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Antibacterial effects of Istanbul thyme (*Origanum vulgare* L. subsp. *hirtum* (Link) letsw.) and Karabaş thyme (*Thymbra spicata* L. var. *spicata*) extracts

Antibakterielle Wirkung der Extrakte aus Istanbul-Thymian (Origanum vulgare L. subsp. hirtum (Link) letsw.) und Karabaş -Thymian (Thymbra spicata L. var. spicata)

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Summary

The most sensitive bacteria against extract concentrations were *Bacillus subtilis, Bacillus cereus* and *Mycobacterium smegmatis.* In addition, *E. coli* 0157: H7 shown resistance against effect of Istanbul thyme extract. While the most sensitive microorganism against Karabaş thyme extract had *Mycobacterium smegmatis, Yersiniae enterocolitica* shown strong resistance. The inhibitory effect of extracts changed depending on concentrations. Most sensitive bacteria against extract concentration had Gram (+) bacteria that *Mycobacterium smegmatis, Bacillus subtilis* and *Bacillus cereus.* The most resistant bacteria is Gram (–) bacteria, *E. coli* O157: H7, *Klebsiella pneumoniae, Staphylococcus aureus* and *Yersiniae enterocolitica* were found. Generally, antibacterial effects of İstanbul extracts obtained by different application and extraction systems were found partly high compared with effects of Karabaş thyme extracts.

Keywords: medicinal plant, extract, Labiate, antibacterial activity, in vitro

Zusammenfassung

Die empfindlichsten Bakterien gegenüber den Extrakt-Konzentrationen waren *Bacillus subtilis, Bacillus cereus* und *Mycobacterium smegmatis. E. coli* 0157: H7 zeigte Resistenzen gegenüber Istanbul-Thymian Extrakte. Während *Mycobacterium smegmatis* der empfindlichste Mikroorganismus gegenüber Extrakte von Karabaş-Thymian war, zeigte *Yersiniae enterocolitica* Resistenzen. Die hemmende Wirkung der Extrakte verändert sich je nach Konzentration. Die empfindlichsten Bakterien gegenüber der Extrakt-Konzentration waren grampositiv: *Mycobacterium smegmatis, Bacillus subtilis* and *Bacillus cereus.* Die resistentesten Bakterien waren gramnegativ: *E. coli* O157: H7, *Klebsiella pneumoniae, Staphylococcus aureus* und *Yersiniae enterocolitica.* Grundsätzlich war die antibakterielle Wirkung, der aus unterschiedlichen Extraktionssystemen gewonnenen İstanbul-Thymian Extrakte höher, als die der Karabaş-Thymian-Extrakte.

Schlüsselwörter: Heilpflanze, Extrakt, Labiate, antibakterielle Aktivität, in vitro

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Introduction

Recently, the biologically active compounds associated with plant natural products were subjected to close investigations throughout advanced analytical techniques that permitted the disclosure of their chemical composition and the evaluation of their biological activities either *in vitro* or *in vivo* (Özcan and Erkmen,2001; Rusenova and Parvanov 2009; Sokovic et al. 2010; Roussenova 2011). Essential oils are distillates of the volatile compounds of a plant's secondary metabolism and may act as phytoprotective agents (Oussalah et al. 2007). Most of the synthetic chemicals used to control microbial deterioration of food commodities are either hazardous to or responsible for altering the palatability of the treated commodity (Özcan and Erkmen 2001; Rusenova and Parvanov 2009). Spices and their derivatives such as essential oils and oleoresins are used with the primary purpose of flavoring foods and beverages although it has long been known that some spices have an antimicrobial activity (Dorman and Deans 2000; Delamare et al. 2007; Eteghad et al. 2009). The antimicrobial properties of extracts and essential oils of *T. serpyllum, T. vulgaris, S. officinalis* and *P. anisum* collected from different places in many countries have been assessed and reviewed (Delamare et al. 2007; Imelouane et al. 2009). Hammad et al. (2007) reported that 20 % aqueous extract of *T. vulgaris* showed the greatest inhibition against *Streptococcus mutans.* For *S. officinalis,* 95 % ethanolic extract inhibited the growth of *S. aureus* (Khalil et al. 2005). Some scientist reported the antimicrobial activity of essential oils from oregano, thyme, sage, rosemary, clove, corianderi garlic and onion against both bacteria and molds (Özcan and Erkmen 2001; Leuschner and Ielsch 2003; Omidbygi et al. 2007; Çelikel and Kavas 2008). High antimicrobial activity of thyme species has been attributed to their phenolic comporents such as thymol and carvacrol (Bassole and Juliani 2012). The general objective of the current study was to determine antibacterial effect of some extracts obtained by two different extraction methods from Istanbul thyme (*Origanum vulgare* L. subsp. *hirtum* (Link) Ietsw.) and Karabaş thyme (*Thymbra spicata* L. var. *spicata*).

