

Antibacterial and antioxidant effects of *Thymus daenensis* essential oil

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ABSTRACT

Thymus daenensis is an Iranian medicinal plant with high antibacterial and antioxidant effects. The present study was done to investigate the antibacterial and antioxidant effects of *T. daenensis* essential oil collected from the Dena mountain, Iran. *T. daenensis* was collected and immediately transferred to the laboratory. After its approval by an expert person, its essential oil was extracted using the Clevenger apparatus. DPPH free radical scavenging activity and antibacterial effects of various concentration of *T. daenensis* essential oil were studied. Higher concentrations of the *T. daenensis* essential oil had higher antibacterial and antioxidant effects. *T. daenensis* essential oil in a concentration of 1 mg/ml had the highest inhibitory effects on the DPPH radicals (96.27±8.60%). The mean diameter of the zone of inhibition for tested bacteria had a range of 6.03±0.28 to 19.29±1.57 millimeter. The highest antibacterial effects were seen for the 1mg/ml concentration of *T. daenensis* essential oil on all tested bacteria and especially on *E. coli* (19.29±1.57 mm) and *K. pneumonia* (18.0±1.69 mm). Bacteria were resistant against some of tested antibiotic agents and especially tetracycline, gentamicin and penicillin G. Essential oil of *T. daenensis* had a higher antibacterial effects on its high concentrations than several types of antibiotic agents. We found that *T. daenensis* essential oil is a good source for production of natural antioxidant and antibacterial agents.

KEY WORDS: ANTIBACTERIAL, PATHOGENIC BACTERIA, ANTIOXIDANT, DPPH RADICALS, *THYMUS DAENENSIS*, ESSENTIAL OIL

INTRODUCTION

Pathogenic agents and especially bacteria are the most important causes of infections in hospitals. *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), *Liste-*

ria monocytogenes (*L. monocytogenes*), *Bacillus cereus* (*B. cereus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Klebsiella pneumoniae* (*K. pneumoniae*) are the most important causes of gastrointestinal, respiratory, burn, wound, soft-tissue, and urinary tract infections

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al-around the world. They are also important causes of food-borne diseases globally (Ruhnke-Trautmann et al. 1989; Woodford and Livermore, 2009 and Kaye and Pogue, 2015).

Bacterial strains of clinical infections harbored the high levels of resistance against the extensive ranges of antibiotics including beta-lactams, macrolides, tetracyclines, cephalosporins, sulfonamides and aminoglycosides antibiotics (Zhang et al., 2006; Huttner et al. 2013). Therefore, much attentions have been done to the prescription of medicinal plants for treatment of various types of infectious diseases (Zhang et al., 2006; Huttner et al. 2013). The genus *Thymus*, member of the *Lamiaceae* family, contains about 400 species of perennial aromatic, evergreen or semi-evergreen herbaceous plants with many subspecies, varieties, subvarieties and forms (Vardar-Unlü et al. 2003; Ulukanli et al. 2011; Jia et al. 2010; Zarshenas and Krenn, et al. 2015).

They are known as "Avishan" in Persian and are well-known aromatic perennial herbs originated from Mediterranean region. Among 215 species of this genus grown in the world, 14 species are distributed in Iranian flora (Vardar-Unlü et al. 2003; Ulukanli et al. 2011; Jia et al. 2010; Zarshenas and Krenn, et al. 2015). *Thymus* species are well known as medicinal plants because of their biological and pharmacological properties. In traditional medicine, leaves and flowering parts of *Thymus* species are widely used as tonic and herbal tea, antiseptic, antitussive, antibacterial, antifungal, antioxidant and carminative as well as treating colds (Vardar-Unlü et al. 2003; Ulukanli et al. 2011; Jia et al. 2010; Zarshenas and Krenn, et al. 2015).

Thymus oils and extracts are widely used in pharmaceutical, cosmetic, and perfume industry, also for flavoring and preservation of several food products (Vardar-Unlü et al. 2003; Ulukanli et al. 2011; Jia et al. 2010; Zarshenas and Krenn, et al. 2015). *Thymus daenensis* (*T. daenensis*) is one of those endemic species in Iran and is widely used as medicine and food additive. It is a source of antibacterial and antioxidant components (Vardar-Unlü et al. 2003; Ulukanli et al. 2011; Jia et al. 2010; Zarshenas and Krenn, et al. 2015). Data on the antibacterial and antioxidant effects of *T. daenensis* are scarce in the world. Therefore, the present study was done to investigate the antioxidant effects of the *T. daenensis* essential oil as well as its antibacterial effects on several types of pathogenic bacteria.

