, respectively (Table 1).

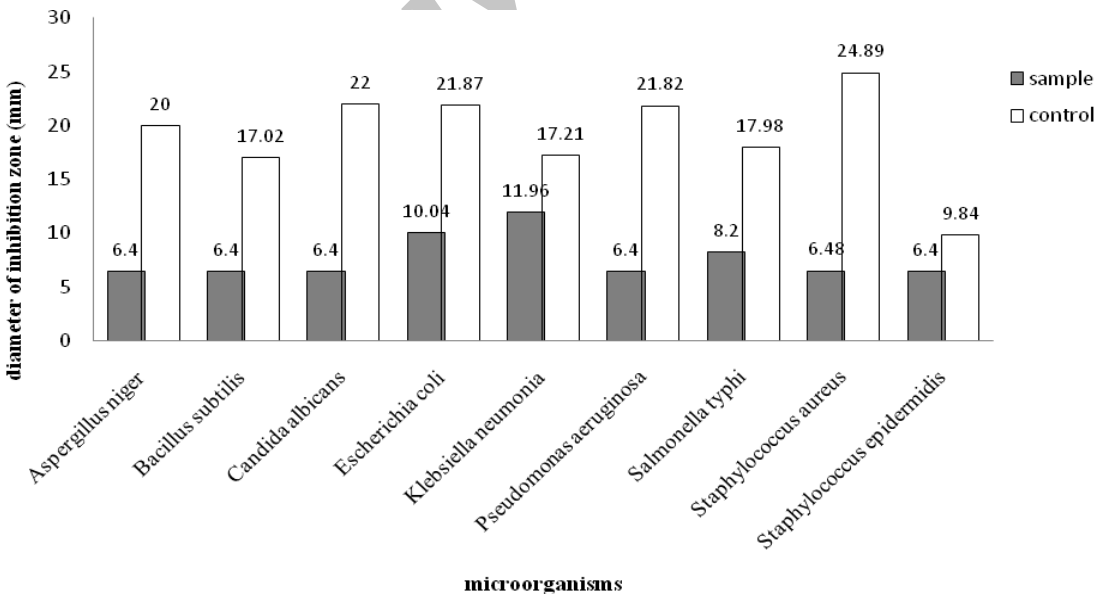

Fig 1. Antimicrobial activity of fennel seedling extracts against examined microorganisms (the numbers show the diameter of inhibition zone). Diameter of the disc was 6.4 mm.

Table 1 Diameter of inhibition zone of fennel seedling extracts and three positive controls against four Gram-negative bacteria (GN), three Gram-positive bacteria (GP) and two fungi (F). Diameter of the disc was 6.4 mm.

Microorganisms	Diameter of inhibition zone of fennel seedling extracts (mm)	Diameter of inhibition zone of Gentamicin (mm)	Diameter of inhibition zone of Ampicillin (mm)	Diameter of inhibition zone of Ketoconazole (mm)
Aspergillus niger (F)	6.40	-	-	20.00
Bacillus subtilis (GP)	6.40	-	17.02	-
Candida albicans (F)	6.42	-	-	22.00
Escherichia coli (GN)	10.04	21.87	-	-
Klebsiella neumonia (GN)	11.96	17.21	-	-
Pseudomonas aeruginosa (GN)	6.40	21.82	-	-
Salmonella typhi (GN)	8.20	17.98	-	-
Staphylococcus aureus (GP)	6.47	-	24.89	-
Staphylococcus epidermidis (GP)	6.40	-	9.84	-

Anwar *et al*. [6] also reported that 30 mg/ml fennel seed extracts (300 µg/disc) showed no antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Aspergillus niger*, *Fusarium solani* and *Rhizopus solani*. According to Al-Ismail *et al*. [13], the ethanolic extract of fennel leaves (2-4 mg/disc) had a weak anti-*Bacillus cereus* activity. The presence of dillapional (a phenyl propanoid derivative) and scopoletin (a coumarin derivative) in the stems of fennel [14] may be responsible for the low antimicrobial effect against Gram-negative bacteria in this experiment.

Conclusion

Antimicrobial effects of fennel seeds have been shown [6] but the results of this study indicate that fennel seedling extract had weak or no inhibitory effect against several tested microorganisms.

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