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RESEARCH ARTICLE

Pistacia terebinthus FRUIT: AN ALTERNATIVE TO PREVENT FOOD SPOILAGE

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ABSTRACT

The aim of this work was to investigate the usage possibilities of *P. terebinthus* fruit, which has a limited usage area, in the food industry. Antimicrobial activity of hexane extract obtained from *P. terebinthus* fruits from Adıyaman (Turkey) was determined by disc diffusion and micro-dilution assays against test microorganisms. In addition, the antibacterial activity of the extract on *Escherichia coli* O157:H7, one of the most important food-borne pathogens, was determined by viable cell count using a macro-dilution assay. The potential for use of the extract with probiotic candidate lactic acid bacteria (LAB) strains was also investigated. The hexane extract presented antimicrobial activity against all the tested microorganisms, with good inhibition zone diameters between 10.51 mm and 18.02 mm. MIC and MFC or MBC values of *P. terebinthus* fruit extract were determined as 5-80 µg/µL against all tested microorganisms. The lowest MIC and MBC values (5 µg/µL) of the extract were obtained against *E. coli* O157:H7. Macro-dilution assay results indicated that the *P. terebinthus* extract at various concentrations (5-10-20 mg/mL) inhibited the growth of *E. coli* O157:H7 more than the control group after all of the incubation hours. No viable *E. coli* O157:H7 cells were detected after 48 hours at all concentrations. The extract showed low antimicrobial activity, and relatively high bactericidal concentration on probiotic candidate LAB strains. This shows that *P. terebinthus* hexane extract at appropriate concentrations can be used together with probiotic strains as a natural preservative and biopreservative to prevent food spoilage and extend shelf life.

Keywords: *Pistacia terebinthus*, Lactic Acid Bacteria, Extract, Antimicrobial, Biopreservative

1. INTRODUCTION

Pistacia terebinthus L., belonging *Anacardiaceae*, is known by different names such as menengiç, bıttım and çedene in some regions of Turkey. *P. terebinthus*, a symbolic plant of the Mediterranean and West Asia, is predominantly found in the southern and western parts of Turkey [1]. *P. terebinthus* is a tree species that grows to a height of 10 meters and grows in the hills and rocks of coastal areas. The fruit, which is pink before ripening, turns green and blue as it matures [2].

P. terebinthus fruit is rich in oil, protein, and dietary fiber and has long been known for its unique taste and aroma properties [2]. The fruits have been the focus of many research due to their anti-inflammatory [3], antimicrobial [4] and antioxidant [5,6] properties. The World Health Organization (WHO) reported in 2018 that 88% of its 194 member states accepted the use of traditional and alternative medicine [7]. In recent years, research has been directed towards finding natural food preservatives due to the potential harms of synthetic preservatives to health [8, 9]. As consumers become more conscious, they are concerned about consuming foods containing these chemical additives. Plants are considered relatively safe for human use and the environment as a source of antimicrobial compounds [10].

Biopreservation refers to the controlled use of microorganisms or their metabolites to prevent microbial spoilage, the growth of pathogenic microorganisms, extend the shelf life of foods, and ensure their microbial safety [11]. The ability of lactic acid bacteria (LAB) to inhibit the growth of pathogenic bacteria and their alternative use in the food industry have been reported in the literature [12, 13]. LAB

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are commonly used as probiotic cultures in various processes that are generally considered safe and have therapeutic effects on the host [14]. LAB and herbal extracts can be used together as natural preservatives to prevent microbial spoilage of food.

In the study, the alternative potential usage of *P. terebinthus* fruit extract to prevent food spoilage and extend shelf life was investigated. Therefore, the antimicrobial activity of the hexane extract against test microorganisms was tested to determine its potential usage as a natural preservative additive in the food industry. It is also aimed at evaluating the potential usage of *P. terebinthus* fruit hexane extract together with LAB in the food industry as a natural bioadditive.

2. MATERIALS AND METHODS

2.1. Preparation of Extract

P. terebinthus fruits were obtained from a herbalist in Adıyaman (Turkey). After the fruit material was washed, it was air-dried at room condition. The fruits were ground and then the powdered sample was extracted with hexane using the soxhlet system. The solvent was then evaporated from the extract by using a rotary evaporator. After dissolving the hexane fruit extract with dimethyl sulfoxide (DMSO) at the concentration of 100 µg/µl, it was sterilized with a 0.45 µm filter.