Material and Methods

Material

Plant materials [*Origanum vulgare* L. subsp. *hirtum* (Link) Ietsw. (Istanbul thyme) and *Thymbra spicata* L. var. *spicata* (Karabaş thyme)] were provided from Çanakkale and Antalya provinces in Turkey. They were collected between May and September during flowering on 2006. About 5 kg was collected for each sample. Collected samples have been dried in room temperature (about 24 °C and 55 % humidity) and in shade. Species have been identified by Dr. Hüseyin Fakir from Süleyman Demirel University and Dr. Ramazan Göktürk from Akdeniz University. Herbarium codes are Leg: 838, and Leg: 1568, respectively, and samples have been kept in Akdeniz and Süleyman Demirel University herbariums.

Method

Soxhlet and ultrasonic water bath extraction

Used solvent mixtures and their quantities were determined with pre-trials. Extraction has been made with individual or different proportions mixtures of solvents. 10 g of ground plant samples (aerial parts) which chopped by mixer are weighed and solvent solutions and samples were extracted with A Soxhlet apparatus for 5h and an ultrasonic water bath device (2 h) and then obtained extracts are filtered by using filter paper (Whatman 42 No). Removal of solvent and water was carried out with rotary evaporator (40 °C+ Vacum). Obtained extracts have been kept at –18 °C until they've been analyzed. Extraction was carried out twice. The codes belonging to application and solvent mixtures are shown in Table 1.

Determination of Antibacterial effect

In this research, *Aeromonas hydrophila* ATCC 7965, *Bacillus cereus* FMC 19, *B. subtilis, Escherichia coli* DM, *E. coli* O157: H7 VT (N), *Klebsiella pneumoniae* FMC 5, *Mycobacterium smegmatis* RUT, *Proteus mirabilis* BC 3624, *Staphylococcus aureus* Cowan 1 and *Yersiniae enterocolitica* bacteria were used. Bacteria were obtained from the Department of Food Engineering, Faculty of Engineering, Erciyes University in Kayseri. Bacteria which to be used in current study were inoculated into nutrient broth culture, and activated for 18 h. Bacteria culture 1 % taken from this activated culture was inoculated into steril nutrient broth, and incubated for 18 h again. *Yersiniae enterocolitica* from the test microorganism was developed in nutrient broth (Acumedia Manufacturers, Inc., Maryland) at 25 °C for 18 hours, and other bacteria were developed in the same nutrient at 37 °C for 18 h. Bacteria were counted using a serial dilution method on nutrient broth (medium) and the test cell concentration of 10^6 to 10^7 cfu was adjusted. The prepared these active cultures were used in the following experiment of antimicrobial activity (Sağdıç and Özcan, 2003; Baydar et al. 2004).

Determining the antimicrobial effect of the extract, agar diffusion

In determining of the antimicrobial effect of the extract, agar diffusion method was used (Özkan et al., 2004). Flasks contained 25 ml of nutrient agar was sterilized at 121 °C for 15 min in autoclave, and after cooling to 43–45 °C, It was inoculated by active bacteria cultures covered above 10^6 to 10^7 cfu / ml with a cell concentration of 1 % (250 l). This nutrient was mixed homogeneously under aseptic, and was allowed to solidify poured into sterile petri dishes of 9 cm diameter. Then, five well in solidified nutrient in petri plate by using steril korkbor in 4 diameter were opened. 50 µl with pipette from 1 %, 2.5 %, 5.0 % and 10.0 % extract solutions prepared with pure ethanol was added into each well. As a control, pure ethanol was used.