MATERIALS AND METHODS

From May to August 2016, aerial parts of *T. daenensis* at the flowering stage were collected from the Dena mountain slopes, Yasuj, Iran. Plants were identified by

an expert person in the field of medicinal plants at the Herbarium of Research Center of Agricultural of Shahrekord city, Iran. *T. daenensis* samples were dried at ambient conditions for 10 days. The dried plant was used to extract the essential oil. Essential oil of the collected *T. daenensis* was extracted by water distillation using the Clevenger apparatus. *T. daenensis* essential oil was prepared at a concentration of 0.25, 0.5, 0.75 and 1 mg/ml. Radical-scavenging activities of *T. daenensis* essential oil were determined against 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. Briefly, 3 mL of *T. daenensis* essential oil was mixed with 1 mL of 1 mM methanolic solution of DPPH (Merck, Darmstadt, Germany). The mixture was vortexed and incubated in the dark at ambient temperature for 30 min. When the DPPH solution was mixed with the sample mixture acting as a hydrogen atom donor, a stable non radical form of DPPH is obtained with simultaneous change of the violet color to pale yellow. The absorbance was then measured at 517 nm. The percentage of DPPH free radical quenching activity was determined using the following equation:

$$\text{Percent of the DPPH scavenging effect} = \frac{\text{Abs of DPPH} - \text{Abs of extract}}{\text{Abs of DPPH}} \times 100$$

Where Abs DPPH is the absorbance value at 517 nm of the methanolic solution of DPPH and Abs extract is the absorbance value at 517 nm for the sample extracts. DPPH radical scavenging of *T. daenensis* essential oil was also compared with the BHT synthetic antioxidant.

Simple disk diffusion method was used to study the diameter of zone of inhibition of several types of bacteria subjected to *T. daenensis* essential oil. Susceptibility of *Staphylococcus aureus* (*S. aureus*) ATCC 25923, *Escherichia coli* (*E. coli*) ATCC 25922, *Listeria monocytogenes* (*L. monocytogenes*) ATCC 19111, *Bacillus cereus* (*B. cereus*) ATCC 14579, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27853 and *Klebsiella pneumoniae* (*K. pneumoniae*) ATCC 43816 (Pasteur Institute, Iran) were determined against the *T. daenensis* essential oil. Guidelines of the Clinical and Laboratory Standard Institute (CLSI) were used to study the antibacterial effects of *T. daenensis* essential oil. Susceptibility of bacterial strains were also studied against tetracycline (30 µg/disk), azithromycin (15 µg/disk), gentamicin (10 µg/disk), penicillin G (10 u/disk), and ciprofloxacin (5 µg/disk) antibiotic disks (Oxoid, UK). Base plates were prepared by pouring Muller-Hinton (MH) agar into sterile Petri dishes and allowed to set. Plant essential oil aliquots of 10 µl were applied per filter paper disc (Whatman No 6 mm diameter). The disc was air-dried and placed onto the seeded top layer of the agar plates. The plates containing the discs were allowed to stand for at least 30 min before incubated at 37°C for 24 h. The diameter of the zone of inhibition produced by each antibiotic disc was

Table 1. Percent of the DPPH radical scavenging of the *T. daenensis* essential oil.

Treatments		Percent of the DPPH radical scavenging
BHT		85.12±7.34b**
<i>T. daenensis</i>	0.25*	55.28±5.01d
	0.5	76.77±7.16c
	0.75	89.92±7.49b
	1	96.27±8.60a

*mg/ml
**Dissimilar capital letters in this column shows significant differences less than 0.05.

measured and interpreted using the CLSI zone diameter interpretative standards (CLSI, 2015). All data were presented as mean ± standard deviation (SD). SPSS. Ver. 20 was used for statistical analysis. Comparison of means were studied using the one-way ANOVA and also post hoc turkey test. *P* value ≤0.05 was determined as significant level. All tests were done 3 times.

RESULTS AND DISCUSSION

We found that increase in the concentration of the *T. daenensis* essential oil caused increase in its antioxidant and also antibacterial effects (*P*<0.05). Table 1 shows the antioxidant effect of *T. daenensis* essential oil against DPPH free radicals. We found that *T. daenensis* essential oil in a concentration of 1 mg/ml had the highest inhibitory effects on the DPPH free radicals (96.27±8.60%). BHT synthetic antioxidant had the higher inhibitory

effects on the DPPH free radicals than 0.25 and 0.5 mg/ml concentration of *T. daenensis* essential oil.

