Some Bioactivities of *Limonium meyeri* Extracts and their Separated Compounds

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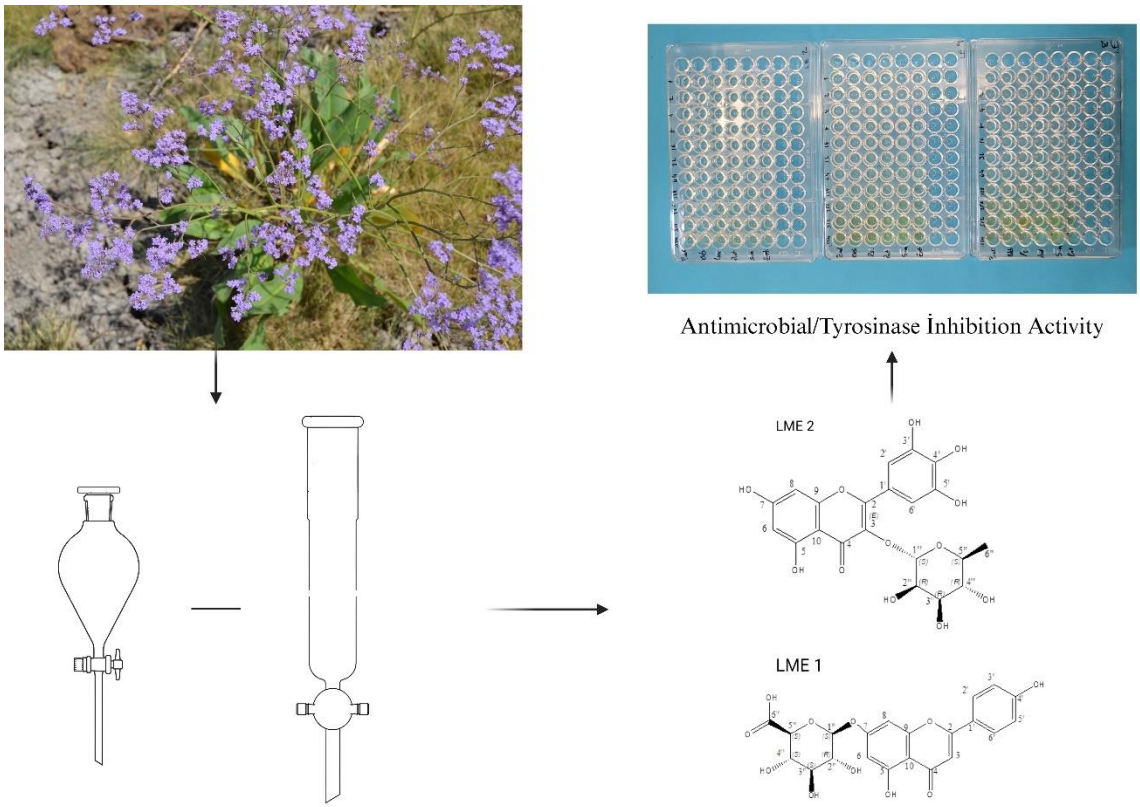
GRAPHICAL ABSTRACT



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The antimicrobial and tyrosinase enzyme inhibition activities of various extracts and isolated compounds of *Limonium meyeri* were investigated. Apigenin-7-O-β-glucuronide and myricetin 3-O-α-rhamnoside were isolated from the ethyl acetate fraction using chromatographic methods. Methanol extract showed 62.0% and ethyl acetate extract showed 57.6% tyrosinase enzyme inhibition activities at 400 µg/mL concentration. Myricetin 3-O-α-rhamnoside (IC_{50} = 27.7 ± 0.6 µg/mL) showed higher tyrosinase enzyme inhibitory effect than apigenin-7-O-β-glucuronide (IC_{50} = 43.0 ± 0.9 µg/mL). n-Hexane extract was found to be effective against *Enterococcus faecalis* (MIC = 1 µg/mL), while methanol extract was effective against *Pseudomonas aeruginosa* (MIC = 16 µg/mL). Myricetin 3-O-α-rhamnoside showed antimicrobial effect against *Enterococcus faecalis* (MIC = 128 µg/mL). Thus, *Limonium meyeri* and its compounds may provide valuable insights for future studies as antityrosinase and antibacterial agents.

