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ANTIBACTERIAL ACTIVITY OF DIFFERENT ESSENTIAL OILS ON *LISTERIA MONOCYTOGENES* STRAINS ISOLATED FROM READY-TO-EAT FOODS

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ABSTRACT
Listeria monocytogenes is one of the food-borne pathogens that cause major health problems worldwide. Application of essential oils (EOs) is used to control this pathogen and reduce microbial levels. The aim of the present study was to investigate the antibacterial activity of 15 different EOs obtained from plants on *L. monocytogenes* strains from ready-to-eat foods. In this study, thyme oil (mean zone 24.850±3.714 mm) showed the highest antibacterial activity against *L. monocytogenes*. Clove oil (mean zone 12.383±2.215 mm) and sage oil (mean zone 11.117±3.170 mm) were also determined high antibacterial activity. Ginger oil and garlic oil did not have any antibacterial activity against *L. monocytogenes* strains. This study shows that using of EOs against food-borne pathogens in food systems could be useful.
Keywords: Essential oils, antibacterial activity, *Listeria monocytogenes*, disc diffusion method

ÇEŞİTLİ UÇUCU YAĞLARIN TÜKETİME HAZIR GIDALARDAN İZOLE EDİLEN *LISTERIA MONOCYTOGENES* SUŞLARI ÜZERİNDEKİ ANTİBAKTERİYEL AKTİVİTESİ

ÖZ
Listeria monocytogenes dünya çapında önemli sağlık sorunlarına neden olan gıda kaynaklı patojenlerden biridir. Uçucu yağların (EOs) uygulanması, patojenlerin kontrol edilmesi ve mikrobiyel seviyelerin azaltılması amacıyla kullanılan yöntemlerden biridir. Bu çalışmanın amacı, bitkilerden elde edilmiş olan 15 farklı EOs'un tüketime hazır gıdalardan izole edilmiş olan *L. monocytogenes* suşları üzerindeki antibakteriyel aktivitesini araştırmaktır. Bu çalışmada, kekik uçucu yağının (ortalama zon çapı 24.850±3.714 mm) *L. monocytogenes*'e karşı en yüksek antimikrobiyel aktiviteyi gösterdiği belirlenmiştir. Karanfil uçucu yağı (ortalama zon çapı 12.383±2.215 mm) ve adaçayı uçucu yağı (ortalama zon çapı 11.117±3.170 mm) ise, diğer yüksek antibakteriyel aktiviteye sahip uçucu yağlardır. Zencefil uçucu yağı ve sarımsak uçucu yağının, *L. monocytogenes* suşlarına karşı antibakteriyel etkisi saptanamamıştır. Bu çalışma, gıda sistemlerinde gıda kaynaklı patojen bakterilere karşı EO'ların kullanılmasının yararlı olabileceğini göstermektedir.
Anahtar kelimeler: Uçucu yağlar, antibakteriyel aktivite, *Listeria monocytogenes*, disk difüzyon yöntemi

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INTRODUCTION

Food safety is becoming an important part of consumers lives from farm to fork (Kuan et al., 2017). Foods may become contaminated with microorganisms at any stage of production, processing, distribution, storage or preparation. Nowadays, recent food trends have focused on unprocessed or natural foods that do not contain chemical preservatives (Oggiano, 2015). Essential oils (EOs) obtained from plants promise as alternative substances for food safety (Sakkas and Papadopoulou, 2017; Imane et al., 2020). Moreover, EOs are also a good alternative to chemical or synthetic preservation agents in food industry (Moreira et al., 2005; Paparella et al., 2008; Gouveia et al., 2016).

