Original Article

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A Study of the Antioxidant and Antimicrobial Effects of Ethanolic Extract of Fennel (Foeniculum vulgare Mill) Seeds

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ABSTRACT

BACKGROUND AND OBJECTIVE: Synthetic additives are used in food industry to prevent contamination of food and reduction of microbial growth, while special attention have been paid to the use of natural anti-bacterial products because of higher food safety. Since plants are potential sources of anti-infective agents, the present study was conducted to study the antioxidant and antimicrobial effects of ethanolic extract of fennel (Foeniculum vulgare Mill) seeds.

METHODS: In this experimental study, the ethanolic extract was prepared after obtaining the fennel seeds. To analyze the antimicrobial effect of the extract, the Micro dilution method was used based on 9 concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39% on food isolates (kept in microbial bank) and standard strains of *Escherichia coli* 1270 PTCC, *Salmonella enterica* 1709PTCC, *Bacillus cereus* 11778ATCC and *Staphylococcus aureus* 1112PTCC. To change the antioxidant effect of the ethanolic extract of fennel, 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used in 7 concentrations of 1000, 500, 400, 300, 200, 100 and 50 ppm.

FINDINGS: *Salmonella enterica* food isolates showed highest level of resistance (2.3 of isolates grew at concentrations above 12.5%) while *Staphylococcus aureus* isolates showed highest level of sensitivity (without growth at concentrations above 12.5%) against ethanolic extract of fennel ($p>0.05$). In all the examined concentrations, the antioxidant effect of the ethanolic extract of fennel seed was reported to be less than synthetic antioxidants BHA (Bytalyted Hydroxy Toluene) ($p<0.05$).

CONCLUSION: According to the results of this study, the ethanolic extract of fennel seed benefits from appropriate antibacterial and antioxidant properties and thus can be used in combination with other preservatives to preserve food against various oxidative systems and microorganisms that cause infection and intoxication.

KEY WORDS: *Fennel, Ethanolic extract, Antimicrobial, Antioxidant*.

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Introduction

Nowadays, food corruption and poisoning caused by microorganisms is still the most important challenge in food industry and for the consumers of these products, even in developed countries (1). To prevent food contamination in food industry, synthetic additives are used to reduce microbial growth and inhibit microorganisms (2).

Because of consumers' sensitivity to the safety of food products that contain synthetic chemicals, more attention have been paid to the use of natural antibacterial products for preserving food in recent years (3). Considering the drug resistance and side effects of the chemical antibacterial drugs, the approaches of scientific researches have inclined to natural resources in recent decades. Several studies have proved the antimicrobial effects of various plants (4).

Overall, it is clear that the growth of microorganisms that cause food corruption and foodborne pathogens reduce the quality of food by reducing the quality of nutrients in food such as fat, protein and carbohydrates, which leads to color change, mildew, biochemical changes, weight loss and toxicity. These changes are dangerous for consumers (2).

Essences and extracts of medicinal plants are suitable for being used as preservatives for raw and processed food because of their antimicrobial, anticancer and antioxidant compounds as well as agents that scavenge free radicals (5).

Fennel (Foeniculum vulgare Mill) is a gramineous and aromatic plant from Umbelliferae family, which looks like Dill with yellow flowers and has oral and therapeutic use (6). This plant has two subspecies; the vulgare subspecies has sweet seeds and is used as flavor in cooked foods such as meat, fish, ice cream, etc. (7). The seed of this plant has anti-flatulence properties, improves eyesight and increases milk in breastfeeding women (8).

Fennel seed has traditionally been used as anti-inflammatory, analgesic, diuretic and antispasmodic plant (9). The methanolic extract of fennel seed has anti-inflammatory and inhibitory effects of delayed hypersensitivity reactions (10). Fennel seed extracts are used for heart diseases (11).

The essence of fennel seeds has shown antibacterial effects against foodborne pathogens such as *Escherichia coli*, *Bacillus megatrium*, *Staphylococcus aureus* (12) and *Listeria monocytogenes* (13). Several studies have been conducted about the antimicrobial effect of the essence and extract of fennel. In a study in Turkey, the most significant antimicrobial effect of the essence of fennel was observed to be on *Staphylococcus aureus* (14).

On the other hand, little antimicrobial activity was reported for the essence of fennel in other studies (15, 16). In a study conducted on some plants, the antibacterial effect of fennel extract was observed on *Bacillus megatrium* (3). In a study in Kerman, the effect of methanolic extract on *Bacillus megatrium* and *Staphylococcus aureus* was reported (17).

Synthetic antioxidants such as Butylated hydroxytoluene (BHT) are not advised to be used in foods because of potential risk of carcinogenesis and concerns regarding food safety (9). In a study, the acetonic extract and essence of fennel showed strong antioxidant activity compared with Butylated hydroxytoluene (18).