2.2. Determination of Antimicrobial Activity

The inhibitory activity of the extract was tested with the disc diffusion method. *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218, *E. coli* O157:H7, *Bacillus cereus* RSKK 863, *Shigella sonnei* Mu:57, *Salmonella enteritidis* ATCC 13076, *Yersinia enterocolitica* ATCC 11175, *Micrococcus luteus* B-4375 and *Staphylococcus aureus* ATCC 25923 were cultured in Nutrient Broth (NB)/Agar. *L. monocytogenes* ATCC 7644 was grown in Tryptic Soy Broth (TSB)/Agar. *Candida glabrata* RSKK 04019 and *C. albicans* ATCC 10231 were cultured in Yeast Extract Peptone Dextrose (YPD)/Agar. De Man, Rogosa and Sharpe (MRS)/Agar was used for *Lactobacillus delbrueckii* MA-9, *Lactobacillus fermentum* MA-8, *L. gasseri* MA-2, *L. gasseri* MA-3, *L. gasseri* MA-4, *L. gasseri* MA-5, *L. fermentum* MA-7, *Lactobacillus vaginalis* MA-10, and *Lactobacillus plantarum* RSKK 1062 as growth medium.

The tested LAB were isolated from human milk in our previous studies and then characterized for probiotic potential [15-17]. *L. plantarum* RSKK 1062 is a commercial LAB strain used as a control.

The test microorganisms (adjusted to 0.5 McFarland, ~$1x10^8$ CFU/mL) were inoculated, and sterile discs (6 mm in diameter) were placed on the agar medium. Then, the extract (20 µL, 2000 µg/disc) was dropped onto the discs and incubated for 24 h at 37°C for bacteria and at 30°C for yeast. After the incubation period, the inhibition zone around the discs was recorded [18]. The solvent (DMSO) of the extract and the antibiotic Gentamicin (CN, 10 µg/disc) were used as negative and positive control groups, respectively. The assays were done in triplicate.

2.3. Micro-Dilution Method

Micro-dilution assay was used to determine the MIC (Minimal Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) or MFC (Minimum Fungicidal Concentration) values of the hexane extract against the tested microorganisms [19]. The microorganisms (0.5 McFarland) were added to a tube containing growth media and extract. After incubation, the concentration of the hexane extract (80, 40, 20, 10, 5 and 2.5 µg/µL) in the liquid medium without growth was recorded as MIC values. The samples from broth media were then inoculated on the agar medium. After the incubation, extract

concentrations in which microbial growth was not observed on solid medium were recorded as MFC or MBC values.

2.4. Macro-Dilution Method

Macro-dilution assay was also performed to determine the antimicrobial activity of *P. terebinthus* fruit extract. The antibacterial activity of the hexane extract on *E. coli* O157:H7 was determined by counting viable cells using the method of Sousa et al. [20] with some modifications. *E. coli* O157:H7 culture (adjusted to 0.5 McFarland) was added into the fruit extract (5-10-20 mg/mL concentrations) and growth medium mixture (total volume 10 mL). The cell suspension without extract was used as a control group. Then, the control group and the bacterial suspensions containing the extract were incubated at 37°C. The samples from the mixture were then diluted and inoculated onto NA medium. After incubation, the viable cells were counted and recorded as $\log_{10}$ CFU/mL.

2.5. Statistical Analysis

Data were analyzed using GNU SPSS version software, and analysis was performed using One Way ANOVA at $p<0.05$. In addition, differences in antimicrobial activity between the tested microorganisms were evaluated using the post-hoc Tukey test. The differences in inhibition zone diameter means were evaluated as statistically significant (*p-value* < 0.05). The analyzed data are reported as the mean and standard deviation (SD) of three replicate results.

3. RESULTS AND DISCUSSION

The antimicrobial activity of the hexane extract from *P. terebinthus* fruit was investigated against test microorganisms. The disc diffusion assay results indicated that the highest two inhibition zone diameters among the test bacteria were 18.02 mm against *L. monocytogenes* ATCC 7644 and 16.38 mm against *P. aueroginosa* ATCC 27853. The highest antifungal activity was obtained against *C. glabrata* RSKK 04019 with an inhibition zone diameter of 17.19 mm. MIC and MFC or MBC values of *P. terebinthus* fruit extract were determined as 5-80 µg/µL against all tested microorganisms (Table 1).