TABLE 1: *Codes belonging to application and solvent mixtures.*

Applications	Solvent mix (h:h%)		Sample (g)	Time (s)
S1	Methanol:acetone:water:acetic acid (55:40:4.5:0.5)	Soxhlet	10	5
S2	Methanol:water: acetic acid (95:4.5:0.5)	Soxhlet	10	5
S3	Acetone: water: acetic acid (95:4.5:0.5)	Soxhlet	10	5
S4	Ethanol: water: acetic acid (95:4.5:0.5)	Soxhlet	10	5
U1	Methanol:acetone: water: acetic acid (55:40:4.5:0.5)	Ultrasonic Benmary	10	2
U2	Methanol: water: acetic acid (95:4.5:0.5)	Ultrasonic Benmary	10	2
U3	Acetone: water: acetic acid (95:4.5:0.5)	Ultrasonic Benmary	10	2
U4	Ethanol: water: acetic acid (95:4.5:0.5)	Ultrasonic Benmary	10	2
U5	Water: acetic acid (95:4.5:0.5)	Ultrasonic Benmary	10	2

TABLE 2: *Inhibitory effect of extracts of İstanbul thyme (mm) * (n:3).*

MO	Concentrations (%)	Application **S1	S2	S3	S4	U1	U2	U3	U4	U5
S. aureus	10.00	17.00±3.00b	15.50±0.50b	21.00±1.00a	12.50±0.50c	17.00±2.00b	21.00±0.00a	16.50±2.50b	14.50±0.50bc	4.50±0.50d
	5.00	11.50±2.50b	10.50±0.50b	17.50±1.50a	7.00±1.00c	11.50±0.50b	17.00±1.00a	11.00±2.00b	9.50±2.50bc	0.00±0.00d
	2.50	8.00±3.00a	4.00±1.00bc	8.00±1.00a	2.00±1.00cd	3.50±0.50bc	6.00±1.00ab	7.00±2.00a	4.00±0.00bc	0.00±0.00d
	1.00	0.00±0.00b	0.00±0.00b	1.50±0.50a	0.00±0.00b	0.50±0.50b	1.50±0.50a	0.50±0.50b	0.00±0.00b	0.00±0.00b
E. coli	10.00	9.00±0.00e	19.00±1.00a	12.50±0.50cd	14.50±0.50bc	13.00±3.00bcd	11.50±1.50d	15.00±1.00b	6.00±0.00f	0.00±0.00g
	5.00	7.00±1.00a	3.50±0.50c	5.50±1.50b	4.50±0.50bc	3.50±0.50c	3.50±0.50c	3.50±0.50c	3.50±0.50c	0.00±0.00c
	2.50	3.50±0.50a	1.00±0.00c	2.00±0.00b	2.00±0.00b	1.50±0.50bc	1.50±0.50bc	1.00±0.00c	2.00±0.00b	0.00±0.00d
	1.00	0.50±0.50b	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	0.00±0.00c	1.00±0.00a	0.00±0.00c
E. coli O157:H7	10.00	6.50±0.50e	9.00±0.00c	11.50±0.50a	9.50±0.50bc	7.50±0.50de	8.50±0.50cd	7.50±0.50de	11.50±1.50a	0.00±0.00f
	5.00	2.50±0.50d	4.50±0.50bc	7.50±1.50a	5.50±1.50b	3.50±0.50cd	5.00±1.00bc	2.50±0.50d	3.50±0.50cd	0.00±0.00e
	2.50	1.00±0.00de	1.50±0.50d	3.00±0.00a	2.50±0.50b	1.00±0.00de	2.00±0.00c	0.50±0.50ef	0.00±0.00f	0.00±0.00f
	1.00	0.00±0.00b	0.00±0.00b	0.50±0.50a	0.75±0.25a	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b
B. subtilis	10.00	24.00±1.00d	30.50±3.50bc	34.50±2.50a	24.50±0.50d	33.00±2.00ab	29.00±1.00c	24.50±2.50d	21.00±1.00d	10.50±0.50e
	5.00	20.00±1.00bc	18.00±1.00cd	29.00±2.00a	18.00±0.00cd	28.00±3.00a	22.50±2.50b	21.00±2.00bc	16.00±1.00d	5.50±0.50e
	2.50	18.50±1.50ab	10.00±5.00d	21.00±2.00a	14.00±1.00c	18.00±1.00abc	18.00±3.00abc	16.00±1.00bc	9.50±0.50d	0.25±0.25e
	1.00	14.50±0.50a	3.00±0.00d	13.50±1.50a	9.00±0.00b	12.50±1.50a	13.00±2.00a	6.00±2.00c	6.00±1.00c	0.00±0.00e