EOs are volatile secondary metabolites that can be extracted from all parts of plants such as buds, gums, blossoms, flowers, leaves, stems, twigs, seeds, fruits, roots, wood, or bark depend on the growing species (Wińska et al., 2019; Cho et al., 2020; Jarzębski et al., 2020). EOs are generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA) (Trinetta et al., 2017; USFDA, 2019). EOs are commonly used in areas such as food, medicine and cosmetics with their properties such as antibacterial, antimycotic, antifungal, antiviral, antiparasitic, antitoxigenic, anticancer, anti-inflammatory and antioxidant activity. These characteristics may be related to the function of parts of plants (Vázquez-Sánchez et al., 2015; Moussaoui and Alaoui, 2016; dos Santos et al., 2018). EOs are complex mixtures containing about 500 compounds, such as alcohols, esters, ethers, terpenoids, aldehydes, phenol and phenol ethers (Stefanakis et al., 2013; Vázquez-Sánchez et al., 2018). However, the chemical composition of EOs may differ. The chemical composition responsible for the antimicrobial properties of EOs is influenced by climate and geographical conditions such as harvesting, storage and isolation techniques (Alexopoulos et al., 2011). There have been more than 3000 EOs, with approximately 300 of which are commercial (Stefanakis et al., 2013; Sakkas and Papadopoulou, 2017). These EOs can be extracted from different parts of the aromatic plants using various methods such as water or steam distillation, solvent extraction, expression under pressure, supercritical fluid and subcritical water extractions. Among of these methods, distillation is the most commonly used (Viuda-Martos et al., 2011; Yagi et al., 2016; Fancello et al., 2020; Imane et al., 2020).

EOs exhibit significant antimicrobial activity against food-borne pathogens such as *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli*, *Salmonella*, and *Bacillus cereus* (Ozogul et al., 2015; Guo et al., 2019; Al-Nabulsi et al., 2020). EOs can also be evaluated against various fungal species (Viuda-Martos et al., 2011; Sakkas and Papadopoulou, 2017). This antibacterial activity can be explained by the damage of EOs to the cell membranes, genetic materials and enzyme systems of bacteria and degradation of phospholipid double layers (Moreira et al., 2005; Viuda-Martos et al., 2011; Güneş and Tıhmınlıoğlu, 2017). Moreover, many EOs are reported to contain antimicrobial compounds such as thymol, carvacrol, eugenol and cinnamaldehyde (Herman et al., 2016; Tariq et al., 2019; Cho et al., 2020; Xiao et al., 2020). Gram positive bacteria are more sensitive to EOs than Gram-negative bacteria because Gram negative bacteria are composed of two layers that protect the cell and give it a hardness to the cell. Therefore, this outer membrane gives the bacterial surface a strong hydrophilic property (Viuda-Martos et al., 2011; Sakkas and Papadopoulou, 2017).

L. monocytogenes is one of the most important foodborne pathogens (Guo et al., 2019) and can cause an important public health problem with high mortality and morbidity rates (Lee et al., 2018; Stratakos, et al., 2020). *L. monocytogenes* infection entitled listeriosis can lead to disease during vulnerable stages of life such as newborn, pregnant woman, elderly and persons with immunocompromising conditions individuals (Gomez et al., 2014). Listeriosis can cause important health problems such as gastroenteritis, septicemia, meningitis, meningoencephalitis, endocarditis, and fetal losses (Zhu et al., 2017; Desai et al., 2019; Nichols et al., 2020). *L. monocytogenes* can be found in various food groups

such as milk and dairy products, meat and meat products, seafood, vegetables and ready-to-eat foods (Lee et al., 2018). *L. monocytogenes* grows between 0.4 and 50 °C (Zhu et al., 2017; Guo et al., 2019). Therefore, the development of this pathogen in refrigerator conditions makes food preservation difficulties. As an alternative to the cooling process for controlling *L. monocytogenes* in foods, non-thermal processing methods can be used as well as traditional methods. Adding natural antimicrobial agents such as bacteriocin, EOs, enzyme, organic acids to foods is one of the most remarkable effective applications in recent years (Nichols et al., 2020).

The purpose of this paper was to provide an overview of the antibacterial activity of EOs considered suitable for application in or on foods. Based on this idea, antibacterial activity of 15 different EOs against *L. monocytogenes* strains previously isolated from ready-to-eat food in Ankara was determined using disc diffusion method.

MATERIALS AND METHODS

Essential Oils

Fifteen different herbal EOs (purity ≥98%, Balen™, Ankara) purchased from herbalists in Ankara were used in this study. All of the EOs used in this study were produced by distillation method by the manufacturer. In addition, the purity and chemical composition of EOs were determined by the manufacturer. Essential oils used in this study were: rosemary (*Rosmarinus officinalis* L), peppermint (*Mentha* x *piperita*), eucalyptus (*Eucalyptus globulus*), thyme (*Thymus vulgaris* L), laurel (*Laurus nobilis* L), juniper (*Juniperus communis* L), St. John's Wort (*Hypericum perforatum* L), ginger (*Zingiber officinale*), black seed (*Nigella Sativa* L), clove (*Syzygium aromaticum*), sage (*Salvia officinalis*), pine turpentine (*Pinus terebenthinae*), garlic (*Allium sativum* L), dill (*Anethum graveolens* L), and cumin (*Cuminum cyminum* L). All of these EOs were stored at room temperature in the dark condition.