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Keywords: Limonium meyeri; Apigenin-7-O-β-glucuronide; Myricetin 3-O-α-rhamnoside; Antimicrobial; Antityrosinase

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INTRODUCTION

Plants have been used for thousands of years to flavor and preserve food and to treat and prevent disease. Secondary metabolites produced by plants are recorded to have many biological effects, including the treatment of infectious diseases (Silva and Fernandes 2010; Qanash *et al.* 2024; Selim *et al*. 2024). It is important to develop technology for the isolation and extraction of bioactive compounds of medicinal value (Das *et al*. 2022). In this context, various traditional and non-traditional procedures such as maceration, infusion, digestion, percolation, decoction, ultrasound extraction, soxhlet extraction, turbo extraction, countercurrent extraction, ultrasound extraction, microwave-assisted extraction, supercritical fluid extraction, solid phase extraction, and column chromatography have been used. Ionic liquid extraction, high-performance gas phase chromatography, liquid chromatography, chiral phase chromatography are among the latest technologies (Kamil Hussain *et al.* 2019).

Plants secrete secondary metabolites such as alkaloids, flavonoids, terpenoids, and steroids. These secondary metabolites have various therapeutic activities. Flavonoids are molecules with activities such as antibacterial, antiviral, antioxidant, anti-inflammatory,

antimutagenic, and anticarcinogenic (Roy *et al.* 2022). In addition, they are used in the food, cosmetics and pharmaceutical industries (Dias *et al.* 2021).

Antimicrobial research is being carried out using various methods to discover synthetic and plant-derived substances that are less harmful to humans, broad-spectrum, and effective at low doses against microorganisms that cause various infections (Abbasoğlu 1996). Drug-resistant bacteria pose a serious risk to human health, and new antimicrobial agents are needed. Natural products are an invaluable source of antimicrobial compounds (Álvarez-Martínez *et al*. 2020; Alghonaim *et al.* 2023; Al-Rajhi *et al.* 2023).

Tyrosinase contains copper. It is a key enzyme in the biosynthesis of melanin, which is found in microorganisms, plants, and animals and is involved in determining the color of mammalian skin and hair. Excessive melanin production causes various pigmentation disorders, such as melasma, age spots, and areas of actinic damage. Additionally, the tyrosinase enzyme causes undesirable browning in plant-based foods, a decrease in nutritional quality, and economic loss of food products. All these reasons increase the need for new potential tyrosinase enzyme inhibitors (Parvez *et al*. 2007). There are natural, synthetic or semi-synthetic compounds used to reduce melanogenesis activity. For example, kojic acid produced from various types of mushrooms, arbutin obtained from the bearberry plant, and vanillin obtained from vanilla beans are used for this purpose. Highly effective and less side-effect tyrosinase inhibitors are needed in the pharmaceutical, food, and cosmetic industries (Hassan *et al.* 2023).

Limonium is a genus belonging to the Plumbaginaceae family. Studies have shown that *Limonium* species are rich in flavonoids, which can be used in the treatment of some diseases such as high fever, hemorrhage, spasm, ulcer, and bladder stones (Lin *et al*. 2000; Temel and Ünver 2012; Kandil *et al*. 2020). Different *Limonium* species have been reported to have antioxidant, antimicrobial, anti-inflammatory, anticancer, antiobesity, antidiabetic, and enzyme inhibition activities (Sami 2022). *Limonium meyeri* is a perennial (rarely annual) herb or subshrub. The leaves are usually in basal rosettes, sometimes in axillary bundles on the stem branches. The spikelets are dense (sometimes capitate) or grouped in spikes, terminating the branches of the inflorescence. The fruits are utriculate or pyxidiate, narrowly ellipsoid and compressed (Doğan *et al*. 2020). As far as the authors have observed, the phytochemical and biological activity studies on *L. meyeri* are insufficient in the literature. Therefore, the antimicrobial and tyrosinase enzyme inhibition effects of methanol, n-hexane, dichloromethane, ethyl acetate, water extracts of *L. meyeri* and isolated apigenin-7-O-β-glucuronide and myricetin 3-O-α-rhamnoside were investigated in this study,

EXPERIMENTAL

Plant Material

Flowering aerial parts (flowers, leaves, branches) of *L. meyeri* was collected from the Erzurum-Dumlu road route (39°58.898'N, 041°18.182'E) in July 2018. The plant was identified by Esen Sezen Karaoğlan. The sample was kept in the Atatürk University Biodiversity Application and Research Center Herbarium (AUEF 1396). Harvested plants were transported with sealed bags. They were dried at room temperature in a dry, airy and clean room away from direct sunlight. After the plants were completely dried, they were stored with sealed bags until the beginning of the study.