P. mirabilis	10.00	15.50±0.50b	16.50±1.50ab	18.00±1.00a	18.00±1.00a	13.00±2.00c	11.00±1.00d	9.00±0.00e	6.00±1.00f	0.00±0.00g
	5.00	10.50±1.50b	10.50±0.50b	12.00±0.00a	11.50±0.50ab	7.50±0.50c	7.00±1.00c	2.00±0.00d	1.50±0.50d	0.00±0.00e
	2.50	5.50±0.50a	2.50±0.50c	6.00±1.00a	5.50±0.50a	4.00±1.00b	1.50±0.50d	0.50±0.00e	0.00±0.00e	0.00±0.00e
	1.00	1.00±0.00de	0.50±0.00ef	2.00±0.00c	1.50±0.50cd	1.50±0.50cd	19.00±1.00a	15.00±1.00b	0.00±0.00f	0.00±0.00f
K. pneumoniae	10.00	7.50±0.50e	10.50±0.50bc	14.00±1.00a	11.00±1.00b	10.50±0.50bc	9.50±0.50cd	8.50±0.50de	9.50±0.50cd	0.00±0.00f
	5.00	2.50±0.50e	4.50±0.50cd	7.50±0.50a	5.00±1.00bc	5.50±0.50b	4.00±0.00d	2.00±0.00ef	1.50±0.50f	0.00±0.00g
	2.50	0.00±0.00	0.00±0.00	0.33±0.58a	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	1.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Y .enterocolitica	10.00	21.00±2.00b	15.50±0.50c	17.50±0.50c	15.50±0.50c	16.00±1.00c	24.00±1.00a	12.50±0.50d	17.00±2.00c	0.00±0.00e
	5.00	12.00±0.00a	10.50±0.50b	10.50±0.50b	10.00±0.00b	10.50±0.50b	12.00±1.00a	7.50±0.50c	8.00±0.00c	0.00±0.00d
	2.50	5.50±0.50a	4.50±0.50b	5.00±0.00b	5.50±0.50a	4.50±0.50b	5.50±1.50a	2.50±0.50c	1.50±0.50c	0.00±0.00c
	1.00	1.25±0.75b	1.50±0.50ab	1.50±0.50ab	2.00±0.00a	1.00±0.00bc	2.00±0.00a	0.50±0.00cd	0.50±0.50cd	0.00±0.00d
A. hydrophila	10.00	9.50±0.50d	15.00±1.00a	13.50±0.50b	11.50±0.50c	12.00±0.00c	10.00±0.00d	9.50±0.50d	9.00±1.00d	0.00±0.00e
	5.00	5.50±0.50ef	9.00±0.00ab	7.50±1.50cd	8.00±0.00bc	9.50±0.50a	6.50±0.50de	4.00±0.00g	4.50±0.50fg	0.00±0.00h
	2.50	3.00±0.00 cd	3.50±0.50bc	4.00±0.00ab	2.50±0.50de	4.50±0.50a	4.00±0.00ab	2.50±0.50de	2.00±0.00e	0.00±0.00f
	1.00	1.00±0.00c	2.00±0.00a	2.00±0.00a	1.50±0.50b	1.50±0.50b	2.00±0.00a	0.75±0.25c	0.00±0.00d	0.00±0.00d
B. cereus	10.00	26.50±1.50de	29.50±0.50b	27.50±0.50cd	25.00±0.00f	34.00±1.00a	28.00±1.00c	25.50±0.50ef	19.50±0.50g	11.00±0.00h
	5.00	19.00±1.00c	17.50±0.50d	19.00±1.00c	19.50±0.50bc	26.00±1.00a	20.50±0.50b	19.50±0.50bc	10.50±0.50e	3.00±0.00f
	2.50	14.00±2.00b	14.00±1.00b	14.50±0.50b	14.50±0.50b	18.50±1.50a	15.00±0.00b	14.50±0.50b	1.00±0.00c	0.00±0.00c
	1.00	9.00±1.00cd	8.00±0.00de	9.50±0.50c	7.00±1.00e	13.50±1.50a	11.00±0.00b	7.50±0.50e	0.00±0.00f	0.00±0.00f
M. smegmatis	10.00	26.00±1.00cd	26.50±0.50c	33.50±0.50a	25.00±0.00d	21.50±1.50e	22.50±0.50e	25.50±0.50cd	29.00±1.00b	0.00±0.00f
	5.00	21.00±0.00b	18.00±1.00d	24.00±1.00a	19.50±0.50bcd	16.00±1.00e	15.00±0.00e	20.00±2.00bc	18.50±0.50cd	0.00±0.00f
	2.50	14.50±1.50b	14.50±1.50b	18.00±2.00a	13.00±1.00b	10.50±0.50d	10.00±0.00d	12.50±0.50bc	11.00±1.00cd	0.00±0.00e
	1.00	8.50±1.50b	8.00±0.00bc	13.00±1.00a	9.00±0.00b	5.50±0.50ef	4.50±0.50f	7.00±0.00cd	6.50±1.50de	0.00±0.00g