Bacterial Strains

In this study, 29 different *L. monocytogenes* strains isolated from ready-to-eat foods in Ankara previously and one reference strain (*Listeria monocytogenes* ATCC 7644) were obtained from the culture collection of Food Microbiology Laboratory, Department of Food Engineering, Ankara University, Ankara, Turkey. These strains inoculated on Tryptic Soy Broth (TSB) (Merck™, Germany) and Brain Heart Infusion (BHI) broth (Merck™, Germany) and incubated at 35°C for 24 h. All of the strains were stored at –20°C in 30% (v/v) glycerol (Merck™, Germany) until tested.

Determination of antibacterial activity

Antibacterial activity of the EOs against *L. monocytogenes* strains was performed using the agar diffusion method as recommended by de Aguiar et al., 2018. As a preliminary step, EOs were sterilized by passing thorough 0.22 µm pore-size membrane filters as described by Cava et al. (2007) and then sterile discs (6 mm diameter white disc) (Oxoid ltd, ES) were impregnated with approximately 15 µL of each steril EOs. All of the discs were soaked for 18 h at room temperature under aseptic condition. For preparation of the bacterial suspension from overnight culture, turbidity was visually adjusted to that of a 0.5 McFarland turbidity standard (~$1.5x10^8$ CFU/mL) using sterile TSB. Ten mL of the bacterial suspensions were added to 90 mL Tryptic Soy Agar (TSA) (Sigma™, Germany). Then, this medium was mixed with a shaker and poured into each sterile petri dishes. After solidification, the discs impregnated with 15 µL of different EOs were placed on the surface of petri dishes. Disks with gentamicin (10 mg) were used as positive control. Negative control discs were soaked with the sterile distilled water. The plates containing discs were kept at room temperature for at least 30 minutes before incubated at 37°C for 24 h. At the end of the incubation times, the inhibition zone diameters were measured by a clean ruler. The sensitivity to the different EOs was classified as follows: not sensitive (-) for diameter less than 8 mm; sensitive (+) for diameter 9–14 mm; very sensitive (++) for diameter 15–19 mm and extremely sensitive (+++) for diameter larger than 20 mm (Ponce et al., 2003). All tests were performed in duplicate.

Statistical analysis

In our study, measurements related to quantitative variables are summarized with mean and standard deviation. The comparisons between groups in terms of measurement averages were tested with variance analysis (ANOVA). When the ANOVA test is found to be significant, sub-sets related to the groups were created with the Post Hoc (Tukey b) test. The significance level was accepted as 5%. The analyzes were carried out using SPSS (Version 25) software.

RESULTS AND DISCUSSION

In this study, the antibacterial effects of 15 different commercially purchased EOs on *L. monocytogenes* strains were investigated. When making measurements, completely transparent diameters formed around the discs were taken into account as the inhibition zone. The formation of the inhibition zone around the disc was shown in Figure 1. The average values of antibacterial inhibition zones for different EOs against *L. monocytogenes* were showed in Table 1. As can be seen in Table 1, when the zone measurement averages were compared, a statistically significant difference was found between the types of EO (F=234,629; p<0,001). The susceptibility of 30 different *L. monocytogenes* strains to EOs was in Table 2. Moreover, zone measurement averages for EO types were shown in Figure 2 with 95% confidence intervals.

