

Bioactive and Antimicrobial Properties of Mastic Tree (*Pistacia lentiscus* L.) Leaves from Turkish Republic of Northern Cyprus

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Abstract

In this research, the leaves of the *P. lentiscus* L. (mastic tree) were subjected to both solvent and ultrasonic extraction techniques, with durations of 30, 40, and 60 minutes. The resulting extracts were analyzed for their color, total phenolic content, antioxidant activity, chlorophyll levels, and antimicrobial effects. The extract obtained through 40-minute ultrasonic extraction exhibited the highest phenolic content, antioxidant activity, and chlorophyll concentration. Ultrasonication also increased color change (ΔE) with longer processing times, with the lowest ΔE observed in the 40-minute ultrasonic extract. While all extracts demonstrated antimicrobial activity against *S. aureus*, no inhibitory effects were detected against *L. monocytogenes* and *E. coli*. Notably, the 40-minute ultrasonic extract produced the largest inhibition zone against *S. aureus*. In summary, the bioactive properties of mastic tree leaves were significantly influenced by the extraction technique and duration.

Keywords: Mastic tree, *Pistacia lentiscus*, phenolics, antioxidant, antimicrobial

1. INTRODUCTION

Pistacia lentiscus, is a plant belonging to the Anacardiaceae family. Frequently referred to as the mastic tree, it secretes a resin known as mastic from its trunk (Gardeli et al., 2008). It exists in two subspecies: the naturally occurring shrub *Pistacia lentiscus* L. and the cultivated variety *Pistacia lentiscus* var. Chia (Cvitković et al., 2021). Mastic trees are dioecious, with male and female growing on separate trees (Ouahabi et al., 2023). This plant exhibits significant variability in its morphological, and chemical, traits, which are influenced by factors such as species type, geographical distribution, and plant gender (Yosr et al., 2018). The mastic tree grows in coastlines together with other maquis vegetation in Mediterranean countries such as Turkey, Northern Cyprus, Greece, Tunisia, Morocco, Spain, and Italy (Akdemir et al., 2015; Ouahabi et al., 2023). Known for its ecological importance, the

mastic tree contributes to the preservation of vulnerable ecosystems due to its high tolerance to drought and frost, rapid regeneration after wildfires, and exceptional resilience to environmental stress (Romani et al., 2002).

Phenolic compounds are a class of secondary metabolites characterized by one or more aromatic rings attached to hydroxyl groups (Dragović et al., 2020). The leaves of the mastic tree are particularly rich in secondary metabolites, including polyphenols, flavonoids and phenolic acids. These bioactive compounds endow the leaves with notable biological activities, such as potent antioxidant, antimicrobial, and anti-inflammatory effects (Bampouli et al., 2015; Ouahabi et al., 2023). Due to their diverse functional properties, phenolic compounds are widely studied for their contributions to both plant resilience and human

health, particularly in diseases related to oxidative stress (Manach et al., 2004; Arranz et al., 2012). Additionally, some studies emphasize the significant role of polyphenols in delaying the development of cancer, diabetes, cardiovascular, and neurodegenerative diseases (Sidor and Gramza-Michałowska, 2015; Sehaki et al., 2023). The phenolic content in mastic tree leaves varies based on genetic origin and several environmental and procedural factors, such as the extraction method, the plant's gender, its geographic location, and the climate, all of which affect the amount and variety of phenolic compounds produced (Romani et al., 2002; Dragović et al., 2020).

The objective of this study was to investigate how different extraction methods influence the total phenolic content, antioxidant capacity, total chlorophyll content, and antimicrobial activity of the leaves of the male shrub form of *Pistacia lentiscus* L., collected in 2024 from Girne, Northern Cyprus. For this purpose, solvent extraction and ultrasonic extraction were employed, with the latter conducted at varying durations of 30, 40, and 60 minutes. The extracts were comparatively analyzed to assess their bioactive properties, providing insight into the efficiency and effectiveness of each method.