*means in the same row with the same letters are not significally different (p>0.05). MO: microorganisms

This prepared petri plates, after incubated in a sterile medium for absorption of extract, *Y. enterocolitica* was incubated at 25 °C, and other bacteria were incubated for 18–24 hours at 37 °C in the reverse position. At the end of this time, the diameters of the inhibition zone were measured as millimeters via caliper. All tests were performed in triplicate.

Statistical analysis

According to completely randomized experiment design was planned (7 different species x 9 different applications x 3 repetition). Obtained datas were statistically evaluated using the SPSS 10.0 statistical program (PASW Statistics Data View Window), importance of differences between groups was determined with variance analysis. Identification of differences between groups was determined with Duncan multiple comparison test (Özdamar 1999).

Results and Discussion

Antibacteral effects of İstanbul thyme extracts obtained by two different extraction methods were determined, and results are given in Table 2. According to analysis results, the inhibitory effect of extracts increased with increasing of extract concentrations. The effective dosage amounts were determined as 10 % > 5 % > 2.5 % > 1 %, respectively. The most sensitive bacteria against extract concentrations were *B. subtilis, B. cereus* and *M. smegmatis.* In addition, *E. coli* 0157: H7 shown resistance against Istanbul thyme extract. Generally, all high concentrations of applications exhibited the antibacterial effect, and followed by U2, U1, S2 and S1 in onder to decreasing. Sağdıç et al. (2002) reported that antibacterial effect of methanol extract of *O. vulgare* changed according to extract concentrations. Şahin et al. (2003) determined the antibacterial effect of methanol and hexan extracts of Çibriska plant on growth of *B. cereus, B. sub-*

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TABLE 3: *Inhibitory effect of extracts of Karabaş thyme (mm) * (n:3).*