Table 1. Average zone diameters (mm) and standard deviation values of herbal EOs*

Essential oils	Mean
Ginger	0.000±0.000[a]
Garlic	0.000±0.000[a]
St. John's Wort	0.100±0.548[a]
Cumin	0.133±0.730[a]
Laurel	0.150±0.822[a]
Juniper	0.267±1.015[a]
Eucalyptus	2.767±2.932[b]
Pine turpentine	3.950±3.687[b]
Black seed	4.233±3.586[b]
Dill	7.433±2.511[c]
Rosemary	8.450±1.936[c]
Peppermint	8.450±3.749[c]
Sage	11.117±3.170[d]
Clove	12.383±2.215[d]
Thyme	24.850±3.714[e]
F: 234.629; p<0.001	

*The difference between the mean values of essential oil types was statistically significant (F=234.629; p<0.001). The smallest significant difference test (LSD: Least Significant Difference) was performed for multiple comparisons to determine the source of the differences. In case of no significant difference between the groups, the mean values were indexed with the same letter.

Table 2. EOs susceptibility of *Listeria monocytogenes* strains

Essential Oils	-[a]		+[b]		++[c]		+++[d]	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Ginger	30	100	0	0.0	0	0.0	0	0.0
Garlic	30	100	0	0.0	0	0.0	0	0.0
St. John's Wort	30	100	0	0.0	0	0.0	0	0.0
Cumin	30	100	0	0.0	0	0.0	0	0.0
Laurel	30	100	0	0.0	0	0.0	0	0.0
Juniper	30	100	0	0.0	0	0.0	0	0.0
Eucalyptus	28	93.3	2	6.7	0	0.0	0	0.0
Pine turpentine	26	86.7	4	13.3	0	0.0	0	0.0
Black seed	24	80.0	6	20.0	0	0.0	0	0.0
Dill	14	46.7	16	53.3	0	0.0	0	0.0
Rosemary	12	40.0	18	60.0	0	0.0	0	0.0
Peppermint	10	33.3	20	66.7	0	0.0	0	0.0
Sage	4	13.3	24	80	2	6.7	0	0.0
Clove	2	6.7	22	73.3	6	20.0	0	0.0
Thyme	0	0.0	0	0.0	3	10.0	27	90.0

- : ≤8 mm (not sensitive)
+ : between 9 mm and 14 mm (sensitive
++: between 15 mm and 19 mm (very sensitive)
+++ ≥20 mm (extremely sensitive)

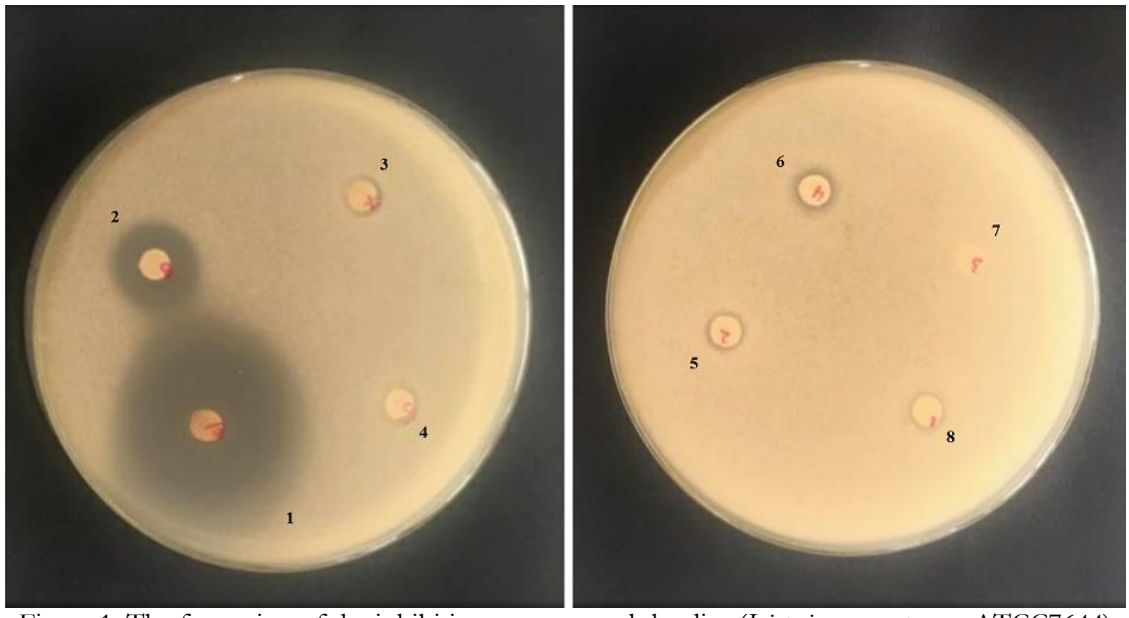

Figure 1. The formation of the inhibition zone around the disc (*Listeria monocytogenes* ATCC7644)
(Disc 1: thyme oil, disc 2: clove oil, disc 3: St. John's Wort oil, disc 4: cumin oil, disc 5: laurel oil, disc 6: Pine turpentine oil, disc 7: garlic oil, disc 8: eucalyptus oil).