Extraction Method

After the plant material (300 g) was dried and powdered, 1000 mL of methanol (Isolab, Wertheim, Germany) was added and left to macerate 24 h. It was filtered with pleated filter paper. Methanol was added to the remaining residual part and extracted (4 times, 800 mL) at 40 °C under reflux in a mantle heater (Barnstead Electrothermal EM5000/C, UK). When the extracts became lukewarm and clear, they were filtered with pleated filter paper. The filtrates were combined and concentrated using a rotavapor (Heidolph, Heizbad Hei-VAP, Germany). The main methanol extract (40 g) was obtained and named LMM. The concentrated extract was dissolved with a 9:1 water:methanol solvent system. Then, it was fractionated using a separating funnel with n-hexane (Sigma Aldrich, St. Louis, MO, USA), dichloromethane (Isolab, Wertheim, Germany), and ethylacetate (Sigma Aldrich, St. Louis, MO, USA) solvents, respectively. Thus, 4.8 g n-hexane (LMH), 1.1 g dichloromethane (LMD), and 3.5 g ethylacetate (LME) subextracts were ontained. The 25.9 g remaining part was used as the aqueous extract and named LMS (Ozgen *et al.* 2024).

Isolation and Structure Determination

About 3 g of ethyl acetate extract was added to the silica gel (Merck, Darmstadt, Germany) column. A solvent system consisting of dichloromethane:methanol:water (90:10:1→50:50:5, v/v) was used for elution. The fractions (120 fractions) were collected into tubes. The fractions in the tube were applied to thin layer chromatography (TLC) plate. The plates were evaluated at UV_{254} / UV_{366} nm wavelength. The spots were evaluated. Then, 1% vanillin/sulfuric acid was sprayed and heated at 110° C. The color of the spots was evaluated. Tubes with similar images were combined (Sami 2022).

Fractions 42 to 68: these fractions were added to the sefhadex (Sigma Aldrich, St. Louis, MO, USA) column. Water:methanol (95:5, 9:1, 5:1, 1:1, v/v) solvent systems were used as eluent. The fractions in the tube were applied to the TLC plate and evaluated. The images suggested that tubes 11 and 12 were pure and clean. These tubes were combined and named LME1 (Sami 2022).

Fractions 19 to 23: these fractions were applied to the sefhadex column. Methanol was used as the mobile phase. The fractions in the tube were applied to the TLC plate and evaluated. Tubes 28 to 35 were combined (383 mg) and applied to the silica gel column. Dichloromethane:methanol:water (90:10:1→50:50:5, v/v) was used as the mobile phase. The fractions in the tube were applied to the TLC plate and evaluated. The images suggested that tubes 51 to 58 were pure and clean. These tubes were combined and named LME2 (Sami 2022).

The structures of the pure substances (LME1 and LME2) were elucidated by 1D-NMR (^{1}H-NMR, ^{13}C-NMR), 2D-NMR (HMQC and HMBC), and Q-TOF-MS measurements. The Q-TOF-MS spectra were recorded on an Agilent 6530 Accurate-Mass Q-TOF-LC/MS (Santa Clara, CA, USA) at Atatürk University Eastern Anatolia Advanced Technology Application and Research Center (DAYTAM), and NMR spectra were taken at Atatürk University Faculty of Science, Department of Chemistry. The NMR spectra were measured on Avance III 600 (Bruker, Karlsruhe, Germany) (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) spectrometers with tetramethylsilane (TMS) as an internal standard. LME1 was dissolved with DMSO, and LME2 was dissolved with CD_3OD.

Antimicrobial Activity Method

Standard bacterial strains of *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Proteus mirabilis* (ATCC 29906), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 29213), and *Enterococcus faecalis* (ATCC 29212) were used. Antimicrobial activities of LMM, LMH, LMD, LME, LMS extracts and LME1 and LME2 compounds were determined by the microdilution method in accordance with the Clinical and Laboratory Standards Institute recommendations (Clinical and Laboratory Standards Institute 2017). Stock solutions were prepared with 10% dimethyl sulfoxide (DMSO) and cationized Mueller-Hinton broth medium (CAMHB). Sulfomerazine was used as standard antibiotic. Test substances and sulfomerazine were prepared in different concentration range by double dilution with CAMHB. Bacterial suspensions were prepared as 0.5 Mc Farland 1×10^8 colony forming unit (CFU)/mL and then diluted 1/100 with CAMHB to ensure the concentration was 10^5 CFU/mL. 50 µL bacterial suspensions were pipetted into plates containing 50 µL diluated extract. Thus, final concentrations in the wells were obtained. The plates were incubated at 35 °C for 24 h. The minimun inhibitory concentration (MIC) values were evaluated based on turbidity in the wells (Ozgen *et al.* 2024).