MO	Concentrations (%)	Application								
		**S1	S2	S3	S4	U1	U2	U3	U4	U5
S. aureus	10.00	12.00±0.00e	17.00±0.00a	15.50±1.50b	13.50±0.50cd	17.50±0.50a	14.00±1.00c	14.50±0.50bc	12.50±0.50de	1.00±0.00f
	5.00	5.50±0.50bc	9.50±0.50a	9.50±1.50a	5.50±0.50bc	9.50±0.50a	6.00±1.00b	4.50±0.50c	5.50±0.50bc	0.00±0.00d
	2.50	2.00±0.00b	2.50±0.50b	4.50±0.50a	1.00±0.00c	2.00±0.00b	2.00±1.00b	0.75±0.25cd	0.25±0.25cd	0.00±0.00d
	1.00	0.00±0.00b	0.00±0.00b	0.25±0.25a	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b	0.00±0.00b
E. coli	10.00	12.50±0.50bc	16.00±1.00a	16.50±0.50a	15.50±0.50a	13.50±0.50b	11.00±1.00c	15.50±1.50a	13.50±1.50b	0.00±0.00d
	5.00	7.50±0.50cd	12.00±2.00a	11.50±0.50a	11.00±1.00a	10.00±1.00ab	6.00±1.00d	6.00±1.00d	8.50±1.50bc	0.00±0.00e
	2.50	3.00±1.00bc	5.00±1.00ab	6.00±1.00a	4.50±0.50abc	6.00±1.00a	3.50±1.50bc	4.00±1.00bc	3.50±1.50bc	0.00±0.00d
	1.00	1.50±0.50b	1.00±0.00bc	2.00±0.00b	4.00±2.00a	3.50±0.50a	1.50±0.50b	1.00±0.00bc	1.50±0.50b	0.00±0.00c
E. coli O157:H7	10.00	11.50±0.50c	13.00±1.00b	15.00±0.00a	15.50±0.50a	9.00±1.00d	8.00±0.00d	11.00±1.00c	13.00±1.00b	0.00±0.00e
	5.00	6.00±0.00e	9.50±0.50b	7.00±0.00d	12.50±0.50a	5.00±0.00f	5.00±0.00f	4.50±0.50f	8.00±1.00c	0.00±0.00g
	2.50	2.00±1.00cd	3.00±1.00bc	2.50±0.50cd	4.00±0.00ab	2.00±1.00cd	1.50±0.50d	2.00±0.00cd	5.00±0.00a	0.00±0.00e
	1.00	0.00±0.00c	0.75±0.25b	0.75±0.25b	1.00±0.00b	0.25±0.25c	0.25±0.25c	0.75±0.25b	2.50±0.50a	0.00±0.00c
B. subtilis	10.00	16.00±3.00c	20.50±1.50b	19.50±2.50b	21.00±2.00b	19.50±0.50b	21.00±1.00b	22.50±0.50b	26.00±1.00a	4.50±0.50d
	5.00	9.50±1.50c	16.50±1.50b	15.50±0.50b	15.50±1.50b	15.50±1.50b	15.00±2.00b	10.50±1.50c	20.00±1.00a	1.00±0.0d
	2.50	6.50±1.50d	9.50±1.50bc	8.50±0.50bcd	8.50±0.50bcd	10.00±1.00b	8.50±2.50bcd	7.50±0.50cd	15.50±0.50a	0.00±0.00e
	1.00	1.50±0.50c	2.50±0.50c	1.50±0.50c	1.50±0.50c	2.00±1.00c	4.00±1.00b	4.00±0.00b	6.00±1.00a	0.00±0.00d
P. mirabilis	10.00	12.50±0.50c	13.50±1.50c	13.00±1.00c	14.00±1.00c	16.00±0.00b	12.50±0.50c	14.00±0.00c	22.00±1.00a	0.00±0.00d
	5.00	7.50±1.50cd	9.00±2.00c	6.50±0.50d	9.00±1.00c	12.00±1.00b	7.00±1.00d	7.50±0.50cd	15.00±0.00a	0.00±0.00e
	2.50	2.50±0.50c	3.00±0.00bc	3.50±0.50b	3.50±0.50b	3.00±0.00bc	3.00±0.00bc	3.00±0.00bc	4.50±0.50a	0.00±0.00d
	1.00	0.25±0.25d	0.75±0.25b	0.75±0.25b	0.75±0.25b	0.50±0.00bc	0.50±0.00bc	0.75±0.25b	2.00±0.00a	0.00±0.00d
K .pneumoniae	10.00	12.00±1.00b	15.50±1.50a	14.50±0.50a	10.50±1.50bc	10.00±0.00c	10.00±1.00c	15.50±0.50a	16.00±1.00a	0.00±0.00d
	5.00	5.50±0.50c	7.00±1.00b	9.50±0.50a	5.50±0.50c	1.50±0.50f	1.00±0.00f	2.50±0.50e	4.50±0.50d	0.00±0.00g
	2.50	0.50±0.50cde	1.50±0.50b	3.50±0.50a	1.00±0.00bc	0.25±0.25de	0.00±0.00e	0.75±0.25cd	1.00±0.00bc	0.00±0.00e
	1.00	0.00±0.00	0.00±0.00	1.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Y. enterocolitica	10.00	11.50±0.50d	15.50±0.50bc	14.00±1.00c	19.50±1.50a	15.50±0.50bc	16.50±0.50b	14.50±0.50c	17.0±0±2.00b	0.00±0.00e
	5.00	7.50±0.50c	8.50±0.50bc	7.50±1.50c	9.50±0.50b	8.50±0.50bc	11.50±0.50a	12.00±1.00a	12.00±0.00a	0.00±0.00d
	2.50	2.00±1.00c	4.50±0.50b	5.00±0.00b	4.00±1.00b	4.50±0.50b	2.50±0.50c	4.50±0.50b	7.00±1.00a	0.00±0.00d
	1.00	0.00±0.00c	0.75±0.25b	1.00±0.00b	0.75±0.25b	1.50±0.50a	0.00±0.00c	1.00±0.00b	1.50±0.50a	0.00±0.00c
A. hydrophila	10.00	11.50±0.50e	13.50±1.50de	17.00±1.00c	24.50±2.50a	22.00±2.00ab	16.00±1.00cd	20.50±2.50b	17.50±2.50c	0.00±0.00f
	5.00	8.00±0.00d	9.00±1.00d	12.50±0.50b	15.00±0.00a	15.50±0.50a	12.50±0.50b	11.00±1.00c	11.00±1.00c	0.00±0.00e
	2.50	3.50±0.50ef	2.50±0.50f	8.50±1.50b	10.50±1.50a	6.50±0.50c	5.50±0.50cd	4.50±0.50de	5.00±1.00cde	0.00±0.00g
	1.00	0.00±0.00f	0.75±0.25e	4.00±0.00a	3.50±0.50a	2.50±0.50b	1.00±0.00de	2.00±0.00bc	1.50±0.50cd	0.00±0.00f
B. cereus	10.00	26.50±0.50b	28.00±1.00a	24.50±0.50c	23.50±0.50c	16.00±1.00f	21.00±0.00d	20.00±0.00e	24.50±0.50c	0.00±0.00g
	5.00	19.50±0.50a	19.00±0.00a	14.50±0.50c	17.50±0.50b	12.00±1.00d	14.50±0.50c	15.00±0.00c	17.500.50b	0.00±0.00e
	2.50	9.50±0.50ab	8.00±0.00bc	8.50±1.50abc	10.00±0.00a	6.00±0.00d	7.50±0.50c	8.50±1.50abc	8.00±1.00bc	0.00±0.00e
	1.00	3.50±0.50b	2.50±0.50c	4.00±0.00ab	4.00±1.00ab	2.00±0.00c	3.50±0.50b	4.50±0.50a	4.50±0.50a	0.00±0.00d

