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Antibacterial Effects of *Thymus daenensis* Celak Ethanolic Extract Against Food-borne Bacteria

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THYMUS daenensis is a native medicinal plant of Iran with high pharmacological effects. The present survey was performed to evaluate the antibacterial effects of *T. daenensis* extract toward *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Pseudomonas aeruginosa* food-borne bacteria. Ethanolic extract of *T. daenensis* was prepared from plant's aerial parts. The diameters of the growth inhibition zones of bacteria against this extract and amoxicillin antibiotic disk was assessed by the disk diffusion test. Minimum inhibitory concentration was assessed using the broth micro-dilution method. The diameter of the growth inhibition zones of different concentrations of *T. daenensis* extract had the ranges between 7.00±0.54 to 15.00±1.09 mm. These findings revealed that the increase in the concentration of *T. daenensis* extract have caused significant increase in the diameter of the growth inhibition zone ($P \leq 0.05$). Antibacterial effects of different concentrations of *T. daenensis* extract were lower than that of amoxicillin disk. *T. daenensis* extract had the highest antibacterial effects against *P. aeruginosa*. Additionally, *T. daenensis* extract had the highest antibacterial effects at lower concentrations (25 mg/mL) toward *S. aureus*. *T. daenensis* extract had the lowest MIC against *S. aureus* (6.00±1.00 mg/mL). The MIC of *T. daenensis* extract toward *P. aeruginosa* was 10.00±1.00 mg/mL. Antibacterial effects of *T. daenensis* ethanolic extract was determined in this survey. According to antibacterial effect of *T. daenensis* extract against food-borne bacteria, its application as a food preservative should potentially assess.

Keywords: Antibacterial, *Thymus daenensis*, Ethanolic extract, Food-borne bacteria.

Introduction

Medicinal plants and herbal extracts have a major role in complementary medicine, particularly against diverse kinds of diseases and disorders in human [1-3]. They mainly contain extensive ranges of significant antibacterial, antifungal, antioxidant, anti-cancer, analgesic, wound and burn healing, sedative, and etc. compounds [4-6]. As a result, their applications have been increased in the folk medicine.

Food-borne diseases are considered as one of the most critical issues mainly caused by food-borne bacteria [7-12]. The most important and prevalent bacteria responsible for severe and lethal cases of food-borne diseases are *Staphylococcus aureus* (*S. aureus*), *Listeria monocytogenes*

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(*L. monocytogenes*), *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) [13-16]. They mainly caused severe food-borne diseases with high outbreak rates which caused severe economic burden due to the coast of treatment and hospitalization of the patients [17-19]. Treatment of these diseases that are occurred due to infection caused by these food-borne bacteria needs antibiotic therapy. However, diverse researches specified incidence of antibiotic resistance of these bacteria toward hazard use of antibiotic agents [20-27]. As a result, antibacterial producing factories are looking to use new herbal compounds as alternatives.

Thymus species, particularly *T. daenensis*, are indigenous medicinal plants of Iran which frequently used for their taste and flavour in food stuffs. Additionally, *T. daenensis* harbors diverse medical, biological and pharmacological activities including antibacterial, anti-fungal, anti-inflammatory, anti-septic, antispasmodic, and antioxidant properties [28]. Furthermore, it is mainly used as carminative, tonic, antitussive, and digestive agent in Iranian folk medicine [28, 29].

Up to now, diverse investigations have been recorded the antibacterial effects of *T. daenensis* [28, 29]. However, none of them were not addressed the antibacterial effects of this medicinal plant on food-borne bacteria. Thus, the present survey was conducted to assess the antibacterial effects of *T. daenensis* extract toward *S. aureus*, *L. monocytogenes*, *E. coli* and *P. aeruginosa* bacteria *in vitro* condition.

Materials and Methods

Plant materials and extract

From June to October 2019, aerial parts of the flowering stage of *T. daenensis* were collected from Shahrekord, Iran. *T. daenensis* samples were identified by a professor who is a member at the Department of Medicinal Plants, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran (Herbarium Number 496). Plants were dried at shed and then were powdered using mill (Culatti DFH48). Then, 25 g of the *T. daenensis* powders were placed in ethanol (70%, Merck, Germany) for about 72 h. The extracts were then filtered (Whatman No. 1) and finally the filtered solution was dried (25 °C) [30].

Bacterial strains

S. aureus ATCC 6538, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 27853 and *L. monocytogenes* ATCC 19111 were used in the present study. These bacterial strains were obtained from the Microbiology Research Center of the Islamic Azad University, Shahrekord Branch. Bacteria were subcultured by inoculation into nutrient broth (Merck, Germany) media and further incubation at 37 °C for about 24 h.