Table 1. Antimicrobial activity of *P. terebinthus* fruit hexane extract

Test Microorganisms	Inhibition Zone Diameters (mm±SD)		MIC (µg/µL)	MBC or MFC (µg/µL)
	Extract	Antibiotic disc CN		
E. coli O157:H7	12.51±0.66[a]	14.07±0.01	5	5
E. coli ATCC 35218	10.52±0.66[c]	10.19±0.02	20	40
L. monocytogenes ATCC 7644	18.02±5.73[b,d]	19.38±0.02	10	10
B. cereus RSKK 863	13.86±0.86	12.97±0.30	40	80
S. sonnei Mu:57	15.39±0.29	11.08±0.80	40	80
S. enteritidis ATCC 13076	13.91±0.63	10.51±0.02	20	40
Y. enterocolitica ATCC 11175	12.76±1.05	19.92±0.01	10	20
M. luteus B-4375	15.55±1.17	10.93±0.01	20	20
P. aueroginosa ATCC 27853	16.38±0.68[e]	16.31±0.02	40	40
S. aureus ATCC 25923	15.78±0.12	13.05±0.02	40	40
E. faecalis ATCC 29212	15.36±0.46	13.48±1.44	40	80
C. albicans ATCC 10231	16.55±1.44[f]	NA	20	20
C. glabrata RSKK 04019	17.19±1.29[f]	NA	40	80

Different successive superscript values in columns differ significantly ($p<0.05$) by one-way ANOVA followed by Tukey's post-hoc test.
NA: No activity

Hacıbekiroğlu et al. [21] investigated the antimicrobial activity of *P. terebinthus* fruit hexane extract (100 mg/mL) using the broth dilution method, however, the hexane extracts did not show any antimicrobial activity against tested microorganisms such as *E. coli*. Durak & Uçak [22] extracted *P. terebinthus* fruit with an acetone-water solvent and investigated its antimicrobial activity using disc diffusion method. The inhibition zone diameters of the extract (0.5 mg/disc) against *S. aureus*, *L. monocytogenes*, *S. typhimurium* and *E. coli* O157:H7 were 9-13 mm, 11.25-14.25 mm, 11.67-15.25 mm and 8.75-11.5 mm, respectively. In current study, the inhibition zone diameters of hexane extract were higher against *L. monocytogenes* ATCC 7644 and *E. coli* O157:H7, and close to the other tested bacteria. In the study of Doğan [23], it was determined that water, ethanol and methanol extracts of *P. terebinthus* fruit did not show antifungal activity on *C. albicans* ATCC 87392 and antibacterial activity against *E. coli* ATCC 1213. The differences among the results may be due to the solvent used in the extraction, the extract concentration differences used, cultivation conditions such as climate and soil characteristics [24].

Today, diseases caused by food-borne pathogens pose a great problem. *E. coli* O157:H7 is a food-borne pathogen frequently associated with disease cases characterized by bloody diarrhea, hemolytic uremic syndrome, and hemorrhagic colitis outbreaks [25]. The hexane extract was used to determine the antimicrobial activity by counting viable cells because it showed the lowest MIC and MBC value (5 µg/µL) on *E. coli* O157:H7 among tested microorganisms. In our study, it was found that the extract of *P. terebinthus* prepared at various concentrations inhibited the growth of *E. coli* O157:H7 more than the control group after all of the incubation hours. As the extract concentration increased, a decrease in the viable cell of *E. coli* O157:H7 was obtained after incubation periods. No viable cells were determined after 48 hours at all tested concentrations (5-10-20 mg/mL) (Figure 1). These results indicate that *P. terebinthus* hexane extract, which has a cidal effect against food-borne *E. coli* O157:H7, may have the potential to prevent food contamination as a natural biopreservative.