*means in the same row with the same letters are not significally different (p>0.05). MO: microorganisms

tilis, E. coli, K. pneumoniae and *S. aureus* by using disc diffusion method, and researchers reported that hexan extract had not antimicrobial effect. In previous study, Amanlou et al. (2004) reported that methanol extract of *Satureja khuzistanica* showed strong antibacterial effect against *Staphylococcus aureus,* but low against *E. coli.* Our results were found partly similar compared with results of Sağdıç et al. (2002), Şahin et al. (2003) and Amonlou et al. (2004). In this application, the most effective dose had been 10 %, and followed by 5 %, 2.5 % and 1 % decreased orders.

Inhibitory effects of extracts of Karabaş thyme were presented in Table 3. The 5 % and 10 % concentrations of Karabaş thyme extracts showed antimicrobial effect against all tested microorganisms. Especially, S1 and U1 applications showed more effect on the growth of tested microorganisms. While the most sensitive microorganism against Karabaş thyme extract had *M. smegmatis, Y. enterocolitica* shown strong resistance. Loziene et al. (2006) reported that extracts of different genotypes of *Thymus puleigioides* shown different antibacterial effect. Similar differences were observed in study carried out by Sökmen et al. (2004) on antimicrobial effect of *Thymus spathulifolius* extract. Our results were found partly similar with antimicrobial effects of *Thymus vulgaris* L., *Thymus serpyllum* L., *T. puleigioides* and *T. spathulifolius* extracts (Sağdıç, 2003; Sökmen et al. 2004; Loziene et al. 2006). Generally, antibacterial effects of İstanbul extracts obtained by different application and extraction systems were found partly high compared with effects of Karabaş thyme extracts. These differences can be probably due to the composition of thyme, the species differences, climatic conditions and the location which they grow.

Conclusion

By increasing of the dose amounts of the extract according to the result of antibacterial effect is determined to increase the antibacterial effect. The effective dosage amounts were determined as 10 % > 5 % > 2.5 % > 1 %, respectively. Most sensitive bacteria against extract concentration had Gram (+) bacteria that *M. smegmatis, B. subtilis* and *B. cereus.* The most resistant bacteria is Gram (–) bacteria,

E. coli O157: H7, *K. pneumoniae, S. aureus* and *Y. enterocolitica* were found. In the vast majority of bacteria, S3, U2 and U1 applications were defined as applications with maximum antibacterial effect. All extracts were effective against bacteria partially.

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Conflict of interest

The authors declare that they respect the journal's ethics requirements and declare that they have no conflict of interest.

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