Table 2 represents the antibacterial effects of the *T. daenensis* essential oil on several types of bacteria. The mean diameter of the zone of inhibition for tested bacteria had a range of 6.03±0.28 to 19.29±1.57 millimeter. We found that the highest antibacterial effects were seen for the 1mg/ml concentration of the *T. daenensis* essential oil on all tested bacteria and especially on *E. coli* (19.29±1.57 mm) and *K. pneumonia* (18.0±1.69 mm). Antibacterial effect of the *T. daenensis* essential oil was not considerable against *S. aureus* and *B. cereus*.

Table 3 represents the antibacterial effects of several types of antibiotic agents on tested bacteria. The mean diameter of the zone of inhibition for tested bacteria had a range of 6.91±0.46 to 14.41±1.38 millimeter. We found that the highest diameter of the zone of inhibition of *S. aureus*, *K. pneumonia*, *P. aeruginosa*, *L. monocytogenes*, *E. coli* and *B. cereus* were seen for azithromycin (15.28±1.22 mm), ciprofloxacin (12.27±1.15 mm), ciprofloxacin (14.41±1.38 mm), azithromycin (13.92±1.26 mm), ciprofloxacin (10.71±0.74 mm) and azithromycin (11.79±1.06 mm). We also found that the essential oil of the *T. daenensis* had a higher antibacterial effects on its high concentrations than several types of antibiotic agents.

The present investigation emerged the high antibacterial and antioxidant effects of the *T. daenensis* essential oil. Results of the present study showed that *T. daenensis* essential oil in concentrations of 0.75 and 1 mg/ml had higher inhibitory effects on DPPH free radicals than BHT synthetic antioxidant. In addition, *T. daenensis* essential oil at mentioned concentration had a higher antibacterial effects on all tested bacteria than tetracycline, gentamicin and penicillin G antibiotic agents. High presence

Table 2. Antibacterial effects of the *T. daenensis* essential oil on several types of bacteria.

Bacteria	Diameter of the zone of growth inhibition (mm)			
	0.25*	0.5	0.75	1
<i>S. aureus</i>	7.33±0.58C**c***	10.31±0.92Cb	12.79±1.23Ba	13.04±1.19Ba
<i>K. pneumoniae</i>	9.25±0.75Bd	12.23±1.04Bc	15.71±1.33Ab	18.0±1.69Aa
<i>P. aeruginosa</i>	9.09 ±0.84Bc	11.89 ±1.09Bb	13.33 ±1.25Bb	16.92 ±1.58Ba
<i>L. monocytogenes</i>	8.85±0.67Bc	11.26±0.72Bb	13.72±1.10Bb	14.85±1.13Ba
<i>E. coli</i>	11.73±1.55Ad	14.25±1.31Ac	16.82±1.44Ab	19.29±1.57Aa
<i>B. cereus</i>	6.03±0.28Cc	8.94±0.75Db	9.21±0.53Cb	11.42±1.16Ca

*mg/ml
**Dissimilar capital letters in each column shows significant differences less than 0.05.
***Dissimilar small letters in each row shows significant differences less than 0.05.

Table 3. Antibacterial effects of the antibiotic agents on several types of bacteria.					
Bacteria	Diameter of the zone of growth inhibition (mm)				
	Tetracycline	Azithromycin	Gentamicin	Penicillin G	Ciprofloxacin
<i>S. aureus</i>	5.21±0.42C*d**	13.28±1.22Aa	6.37±0.54Cd	12.88±1.07Ab	9.15±0.39Dc
<i>K. pneumoniae</i>	7.38±0.67Bc	12.13±1.15Ca	9.64±0.71Bb	10.13±1.07Bb	12.27±1.15Ba
<i>P. aeruginosa</i>	7.52 ±0.65Bc	11.33 ±1.05Cb	12.27 ±1.21Ab	13.14 ±1.26Aa	14.41±1.38Aa
<i>L. monocytogenes</i>	7.20±0.62Bc	13.92±1.26Ba	7.14±0.45Cc	11.85±1.08Ab	10.52±0.84Cb
<i>E. coli</i>	8.31±0.65Ab	10.71±1.00Da	9.24±0.55Bb	8.33±0.36Cb	10.79±0.74Ca
<i>B. cereus</i>	7.25±0.31Bc	11.79±1.06Ca	6.91±0.46Cc	10.21±1.05Ba	8.22±0.67Db

*Dissimilar capital letters in each column shows significant differences less than 0.05.
**Dissimilar small letters in each row shows significant differences less than 0.05.

of antioxidant, flavonoid, phenol, carotenoid and volatile components are the main factor which cause high antioxidant effects of *T. daenensis* essential oil. In fact, essential oil of the *T. daenensis* is a source of 1,8-cineole, linalool, α -terpineol, geraniol, *trans*-thujan-4-ol, terpinen-4-ol, thymol and carvacrol which have potential antibacterial and antioxidant effects (Diaz-Maroto et al. 2006).