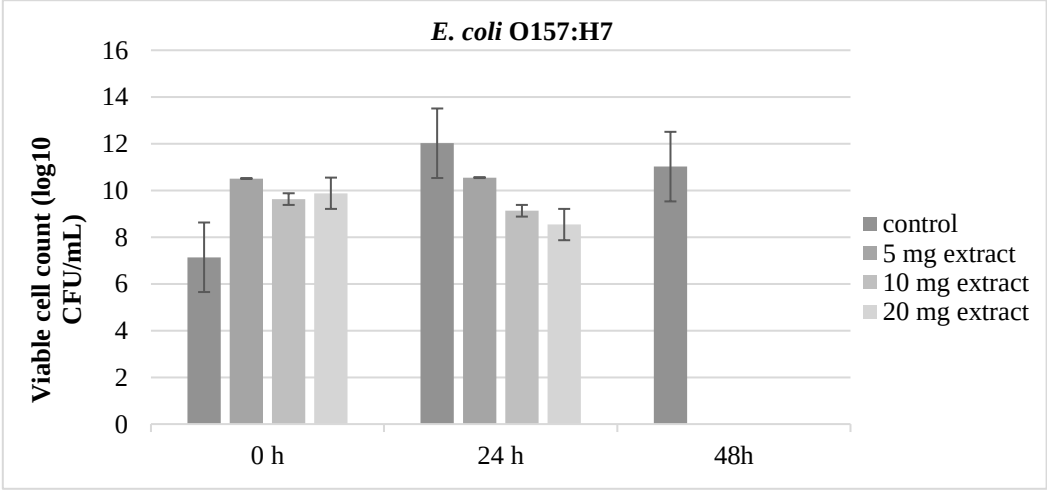

Figure 1. Viable cell counts of *E. coli* 0157:H7

In a study, the antimicrobial activity of *P. terebinthus* seed essential oil (MEO) against *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 11778, and *Staphylococcus aureus* ATCC 29213 was investigated. The inhibition zone diameters of MEO were recorded as 2.0 mm, 3.5 mm, and 3.5 mm, respectively [26]. In a study conducted by Çoban et al. (2017), it was reported that *P. terebinthus* methanol, ethyl acetate, water, pure methanol, pure ethyl acetate, distilled water fruit extracts did not show antimicrobial

activity on *Klebsiella pneumoniae* ATCC 13882, *C. albicans* ATCC 10231 and *C. glabrata*. In our study, *P. terebinthus* fruit hexane extract exhibited antifungal activity with good inhibition zones on *C. albicans* ATCC 10231 and *C. glabrata* RSKK 04019 [27]. In another study, the inhibition zone diameters of *P. terebinthus* fruit methanol extract were determined as 9 mm for *E. coli* ATCC 25922, 13 mm for *S. aureus* ATCC 25923 and 12 mm for *P. aereginosa* ATCC 9027. The inhibition zone diameters obtained as a result of this study were found to be higher for these three bacteria [28]. This difference may be due to the difference in the solvent and method used in extraction.

LAB, the most common probiotic microorganism, is found in human milk, the gastrointestinal tract, or the urogenital tract [29]. The antimicrobial activity of the hexane extract against LAB obtained from breast milk is presented in Table 2. The results indicated that the hexane extract had inhibitory activity on all the tested LAB strains. The data of the disc diffusion test showed the lowest inhibition zone diameters of 10.48 mm on *L. delbrueckii* MA-9. The MIC values of the hexane extract on the tested LAB were determined as 20-40 µg/µL, and the MBC values were determined as 20-80 µg/µL. The extract inhibited all the tested LAB, however, with high MIC and MBC values. Therefore, appropriate concentrations of the hexane extract together with the probiotic candidate LAB strains may be used as natural bioadditives in the food industry.

Table 2. Inhibitory activity of *P. terebinthus* fruit hexane extract on probiotic candidate LAB originated from human milk

Test Microorganisms	**Inhibition Zone Diameter (mm±SD)**	**MIC (µg/µL)**	**MBC (µg/µL)**
L. gasseri MA-2	$11.82\pm0.49^{a,n}$	20	20
L. gasseri MA-3	$10.96\pm0.65^{c,f,h,j,l}$	20	20
L. gasseri MA-4	$12.77\pm0.30^{d,e,n}$	40	40
L. gasseri MA-5	$12.89\pm0.39^{d,g,n,s}$	20	20
L. fermentum MA-7	$12.47\pm0.23^{d,I,n}$	20	40
L. fermentum MA-8	$12.33\pm0.54^{d,k,n}$	40	40
L. delbrueckii MA-9	$10.48\pm0.65^{b,f,h,j,l,m,p}$	20	40
L. vaginalis MA-10	$11.85\pm0.35^{n,o}$	20	20
L. plantarum RSKK 1062	$11.59\pm0.17^{h,r}$	40	80

Different successive superscript values in columns differ significantly ($p<0.05$) by one-way ANOVA followed by Tukey's post-hoc test.

The statistical analysis results indicated a statistically significant variation in inhibition zone diameter averages of the extract for test microorganisms as well as LAB ($p<0.05$). Multiple comparison analysis using by the Tukey test was performed to determine the microbial strains that caused the difference and is presented in Table 1 and Table 2.

4. CONCLUSION

Synthetic additives, the use of which has increased with the development of technology, have many side effects on health. In addition, it is an undeniable fact that microorganisms form resistance to synthetic antimicrobials that are used unconsciously. For such reasons, the use of plant extracts has come to the fore again, and studies on the development of the use of these products in many areas have been accelerated. The study demonstrated the antimicrobial activity of the hexane extract of *P. terebinthus* fruit. The extract with high antimicrobial activity may have potential use as a natural antimicrobial agent. The study revealed the promising potential of using *P. terebinthus* fruit, which has a very limited usage area, in the food industry to prevent food spoilage and extend shelf life.

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CONFLICT OF INTEREST

The author stated that there are no conflicts of interest regarding the publication of this article.

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