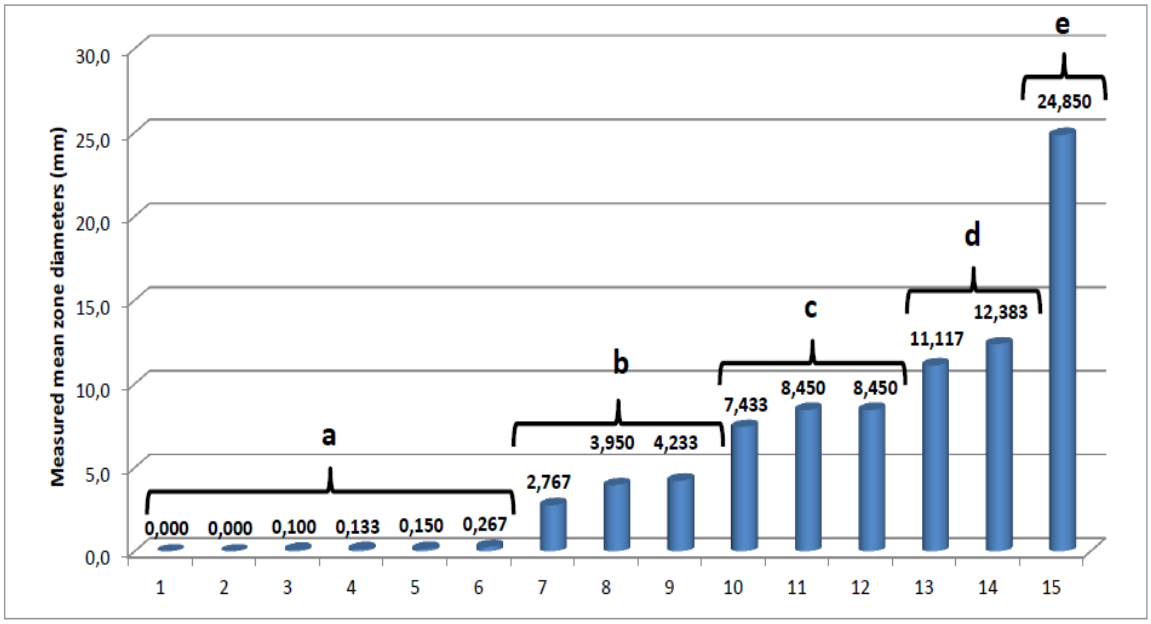

Figure 2. Subsets for different Eos types and comparison of mean zone diameters (with 95% confidence interval)

Group a: Ginger oil (1), Garlic oil (2), St. John's Wort oil (3), Cumin oil (4), Laurel oil (5), Juniper oil (6)
Group b: Eucalyptus oil (7), Pine turpentine oil (8), Black seed oil (9)
Group c: Dill oil (10), Rosemary oil (11), Peppermint oil (12)
Group d: Sage oil (13), Clove oil (14)
Group e: Thyme oil (15)

According to the results, the most effective antibacterial activity was found to be against thyme oil (mean zone 24.850 ± 3.714 mm). This antibacterial effect was followed by clove oil (mean zone 12.383 ± 2.215 mm) and sage oil (mean zone 11.117 ± 3.170 mm). In this study, the lowest antibacterial activity of EOs were St. John's wort oil (mean zone 0.100 ± 0.548 mm), cumin oil (mean zone 0.133 ± 0.730 mm), laurel oil (mean zone 0.150 ± 0.822 mm), and juniper oil (mean zone 0.267 ± 1.015 mm), respectively. In addition, ginger oil and garlic oil did not show any antibacterial activity against the *L. monocytogenes* strains used in the study.