2. MATERIALS AND METHODS

2.1. Materials and chemicals

The leaves from the shrub form of *Pistacia lentiscus* L. (mastic tree) were collected in 2024 from Girne, located in the Turkish Republic of Northern Cyprus. Reagents including Folin-Ciocalteu phenol, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium persulfate, gallic acid, and sodium carbonate were supplied from Sigma-Aldrich (St. Louis, MO, USA) as analytical grade. The test microorganisms (*Staphylococcus aureus* ATCC 13565 (Gram-positive), *Escherichia coli* NCTC 12241 (Gram-negative), and *Listeria monocytogenes* ATCC 7644 (Gram-positive)) used in the study were supplied by the Department of Food Technology at

the Faculty of Kadirli Applied Sciences, Osmaniye Korkut Ata University, Osmaniye, Türkiye.

2.2. Methods

2.2.1. Phenolic extraction of leaves

The mastic tree leaves were subjected to two extraction techniques: solvent (S) and ultrasonic (U). Solvent extraction (S) of mastic tree leaves was carried out following the method of Botsaris et al. (2015) with some modifications. Freshly ground leaf samples (approximately 2 g) were mixed with 20 ml of 85% ethanol solution. The mixture was then centrifuged at 5000 rpm for 10 minutes and filtered using coarse filter paper.

Ultrasonic extraction of mastic tree leaves was conducted with modifications to the method of Botsaris et al. (2015), employing three different extraction durations: 30 (U-30), 40 (U-40), and 60 (U-60) minutes. Ground leaf samples (approximately 2 g) were mixed with 20 ml of 85% ethanol solution and subjected to ultrasonic treatment in an ultrasonic bath for the specified times. After extraction, the samples were centrifuged at 5000 rpm for 10 minutes and filtered through coarse filter paper.

2.2.2. Color properties of phenolic extracts

The CIE Lab color parameters (L^* , a^* , and b^*) of phenolic extracts from mastic tree leaves, produced using various extraction techniques and conditions, were assessed using a chromameter (Hunterlab ColorQuestXE, USA) (Guclu et al., 2022). In these color models, L^* values represent color brightness (0: dark, 100: bright), $+a^*$ indicates redness, $-a^*$ signifies greenness, $+b^*$ shows yellowness, and $-b^*$ represents blueness. The total color difference (ΔE^*) values were determined using the following equation:

$$\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

2.2.3. Analysis of total phenolic content of leaves

The total phenolic content of mastic tree leaves was determined using the Folin-Ciocalteu approach, as described by Singleton et al. (1999). The absorbance values obtained from the analysis

were calculated using a gallic acid calibration curve (50–500 mg/kg), and the total phenolic content of the samples was expressed as mg Gallic Acid Equivalent (mg GAE/g).

2.2.4. Analysis of antioxidant activity

The antioxidant activity of mastic tree leaves was evaluated using two distinct methods: DPPH and ABTS. For the analysis, phenolic extracts of fresh leaf samples were diluted at a 1:1000 ratio. The DPPH method involved tracking the time-dependent reduction of the DPPH radical in methanol, measured at 515 nm with a UV-Vis spectrophotometer (PG Instruments, T60 UV-Visible Spectrophotometer, United Kingdom) (Brand-Williams et al., 1995). The antioxidant activity results were expressed as scavenging activity (%) using the formula below:

$$\text{Scavenging Activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

The ABTS method activates metmyoglobin in the presence of hydrogen peroxide, leading to the production of a colored radical solution. The absorbance of the sample was measured at 734 nm (Guclu and Selli, 2022). Trolox was used as the standard for calibration, and the results were expressed as μmol Trolox equivalents per g of the sample (μmol Trolox/g) based on the obtained calibration curve.

2.2.5. Analysis of chlorophyll content

The determination of chlorophyll content in the leaf extracts was carried out according to the method outlined by Lichtenthaler and Buschmann (2001). The chlorophyll levels were assessed using the phenolic extracts prepared with 85% ethanol. Absorbance measurements for both the extracts and the blank solution (85% ethanol) were taken at wavelengths of 649 nm and 664 nm with a spectrophotometer (PG Instruments, T60 UV-Visible Spectrophotometer, United Kingdom). Based on these absorbance values, chlorophyll a, chlorophyll b, and total chlorophyll concentrations were computed using the following calculation formulas:

$$\text{Chlorophyll a (mg/l)} = (13.36 \times A_{664}) - (5.19 \times A_{649})$$

$$\text{Chlorophyll b (mg/l)} = (27.43 \times A_{649}) - (8.12 \times A_{664})$$

$$\text{Total chlorophyll (mg/l)} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