The aqueous and ethanolic extract of fennel seed also showed antioxidant activities in other researches. 100 µg ethanolic extract of fennel inhibited 77.5% peroxidation from linoleic acid system and this effect was more than the same amount of alpha-tocopherol (36.1%) (19). This study aims to analyze the antioxidant and antibacterial effects of ethanolic extract of fennel seed.

Methods

Sampling and extraction: In this experimental study, fennel (Foeniculum vulgare Mill) seeds were obtained from Medicinal Plant Centers in Maragheh (April 2015). Maceration method and ethanol solvent were used for seed extraction. For this purpose, 300 g dried seeds of fennel plant were poured into 7.5 liters ethanol (at 85 ˚C) in three turns and they were kept at lab temperature for 48 hours each time. The solution was filtered with filter paper and then the filtered extracts were evaporated in a rotary device.

Preparing the isolates and bacterial strains: 3 isolates of *Escherichia coli* (isolated from local cheese), *Salmonella enteric* (isolated from red meat), *Bacillus cereus* (isolated from rice) and *Staphylococcus aureus*

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(isolated from local cheese), which were previously identified and kept in the Microbial Bank of Microbiology Laboratory in Azad University of Tabriz, were used to analyze the antimicrobial effect of the ethanolic extract of fennel seed. The standard strains of *Escherichia coli* PTCC 1270, *Salmonella enteric* PTCC 1709, *Bacillus cereu*s ATCC 11778 and *Staphylococcus aureus* PTCC 1112 were used as control.

Measuring the antimicrobial activity of the ethanolic extract using Microdilution method: Microdilution method, that is the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), was used to analyze the antimicrobial effect of the ethanolic extract of fennel seed. 100 µl brain-heart infusion medium (BHI) was poured into one row of microplate wells (except for the first well) and then, 100 µl ethanolic extract was poured into the 1st and 2nd well and 100 µl of second well contents was poured into the 3rd well and this action continued until the 9th well and finally, 100 µl of the 9th well content were poured away. Therefore, 9 concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39% ethanol extract of fennel seed were found in the 1st to the 9th well. 0.5 McFarland standard was prepared from the new bacterial culture and 100 µl of 1.100 dilution was added to all wells except for the 11th and 12th wells.

In the next step, 30 µl resazurin reagent (blue or purple) was added to all wells. The 10th well was used as bacteria control, the 11th well was used as culture medium control and the 12th well was used as extract control.

Then, one well with intermediate color change (considered as the minimum inhibitory concentration [MIC]) along with 2 wells before and 2 wells after that well were cultured in Brain Heart Infusion Agar and were incubated after 24 hours at 37 ˚C. Each plate with wells in which no bacterial growth occurred is considered as the minimum bactericidal concentration (MBC) (20).

Measuring the antioxidant activity (free radical scavenging activity) using DPPH method: Measuring the antioxidant activity by deactivating the free radicals was done using 2,2-diphenyl-1-picrylhydrazyl (DPPH) material and decolorizing the purple color of this material (21 , 22). First, 500 µl of methanolic solution of DPPH was prepared. Then, various concentrations of BHT were prepared as reference antioxidant and 4 ml of each concentration were transferred to foiled test tubes and were mixed with 1 ml DPPH. Finally, 30 minutes after solution absorbance at a wavelength of 517 nm, it was measured using spectrophotometer (23). The test was done for the synthetic antioxidant of Butylated hydroxytoluene and 7 concentrations of extract were prepared (concentrations of 1000, 500, 400, 300, 200, 100 and 50 ppm) and free radical scavenging activity (RSA%) was calculated according to the following formula:

$$RSA\% = (A_c - A_s)/A_c \times 100$$

Results

In 12.5% and higher concentrations of the ethanolic extract of fennel, only one isolate of the three *Salmonella enteric* isolates was not able to grow (two isolates grew), whereas none of the studied bacteria isolates were able to grow at concentrations higher than 12.5%, indicating higher resistance of *Salmonella enteric* isolates compared with other tested isolates. *Salmonella enteric* isolates in food revealed the highest significant level of resistance to the ethanolic extract of fennel compared with other studied bacteria ($p>0.05$). Furthermore, *Staphylococcus aureus* isolates in food revealed the highest significant level of susceptibility against the ethanolic extract of fennel compared with other studied bacteria ($p>0.05$).

Escherichia coli and *Bacillus cereus* bacteria isolates revealed similar level of susceptibility against the ethanolic extract of fennel. The standard strains of *Escherichia coli*, *Salmonella enteric*, *Staphylococcus aureus* and *Bacillus cereus* revealed highest to lowest level of resistance to the ethanolic extract of fennel, respectively.