Several investigations focused on the antibacterial and antioxidant effects of *T. daenensis* essential oil (Sabahi et al. 2013; Damjanović-Vratnica et al. 2015; Amiri 2012). Sabahi et al. (2013) showed that the antioxidant potential of crude extract of *T. daenensis* by DPPH free radical scavenging ($IC_{50} = 194 \pm 0.02 \mu\text{g/mL}$), nitric oxide scavenging ($74 \pm 0.0 \%$) and hydroxyl radical scavenging ($60.2 \pm 0.27 \%$) was more active than its essential oil. In nitric oxide scavenging crude extract was more active than the antioxidant standard BHT, 42 ± 0.79 . They showed that high presence of Geraniol (66.8%), Geranyl acetate (13.9%) and Beta caryophyllene (9.6%) in the *T. daenensis* essential oil warrant its high antioxidant effects.

Damjanović-Vratnica et al. (2015) showed that geraniol (25.66%), geranyl-acetate (20.34%), linalool (10.89%) and caryophyllene oxide (9.89%) were the most commonly detected chemical components in the *T. vulgaris* essential oil. They showed that the essential oil of the *T. vulgaris* had considerable antimicrobial effects on *S. aureus*, *E. coli*, *Candida albicans* and *K. pneumoniae*. Amiri (2012) reported that antioxidant activity of polar subfraction of *T. daenensis subsplancifolius* (Celak) Jalaš was found to be higher than other thymus species in

DPPH assay. He showed that the inhibitory effects of the *T. daenensis subsplancifolius*, *T. daenensis subsplancifolius* polar sub-fraction and *T. daenensis subsplancifolius* non-polar sub-fraction essential oils on DPPH free radical were 99.6 ± 0.5 , 19.1 ± 0.1 and $248.7 \pm 1.6 \mu\text{g/mL}$, respectively. *T. tosevii* var. *tosevii*, *T. tosevii* var. *degenii*, *T. tosevii* var. *longifrons*, *T. tosevii* ssp. *substriatus*, *T. longidens* var. *lanicaulis* and *T. caramanicus* are other some *Thymus* species with high antioxidant and antibacterial effects (Vardar-Unlü et al. 2003; Ulukanli et al. 2011; Jia et al. 2010; Zarshenas and Krenn, et al. 2015).

Nikolić et al. (2013) revealed the high antimicrobial, antioxidant and antitumor activity of *T. serpyllum* L., *T. algeriensis* Boiss. and Reut and *T. vulgaris* L. essential oils. They showed that Thymol was a major chemical component in all tested thymus species. Difference in the species of plant, method of sampling, part of plant used for essence extraction, method of extraction and geographical area caused difference in the chemical composition of thymus species and therefore variation in their antibacterial and antioxidant effects.

Bacterial strains of our investigation harbored the high levels of resistance against tetracycline, azithromycin, gentamicin, penicillin G, and ciprofloxacin antibiotic agents. It may be due to the irregular and unauthorized prescription of these antibiotics in veterinary and also medical fields in Iran. High prevalence of resistance against these antibiotics have also been reported by other researchers (Momtaz et al. 2013; Dehkordi et al. 2014; Shahrani et al. 2014; Ranjbar et al. 2017) which showed an emerging demand to use from natural antibacterial agent's like *T. daenensis*.

CONCLUSION

In conclusion, we identified a considerable inhibitory effects of the *T. daenensis* essential oil on DPPH free radicals and also high antibacterial effects of this plant against Gram-negative and Gram-positive bacteria. Results showed that *T. daenensis* essential oil in high concentrations had higher antibacterial and antioxidant effects. According to results, production of drug from the essential oil of the *T. daenensis* at 0.75 and 1 mg/ml concentration is a good approach for treatment of the cases of *E. coli*, *K. pneumonia*, and *P. aeruginosa* bacteria. Judicious prescription of antibiotic according to the results of the disk diffusion method can eliminate the risk of occurrence of antibiotic resistance.

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