2.2.6. Analysis of antimicrobial activity

The antimicrobial properties of the leaf extracts were evaluated using a modified version of the well diffusion technique described by Benabbou et al. (2009). The test microorganisms included *Staphylococcus aureus* ATCC 13565 (Gram-positive), *Escherichia coli* NCTC 12241 (Gram-negative), and *Listeria monocytogenes* ATCC 7644 (Gram-positive). Initially, bacterial strains were cultivated on Tryptic Soy Agar (TSA) at 37°C for 24 h. A single colony from each strain was then transferred into Tryptic Soy Broth (TSB) to prepare overnight cultures at the same temperature. For the assay, 0.1 ml of each overnight culture was evenly spread onto the surface of TSA plates using the spread plate method. Wells were carefully created in the solidified agar, and 400 μl of the leaf extract was added to each well. The plates were incubated aerobically at 37°C for 24–48 hours, after which the presence and size of inhibition zones surrounding the wells were assessed to determine antimicrobial activity.

2.2.7. Statistical analysis

One-way Analysis of Variance (ANOVA) was used to statistically analyze the analysis results using SPSS software (v.20.0, SPSS Inc., Chicago, IL). The Duncan Multiple Range test was used to further analyze significant differences found by ANOVA. Based on two replicates, the results were presented as mean $\pm$ standard deviation.

3. RESULTS AND DISCUSSION

3.1. Color properties of leaf phenolic extracts

The color properties of the mastic tree leaf extracts are displayed in Table 1. It was observed that the extraction technique and durations caused statistically significant color changes ($p < 0.05$). The mean color brightness (L^*) value was 32.99 for the S extract and 33.40 for the U-30 extract. However, it decreased as the ultrasonication time increased.

That is to say, the lowest L^* value was observed in the U-60 extract indicating that the U-60 extract was darker than the other extracts. The highest a^* value (0.75 and 0.74) was determined in the U-30 and U-40 extracts, while the lowest a^* value (0.14) was determined in the S extract. Conversely, the highest b^* value (7.59) was in the S extract, as the lowest b^* values (1.99) were observed in the U-60 extract. It was found that the ultrasonication caused the redness value to increase and the yellowness value to decrease. In terms of color difference values (ΔE), it was observed that ultrasonication caused more color change as the

processing time increased. The lowest color difference was detected in the U-40 extract. A study on strawberry juice reported that there were similar increases in $\Delta E'$ over time as the ultrasonication time increased (Wang et al., 2019). It has been reported in studies that ultrasonication, due to the sensitivity of plant pigments to time and temperature, may lead to structural degradation of the plant as the parameters such as processing time and temperature increase, thereby affecting various characteristics, including color (Žlabur et al., 2016; Kumar et al., 2023).

Table 1. Color properties of leaf extracts

	L^*	a^*	b^*	ΔE^*
S	32.99 ^b ± 0.02	0.14 ^b ± 0.01	7.59 ^a ± 0.07	-
U-30	33.40 ^a ± 0.02	0.74 ^a ± 0.03	2.87 ^c ± 0.06	4.78 ^b ± 0.01
U-40	30.21 ^c ± 0.03	0.75 ^a ± 0.05	3.97 ^b ± 0.02	4.60 ^c ± 0.07
U-60	28.42 ^d ± 0.02	0.72 ^a ± 0.04	1.99 ^d ± 0.02	7.25 ^a ± 0.05

^{a-d}: Significant differences indicated by different letters within the same column ($p < 0.05$).