Disk diffusion test

In order to assess the antibacterial effects of *T. daenensis* extract, instructions of Clinical and Laboratory Standard Institute (CLSI) were used [31]. For this purpose, simple disk diffusion method was applied. Diverse concentrations of *T. daenensis* extract including 6, 12.5, 25, 50 and 75 mg/mL were prepared. Additionally, amoxicillin disk (25 µg/disk) was used for comparison of dimeter of the growth inhibition zone of growth bacteria. Bacterial strains (0.5 McFarland standard concentration) were cultured on to Mueller-Hinton Agar (MHA, Merck, Germany) media. Sterile paper discs (6 mm diameter) were separately impregnated with 10 µL aliquots of *T. daenensis* extract concentrations and placed onto the seeded top layer of the inoculated MHA plates. Moreover, amoxicillin disk was placed in each plates contained bacteria. Plates contained the discs were allowed to stand for at least 30 min before incubated at 37°C for 24 h. Data interpretation was performed according to the diameter of the growth inhibition zone of bacteria toward different concentrations of *T. daenensis* and also antibiotic disk according to CLSI [31]. Distilled water was used as negative control in all reactions.

Minimum Inhibitory Concentration (MIC)

Broth micro-dilution method was used to assess the MIC values of *T. daenensis* extract against *S. aureus*, *L. monocytogenes*, *E. coli* and *P. aeruginosa* bacteria according to standard method [32]. A 96-wells micro-plate was used for this purpose. Nutrient broth (Merck, Germany) was used as test medium. A final concentration of 1.5×10^6 CFU/ml of all bacteria were prepared spectrophotometrically (Shimadzu, Japan) by regulating the optical density to 0.1 at 600 nm. At that time, 100 µL of each concentration of *T. daenensis* extract (4, 6, 8, 10 and 12 mg/mL) was added in wells contained 95 µL of nutrient broth and 5 µL of 1.5×10^6 CFU/mL of each bacterium, separately. At that time, micro plate was incubated at 37°C for 24 h. The lowest concertation of *T. daenensis* extract which can inhibit growth of bacteria was considered as MIC.

Statistical analysis

Data were presented as mean ± standard deviation (SD). Then, data were transferred to Excel software and the SPSS. Ver. 21 (Chicago, USA) was used for statistical analysis. Comparison of means were assessed by the one-way analysis of variance (ANOVA) and turkey test. Finally, *P* value ≤0.05 was determined as significant level.

Results

Disk diffusion test

The dimeter of growth inhibition zones of *S. aureus*, *L. monocytogenes*, *E. coli* and *P. aeruginosa* bacteria treated with different concentrations of *T. daenensis* extract and also amoxicillin were measured. Table 1 describes the dimeter of growth inhibition zones of bacteria toward *T. daenensis* extract and amoxicillin. According to obtained data, increase in the concentration of *T. daenensis* extract caused significant increase in the diameter of the growth inhibition zone (*P* ≤0.05). However, antibacterial effects of different concentrations of *T. daenensis* extract were lower than amoxicillin disk. Findings revealed that *T. daenensis* extract had the highest antibacterial effects against *P. aeruginosa*. Additionally, *T. daenensis* extract had the highest antibacterial effects at lower concentrations (25 mg/mL) toward *S. aureus*. The diameter of the growth inhibition zones of different concentrations of *T. daenensis* extract had the ranges between 7.00±0.54 to 15.00±1.09 mm. However, the diameter of the growth inhibition zones of amoxicillin had the ranges between 14.00±0.71 to 16.00±1.24 mm.

MIC values

Table 2 describes the MIC values of *T. daenensis* extract against examined bacteria. Obtained data showed that *T. daenensis* extract had the lowest MIC against *S. aureus* (6.00±1.00 mg/mL). The MIC of *T. daenensis* extract toward *P. aeruginosa* was 10.00±1.00 mg/mL. However, MIC values were not obtained for *E. coli* and *L. monocytogenes* bacteria.

Discussion

The present survey was conducted to assess the antibacterial effect of *T. daenensis* extract against diverse food-borne pathogens including *S. aureus*, *L. monocytogenes*, *E. coli* and *P. aeruginosa in vitro* condition. Findings revealed that *T. daenensis* extract harbored considerable

TABLE 1. Dimeter of growth inhibition zones of bacteria toward *T. daenensis* extract and amoxicillin.