Tyrosinase Enzyme Inhibition Method

The tyrosinase inhibitory effects of LMM, LMH, LMD, LME, LMS extracts and LME1 and LME2 compounds were determined following the reported method using 3,4-dihydroxyl-L-phenylalanine (L-DOPA) (Sigma Aldrich, St. Louis, MO, USA) as a substrate (Masuda *et al.* 2005). Kojic acid (Sigma Aldrich, St. Louis, MO, USA) was used as a positive control. A mixture consisting of 40 µL of test solution at different concentrations, 40 µL of tyrosinase solution, and 80 µL of phosphate buffer (pH = 6.8) were pipetted into a 96-well microplate (Group C). Group A contains phosphate buffer (120 µL) and enzyme (40 µL), group B contains only phosphate buffer (160 µL), and group D contains phosphate buffer (120 µL) and sample (40 µL). The groups were replicated three times for each test sample. It was incubated for 10 min at 23 °C. Then, 40 µL L-DOPA was added to all wells and incubated at 23 °C for 10 min, and the absorbance was measured at 490 nm with a microplate reader (Bio Tek EL X 800). Percentage of inhibition (%) was calculated with the formula $[[(A - B) - (C - D)] / (A - B)] \times 100$ (Karaoglan *et al.* 2023).

RESULTS AND DISCUSSION

Isolation and Structure Determination Results

The compounds LME1 and LME2 appeared yellow on the TLC plate, as a stain at UV_{254} nm, dark purple at UV_{366} nm, and bright yellow when heated with vanillin/H_2SO_4 reagent at 110 °C, indicating that they may be flavonoids. The ^{1}H-NMR, ^{13}C-NMR, HMQC, HMBC, and Q-TOFF results and literature comparison of results showed that LME1 was apigenin-7-O-β-glucuronide (Lin *et al*. 2001; Ma *et al*. 2017) and LME2 was myricetin 3-O-α-rhamnoside (Hwang and Chung 2018; Katlego *el al*. 2020). Phytochemical analysis results of LME1 and LME2 are given below.

Apigenin-7-O-β-glucuronide (LME1)

Q-TOFF-MS: (447 $[M+H]^+$, $C_{21}H_{18}O_{11}$; ^{1}H-NMR Spectrum (δ, ppm): 6.78 (s, H-3), 6.38 (d, *J* = 1.8 Hz, H-6), 6.76 (d, *J* = 2.2 Hz, H-8), 7.86 (d, *J* = 8.8 Hz, H-2', 6'), 6.87 (d, *J* = 8.8 Hz, H-3', 5'), 5.03 (d, *J* = 7.3 Hz, H-1"), 3.15 to 3.60 (H-2", 3", 4", 5"); ^{13}C-NMR Spectrum (δ, ppm): 164.9 (C-2), 103.4 (C-3), 182.6 (C-4), 161.6 (C-5), 100.3 (C-6), 163.7 (C-7), 103.4 (C-8), 157.6 (C-9), 105.9 (C-10), 121.2 (C-1'), 129.1 (C-2'), 116.7 (C-3'), 162.4 (C-4'), 116.7 (C-5'), 129.1 (C-6'), 100.1 (C-1"), 73.5 (C-2"), 74.3 (C-3"), 72.6 (C-4"), 77.1 (C-5"), and 172.9 (C-6"). The molecular structure of the compound (LME1) is shown in Fig. 1.

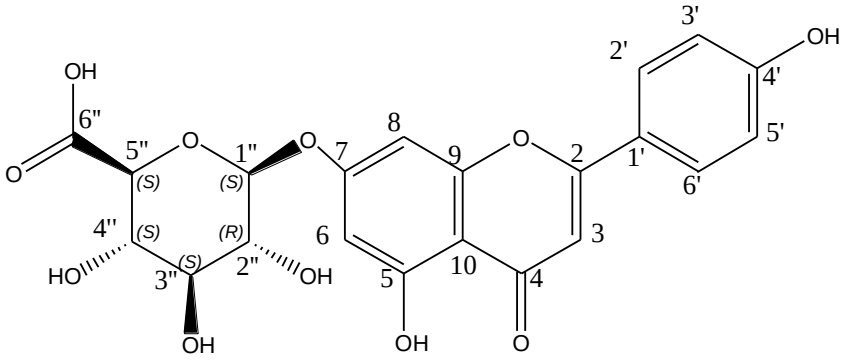

Fig. 1. Apigenin-7-O-*β*-glucuronide (LME1)