3.2. Total phenolic content and antioxidant properties

The total phenolic content and antioxidant activity values of the resulting extracts are summarized in Table 2. The findings demonstrated that the U-40 extract had the highest total phenolic content, whereas the S extract resulted in the lowest values. It was observed that the choice of extraction method and duration significantly influenced total phenolic content. Within the ultrasonic extractions, U-30 showed the lowest total phenolic content, which increased with longer durations before showing a decline. Variance analysis indicated that both the extraction technique and duration had statistically significant effects on total phenolic content ($p < 0.05$). In two different studies where *P. lentiscus* L. leaves collected from various regions of Morocco were extracted using different solvents (water, ethanol, methanol, ethyl acetate, and hexane), the total phenolic contents were reported to range from 67.83 to 345.95 mg GAE/g (Barbouchi et al., 2020) and from 30.94 to 125.02 mg GAE/g (Labhar et al., 2023), which are similar to

our findings. In other previous studies, the total phenolic contents of *P. lentiscus* L. leaves subjected to ultrasonic extraction were reported as 51.3 mg GAE/g (Detti et al., 2020) and 130.17 mg GAE/g (Zaidi et al., 2024).

The antioxidant activities of the leaf extracts were assessed using two distinct methods, DPPH and ABTS, with the results shown in Table 2. Both methods revealed that the U-40 extract exhibited the highest antioxidant activity, consistent with the total phenolic content findings. Statistical analysis indicated that the extraction methods and durations had a significant effect ($p < 0.05$) on the antioxidant activity values of the extracts. In an earlier study, the antioxidant activity values of fresh and freeze-dried *P. lentiscus* var. Chia leaves were examined by Bampouli et al. (2015) using different extraction methods (Soxhlet, microwave, and ultrasound-assisted extraction) according to the DPPH method. The highest antioxidant activity values were achieved in both leaf samples when the ultrasound-assisted extraction method was

employed. In another previous study, similarly, the antioxidant activity of *P. lentiscus* L. leaves obtained from Croatia was determined to be 386.82 μmol Trolox/g (Elez Garofulić et al., 2020). In a previous study where *P. lentiscus* L. leaves sourced from Morocco were extracted using different solvents (hexane, ethyl acetate, ethanol, and water) and their antioxidant contents were investigated, the aqueous and ethanolic extracts were reported to exhibit high antioxidant activity due to their rich total phenolic content (Labhar et al., 2023).

The literature review reveals that varying results have been reported in studies on the total phenolic content and antioxidant activity of *P. lentiscus* leaves. These differences are thought to arise from factors like the genetic origin of the plants, environmental conditions, harvest timing, as well as the extraction methods and solvents employed (Dragović et al., 2020; Elez Garofulić et al., 2020).

Table 2. Total phenolic content, antioxidant activity values and chlorophyll contents of leaf extracts

	S	U-30	U-40	U-60
Total phenolic content (mg GAE/g)	52.42 ^d ± 0.06	59.19 ^c ± 0.13	65.19 ^a ± 0.13	63.01 ^b ± 0.00
DPPH (%)	77.84 ^b ± 0.46	53.06 ^c ± 0.00	85.15 ^a ± 0.31	42.03 ^d ± 1.39
ABTS (μmol Trolox/g)	548.71 ^d ± 11.11	596.57 ^b ± 2.02	605.14 ^a ± 1.01	570.14 ^c ± 1.01
Chlorophyll a (mg/l)	3.47 ^b ± 0.00	3.51 ^b ± 0.00	3.95 ^a ± 0.09	3.35 ^c ± 0.01
Chlorophyll b (mg/l)	2.15 ^a ± 0.02	2.01 ^b ± 0.02	2.10 ^{ba} ± 0.07	1.84 ^c ± 0.01
Total chlorophyll (mg/l)	5.62 ^b ± 0.02	5.52 ^c ± 0.02	6.05 ^a ± 0.02	5.18 ^d ± 0.02

^{a-d}: Significant differences indicated by different letters within the same line ($p < 0.05$).

3.3. Chlorophyll content

The chlorophyll a, b, and total chlorophyll contents of the mastic tree leaf extracts are shown in Table 2. A comparison of all samples revealed that the chlorophyll a content was higher than that of chlorophyll b. Similarly, Ayaz Tilkat et al. (2019) found that the chlorophyll a levels in *P. lentiscus* L. leaves were higher. As shown in Table 2, the U-40 extract exhibited the highest chlorophyll a and total chlorophyll contents. The S extract, on the other hand, had the highest chlorophyll b content, with the U-40 sample showing the second-highest level. Chlorophyll molecules occur in different forms in nature, with chlorophyll a and b being the most prevalent. Chlorophyll a is typically associated with plants that receive higher light exposure. However, it has also been noted that the concentrations of both chlorophyll a and b are influenced by environmental factors, including the plant's age, light intensity, temperature, and relative humidity (Cvitković et al., 2021). In a study examining the effects of traditional and ultrasonic extraction methods on the chlorophyll (chlorophyll a and b) content of aqueous extracts from lemon balm leaves, it was found that the samples obtained through ultrasonic extraction contained higher total chlorophyll compared to those from traditional extraction (Žlabur et al., 2016).