Bacteria and antibiotic agent	Diameter of growth inhibition zone (mm) of different concentrations (mg/mL) of *T. daenensis* extract					Amoxicillin	*P* value*
	6	12.5	25	50	75		
S. aureus	-	-	7.00±0.54 b	11.00±0.85 a	13.00±1.14 a	15.00±0.87	0.536
E. coli	-	-	-	8.00±0.51 b	13.00±1.22 a	15.00±1.26	0.354
P. aeruginosa	-	-	-	11.00±0.92 a	15.00±1.09 a	16.00±1.24	0.154
L. monocytogenes	-	-	-	-	7.00±0.38	14.00±0.71	0.000

Small letters in each rows shows significant statistical differences about *P* ≤0.05.
*****P* value between extracts and amoxicillin.

TABLE 2. MIC values of T. daenensis extract against examined bacteria.

Bacteria	MIC values (mg/mL)
S. aureus	6.00±1.00
E. coli	-
P. aeruginosa	10.00±1.00
L. monocytogenes	-

antibacterial effects against *S. aureus*. Additionally, the lowest MIC value of extract was found for the *S. aureus* (6.00±1.00 mg/mL). In keeping with this, the antibacterial effect of amoxicillin was higher than *T. daenensis* extract. However, the antibacterial effect of amoxicillin was significantly higher than the *T. daenensis* extract only against *L. monocytogenes*.

A probable reason for the antibacterial effect of examined plant is the presence of certain antibacterial components, particularly thymol, carvacrol, terpinene, terpinene, myrcene and p-cymene, in the *T. daenensis* extract [33]. It seems that the *T. daenensis* extract had the higher antibacterial effects against Gram-positive bacteria. The probable reason of this findings is the presence of outer lipopolysaccharide membranes in Gram-negative which caused them to be more resistant to antibacterial compounds. This barrier also decreases the penetration of plant extract into the inner layers of the bacterial cell, particularly due to the presence of hydrophilic materials [2, 4].

Several investigations evaluated the antibacterial effects of thymus species [34, 35]. Khiabani *et.al.* (2016) [36] reported that the MIC values of *T. daenensis* essential oil against *S. aureus*, *Bacillus cereus*, *Salmonella entrica* and *E. coli* bacteria were 0.0001, 0.0001, 0.0004 and 0.0005 %v/v, respectively. They showed that *E.coli* had the lower sensitivity against *T. daenensis* because of the presence of lipopolysaccharide membranes. In another research, the methanolic extract of the *T. daenensis* harbored potential antibacterial effects against *Micrococcus luteus, S. aureus*, *Streptococcus pyogenes* and *Entrococcus faecalis* (Gram-positive bacteria) but similar to our findings, it didn't have any significant antibacterial effects against Gram-negative bacteria (*Klebsiella pneumoniae* and Salmonella) [37]. Mahboubi *et.al.* (2017) [34] disclosed that the *T. kotschyanus*, *T. vulgaris*, and *T. pubescens* had the highest antibacterial effects amongst all examined thyme species. Antibacterial effects of diverse thymus species cultivated in Italy [38], Iran [39], Brazil [39], and Hungary [40] were reported in the literature. In keeping with this, further researches are required to assess the antibacterial effects of all types of the *T. daenensis* extract against diverse kinds of food-borne bacteria.

According to the comestible nature of the *T. daenensis* ethanolic extract and also its routine use in Iranian folk medicine as therapeutic agent and additive and rendering the high distribution of food-borne pathogens [41-59] among foods with animal origins, thus, the application of *T. daenensis* ethanolic extract as a food preservative has been recommended. *T. daenensis* ethanolic extract can use in both forms of food additive and also edible coating or film. However, some additional researches should perform to found more details about its application as food additive and edible coating or film. In this case, several investigations confirmed the antibacterial effects of thyme family as an edible food additive [60, 61].

Conclusion

The present research is an initial report of antibacterial effects of *T. daenensis* extract against food-borne bacteria. Findings showed potential antibacterial effect of *T. daenensis* extract against Gram-positive bacteria, particularly *S. aureus*. However, the antibacterial effect of *T. daenensis* extract was lower than amoxicillin antibiotic agent. Thus, further surveys are required to assess all impacts of antibacterial effects of *T. daenensis* extract against diverse bacteria. However, according to the antibacterial effect of *T. daenensis* extract and also it's a perfect taste and odour, its application has been recommended to assess as a food preservative in diverse kinds of food stuffs.

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Conflict of interest

The authors declared that no conflict of interest.

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