When the antibacterial activity of EOs was examined, it was possible to form five homogeneous EOs groups (Figure 2). In our study, thyme oil samples were showed the highest antibacterial activity against to all *L. monocytogenes* stains with an average zone diameter of 24.850±3.714 mm. Thyme oil was classified as group "e" in Figure 2. We found that 90% (27/30) of *L. monocytogenes* strains were extremely sensitive to thyme oil and 10% (3/30) were very sensitive. Our findings were similar to previously published report. Viuda-Martos et al. (2011) reported that thyme oil (20 µL) had high inhibitory effects on three bacteria tested: 38.00 mm, 16.50 mm and 19.83 mm for *L. innocua*, *Serratia marcescens* and *Pseudomonas fluorenscens*, respectively. Similarly, Hu et al. (2018) investigated the antibacterial effect of thyme oil on *L. monocytogenes*, *S.* Typhimurium, *Stap. aureus* and *E. coli* O157: H7 and found average zone diameters of 23.60 mm, 20.01 mm, 14.07 mm and 22.47 mm, respectively. Moreover, Canberi et al. (2020) found that thyme oil had high antibacterial effect against *Stap. aureus* strains with an average zone diameter of 23.203 mm. However, in contrast to our findings, Moreira et al. (2005) reported that thyme oil showed lower antibacterial activity against *E. coli* strains and obtained zone diameters between 10-12 mm. High antibacterial activity of thyme oil may be related to the high thymol and carvacrol content, which can form hydrogen bonds with the active

site of many enzymes due to the acidic nature of the hydroxyl group (Wińska et al., 2019).

Clove oil and sage oil used in our study were in the second group entitled "d" in Figure 2 with the highest antibacterial activity against *L. monocytogenes* strains. Clove oil was found to be the most effective EO after thyme oil with an inhibition zone diameter of 12.383±2.215 mm. In this study, while 20% (6/30) of *L. monocytogenes* strains were very sensitive to clove oil, 73.3% (22/30) of *L. monocytogenes* strains were sensitive to that. In addition to these results, 6.7% (2/30) of *L. monocytogenes* strains were not sensitive to clove oil. Consistent with our results, de Aguiar et al. (2018) reported that clove oil showed an antibacterial activity against *Streptococcus suis* isolates with an inhibition zone diameter of 6.0-28.0 mm (mean zone15.8±5.0 mm). In contrast to our study, Condò et al. (2020) found that clove oil had a weak antibacterial effect against *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Stap. aureus* and *S. epidermidis* and also no antibacterial activity of clove oil was detected against *Ent. faecalis* and *Streptococcus pyogenes*. In our study, antibacterial activity of sage oil against *L. monocytogenes* strains was similar to antibacterial activity of clove oil. The average value of the inhibition zone diameter measured for sage oil was 11.117±3.170 mm. The measurements of the inhibition zones were lower than that reported by Radünz et al. (2019) who investigated the antibacterial activity of clove oil on *L. monocytogenes* and reported the average inhibition zone diameter as 24.7 mm. Moreover, higher antibacterial activity of sage oil was published by Miladinović and Miladinović (2000) who reported that the antibacterial effect of sage oil against *Stap. aureus, E. coli,* and *B. subtilis,* and measured inhibition zone diameters of 19.05±0.23 mm, 19.20±0.57 mm and 20.20±0.84 mm, respectively. Eugenol, the main component of clove oil, has an inhibitory effect on *L. monocytogenes* (Tariq et al., 2019). The antibacterial activity of sage oil can be explained by the presence of components such as camphor, thujone and 1,8-cineole (Ghorbani and Esmaeilizadeh, 2017).