3.4. Antimicrobial activity values

The antimicrobial activities of extracts from mastic tree leaves, obtained through different extraction techniques, was assessed via the well diffusion

method using *S. aureus*, *E. coli*, and *L. monocytogenes* as test microorganisms. The inhibition zone diameters resulting from the antimicrobial activity of the samples against the tested microorganisms are summarized in Table 3. The findings revealed that *S. aureus* exhibited greater sensitivity, while no antimicrobial activity was observed against *E. coli* and *L. monocytogenes*. The results indicated that the U-40 extract produced the highest inhibition zone against *S. aureus*, measuring 20.47 mm, whereas the lowest inhibition zone, measuring 19.75 mm, was observed with the S extract. Variance analysis revealed a statistically significant difference ($p < 0.05$) in the antimicrobial activity of the leaf extracts. In a prior investigation, significant antibacterial effects of mastic tree leave against *S. aureus* were demonstrated using the disc diffusion method, while no such effects were observed against *E. coli*, similar to the present study. (Missoun et al., 2017). Another study comparing aqueous and methanolic extracts revealed that aqueous extracts showed no inhibitory effect against *E. coli*, and *S. aureus*, whereas methanolic extracts formed inhibition zones of 8 mm and 11 mm, respectively (Al-Zaben et al., 2023). In a previous study, it was reported that *P. lentiscus* L. leaf extract created the largest inhibition zone (20 mm) against *S. aureus* (Ouahabi et al., 2023). The antimicrobial activity of plants is often attributed to their phenolic compounds, with monoterpenes such as α -pinene and β -pinene, along with flavonoids, being identified as the likely active components (Djebari

et al., 2021). It has been reported that quercetin-3-O-glucoside is one of the key compounds responsible for antimicrobial activity (Selim et al., 2022).

Table 3. Inhibition zone diameters of leaf extracts (mm)

	Inhibition zone diameters (mm)			
	S	U-30	U-40	U-60
<i>Staphylococcus aureus</i>	19.75 ^b ± 0.23	19.81 ^b ± 0.09	20.47 ^a ± 0.44	19.79 ^b ± 0.17
<i>Escherichia coli</i>	NI	NI	NI	NI
<i>Listeria monocytogenes</i>	NI	NI	NI	NI

NI: No inhibition observed.
a, b: Significant differences indicated by different letters within the same line (p<0.05).

4. Conclusion

In this research, mastic tree leaves (*P. lentiscus* L.), known for their richness in bioactive compounds and ecological significance, were subjected to various extraction techniques, including solvent extraction and ultrasonic extraction for durations of 30, 40, and 60 minutes. The resulting extracts were analyzed and compared for color, total phenolic content, antioxidant activity, chlorophyll levels, and antimicrobial properties. The extract obtained using ultrasonic extraction for 40 minutes was found to have the highest total phenolic content, antioxidant activity, and total chlorophyll levels while also exhibiting the least color changes, highlighting its potential for improved quality. Antimicrobial activity was detected in all extracts against *S. aureus*, while no activity was noted against *E. coli* and *L. monocytogenes*. The largest inhibition zone against *S. aureus*, measuring 20.41 mm, was observed in the sample subjected to ultrasonic extraction for 40 minutes. The findings revealed that ultrasonic extraction yielded superior results compared to solvent methods; however, prolonged extraction times led to a decline in bioactive properties. This highlights the importance of conducting optimization studies in future research to establish the most effective extraction conditions, ensuring the preservation of bioactive compounds while maximizing extraction efficiency.

Note:
The total phenolic content and antioxidant properties presented in this paper were submitted as a full-text presentation at the TURK-COSE 2024: VI. International Turkic World Congress on Science and Engineering.

Conflict of Interest Statement

No potential conflict of interest was reported by the authors.

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