The standard strain of *Escherichia coli* revealed more resistance to the ethanolic extract of fennel compared to *Escherichia coli* isolates in foods. The standard strain of *Bacillus cereus* revealed more susceptibility to the ethanolic extract of fennel compared to *Bacillus cereus* isolates in foods (table 1). A comparison between the antixodiant properties of ethanolic extract of fennel with BHT demonstrated that in identical concentrations, the antioxidant effect of BHT is much stronger than ethanolic extract of fennel ($p<0.05$). As the concentration of the ethanolic extract of fennel increased, its antioxidant effect also increased (table 2).

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Table 1. The antimicrobial effect of the ethanolic extract of fennel on the studied bacteria

Extract (%) Bactria	50≤	25	12.5	6.25	3.12	1.56≤
*E. coli*PTCC 1270	+	+	+	+	+	+
E. coli	-3	-3	-1	+3	+3	+3
S. enteric PTCC 1709	-	-	+	+	+	+
S. enteric	-1	-1	-1	+3	+3	+3
S. aureus PTCC 1112	-	-	-	-	-	+
S. aureus	3-	3-	-2	-2	-1	+3
B. cereus ATCC 11778	-	-	-	-	-	-
B. cereus	-3	-3	-1	+3	+3	+3

Bacterial growth (+), Lack of bacterial growth (-)

Table 2. Comparing the antioxidant properties of the ethanolic extract of fennel and BHT

Extract concentration	1000 ppm	500 ppm	400 ppm	300 ppm	200 ppm	100 ppm	50 ppm
Extract (%)	31.42	17.33	15.92	12.63	6.99	3.71	1.83
BHT (%)	93.75	93.51	93.24	92.86	92.53	90.41	77.97

Discussion

Results of the present study demonstrated that *Salmonella enterica* and *Staphylococcus aureus* isolates had the highest and lowest level of resistance to the ethanolic extract of fennel, respectively, which is consistent with the results of a study by Shahat et al., which reported that *Staphylococcus aureus* has highest level of susceptibility against fennel essence (19). Manonmani et al. reported that the ethanolic extract of fennel does not have antibacterial effects on *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* (11).

Gulfraz et al. demonstrated that the antimicrobial effect of ethanolic extract of fennel on gram positive bacteria was more than gram negative bacteria, which is consistent with the present results (25).

Anwar et al. also reported that the antimicrobial effect of the fennel essence is more on gram positive bacteria and *Escherichia coli* revealed to be more resistant to the fennel essence compared with *Bacillus cereus* (9). Overall, gram negative bacteria are more resistant to the antimicrobial effects of herbal essences compared with gram positive bacteria, which is due to their lipopolysaccharide (LPS) layer (25).

In another study about the effect of the ethanolic extract of fennel on 15 microorganisms including *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*, no developmental inhibitor effect was observed (26), which is not consistent with the present study. These differences may be due to differences between the studies isolates in term of geographic or ecologic origin.

The standard gram negative bacteria strains showed more resistance to the ethanolic extract of fennel compared with gram positive strains, which also applied to the tested bacteria isolates. Overall, the higher the level of phenolic materials is, the more antibacterial effects on pathogenic bacteria will be (27).

In this study, the antimicrobial effect of the ethanolic extract of fennel was analyzed using Microdilution method. A review of previous studies show that Microdilution method is the most common method for measuring the antibacterial effects of the extract and essence of various plants (20, 24).

In the present study, comparing the antioxidant properties of the ethanolic extract of fennel with BHT demonstrated that at similar concentrations, the antioxidant effect of BHT is much stronger than the ethanolic extract of fennel and as the concentration increases, the antioxidant activity of the extract increases.

Anwar et al. demonstrated that 80% ethanolic extract of fennel had the strongest antioxidant activity compared with its essence and as the concentration increased, the antioxidant activity of the extract increased. Moreover, the ethanolic extract of fennel revealed less antioxidant activity compared with BHT (9), which is consisted with the present study.

Shahat et al. demonstrated that the extract of vulgare variety of fennel has less antioxidant activity compared with synthetic BHT (19), which is consistent with the results of the present study. Mata et al. reported that the antioxidant activity of the ethanolic extract of fennel is more than BHT (28), which is not consistent with the results of the present study.

Lack of consistency between two independent studies may indicate the differences in chemical compounds that constitute the essence and extract of a

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particular plant in different regional, climatic, geographic and age conditions. Bagamboula et al. believe that differences in the constituents of the essence of a particular plant may depend on geographical region of the plant, changes in soil, climatic changes, age of the plant, harvest season, the parts used for essence extraction, method of essence extraction and the type of solvent used (29). Considering the antimicrobial and antioxidant effects of the ethanolic extract of fennel in Maragheh region, the efficient ratios of these compounds can be used as antimicrobial and antioxidant material as well as a flavor for foods.

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