The third group in Figure 2 consists with peppermint oil, rosemary oil and dill oil. In our study, the average zone diameters measured for rosemary oil, peppermint oil and dill oil were 8.450±3.749 mm, 8.450±1.936 mm and 7.433±2.511 mm, respectively. Additionally, 66.7% (20/30) of *L. monocytogenes* strains were found to be sensitive to peppermint oil, 60.0% (18/30) to rosemary oil and 53.3% (16/30) to dill oil. On the other hand, 33.3% (10/30) of *L. monocytogenes* strains were not sensitive to peppermint oil, 40.0% (12/30) to rosemary oil and 46.7% (14/30) to dill oil. Similar results has been previously reported by Hu et al., (2018) who determined the antibacterial activity of rosemary oil against *L. monocytogenes* strains and obtained the average zone diameters of 9.87 mm and 6.83 mm. In addition, they did not find antibacterial effect of peppermint oil on *L. monocytogenes*. de Aguiar et al. (2018) were also obtained that peppermint oil showed antibacterial effects against *Strep. suis* isolates with a zone diameter of 16.4 mm. Esmael et al., (2020) found that the antibacterial effect of rosemary oil against *S. aureus* and *Stap. epidermidis* and measured inhibition zone diameters of 12.5 mm and 15.18 mm, respectively. Opposite result has been previously reported by Elgayyar et al. (2001) who found that *L. monocytogenes* was not sensitive to rosemary oil and dill oil. In consistent with our results, the antibacterial activity of dill oil against *B. cereus, Stap. aureus, E. coli* and *Salmonella typhi* has also been reported by Hojjati (2017) who revealed that inhibition zone diameters measured for dill oil was found to be 1.33±0.31 cm, 1.30±0.08 cm, 1.23±0.82 cm, and 1.44±0.06 cm, respectively. According to statistical data, the group "b" containing black seed oil, pine turpentine oil and eucalyptus oil showed low antibacterial activity against pathogen bacteria in this study. While 20% (6/30) of *L. monocytogenes* strains were sensitive to black seed oil (4.233±3.586 mm), 80% (24/30) of the strains were not sensitive to black seed oil. Inhibition zone diameters measured for pine turpentine oil and eucalyptus oil were found to be 3.950 ± 3.687 mm and 2.767±2.932 mm, respectively. Moreover, 13.3% (4/30) of *L. monocytogenes* strains were sensitive to pine turpentine oil, and 6.7% (2/30) of strains were sensitive to eucalyptus oil.

In accordance with our results, Nair et al., (2005) reported that antibacterial activity of black seed oil with average inhibition zone diameters ranging from 28.20±2.00 mm to 39.50±1.10 mm was detected against *L. monocytogenes* strains. Similarly, Viuda-Martos et al., (2011) found that black seed showed antibacterial activity with an average 19 mm inhibition zone diameter against *L. innocua*. Finally, Ozogul et al., (2015) investigated the antibacterial activity of eucalyptus oil and pine oil against *E. coli* strains, and determined the average inhibition zone diameters of 2.75±0.07 mm and 5.25±0.35 mm, respectively.

The group entitled "a" with the lowest antibacterial activity in our study included juniper oil, laurel oil, cumin oil, St. John's Wort oil, garlic oil and ginger oil. We found all *L. monocytogenes* strains showed no sensitivity to garlic oil and ginger oil. At the same time, the average inhibition zone diameters for juniper oil, laurel oil, cumin oil, and St. John's wort oil were measured as 0.267±1.015 mm, 0.150±0.822 mm, 0.133±0.730 mm and 0.100±0.548 mm, respectively. In this group, we can say that these EOs did not have a good antibacterial activity on *L. monocytogenes* strains. In contrast to our findings, Canberi et al. (2020) examined the antimicrobial activity of juniper oil, laurel oil, cumin oil, St. John's Wort oil, garlic oil and ginger oil against *S. aureus* strains and found average zone diameters 6.49 mm, 6.33 mm, 6.51 mm, 6.21 mm, 6.27 mm and 6.25 mm, respectively. Moreover, antibacterial activity of garlic oil against *Stap. aureus* was measured by Khashan (2014) who reported that the zone of inhibition was 23 mm. When our results are compared with the results obtained Khashan (2014) and Canberi et al. (2020), garlic oil was more effective on *S. aureus*.

CONCLUSIONS

In this study, the antibacterial activity of some EOs against *L. monocytogenes* strains from ready-to-eat foods has been demonstrated using disc diffusion method. Thyme oil is particularly promising by inactivating *L. monocytogenes* strains. Consequently, the EOs could be used alone or in combination with antibacterial agents to control food-borne bacteria, although more in vivo studies on the safety and the effect of EOs are needed.

CONFLICT OF INTEREST

The authors express no conflict of interest associated with this work.

AUTHORS' CONTRIBUTIONS

PŞ designed the research. SA, HAC, and EŞ, carried out microbiological analyzes of the research and also made statistical analyzes. SA and PŞ wrote the paper. All authors contributed to the article and approved the submitted version.

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