Myricetin 3-O-α-rhamnoside (LME2)

Q-TOFF-MS: (465 $[M+H]^+$, $C_{21}H_{20}O_{12}$; ^{1}H-NMR Spectrum (δ, ppm): 6.19 (d, *J* = 2.0 Hz, H-6), 6.36 (d, *J* = 2.0 Hz, H-8, 6.95 (s, H- 2', 6'), 5.30 (d, *J* = 1.6 Hz, H-1"), 4.21 (m, H-2"), 3.78 (dd, *J* = 9.4 Hz, *J* = 3.5 Hz, H-3"), 3.23 to 3.52 (H-4", 5"), 0.95 (d, *J* = 6.2 Hz, H-6"); and ^{13}C-NMR Spectrum (δ, ppm): 158.3 (C-2), 135.1 (C-3), 178.5 (C-4), 162.0 (C-5), 98.7 (6), 164.8 (7), 93.5 (8), 157.3 (9), 104.6 (10), 120.7 (1'), 108.4 (2'), 145.7 (3'), 136.7 (C-4'), 145.7 (C-5'), 108.4 (C-6'), 102.4 (C-1"), 70.9 (C-2"), 70.9 (C-3"), 72.1 (C-4"), 70.7 (C-5"), and 16.5 (C-6"). The molecular structure of the compound (LME2) is shown in Fig. 2.

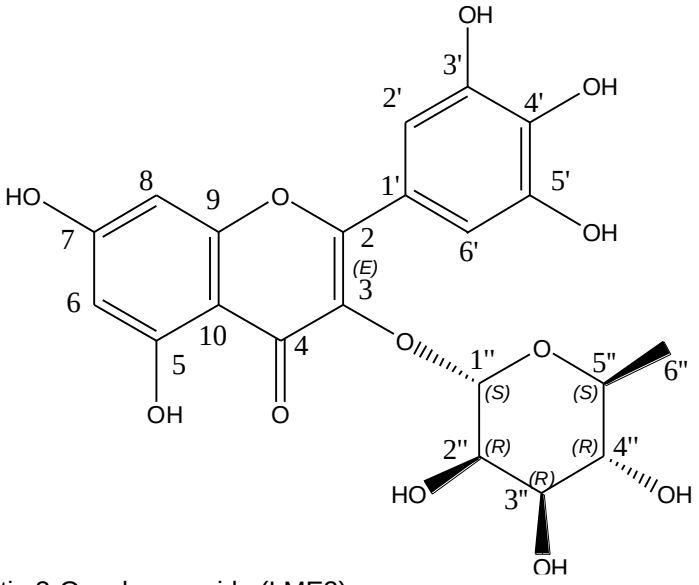

Fig. 2. Myricetin 3-O-*α*-rhamnoside (LME2)

As far as we have observed, the apigenin-7-O-β-glucuronide has not been commonly found in *Limonium* species in previous studies. It was recorded that myricetin 3-O-α-rhamnoside compound was isolated from *Limonium* species such as *L. bicolor*, *L. sinense*, *L. gmelinii*, *L. myrianthum*, *L. leptophyllum*, and *L. aureum* (Lin and Chou 2000; Ye and Huang 2006; Kozhamkulova *et al.* 2010; Chen *et al.* 2017; Gadetskaya *et al.* 2017). Apigenin-7-O-β-glucuronide is a compound with a flavone structure consisting of 21 carbon atoms. Myricetin 3-O-α-rhamnoside is a compound with a 21-carbon flavonol structure. Flavonoids are effective in the treatment of diseases due to their high antioxidant and free radical scavenging activities (Chen *et al.* 2023). The isolation studies related to *L. meyeri* are insufficient in the literature. In this context, we believe that isolating apigenin-7-O-β-glucuronide and myricetin 3-O-α-rhamnoside from *L. meyeri* in this study is valuable.

Antimicrobial Activity Results

The LME showed antimicrobial activity against *E. coli* (MIC= 256 µg/mL), *P. aeruginosa* (MIC= 256 µg/mL), *E. faecalis* (MIC= 512 µg/mL); LMH against *P. mirabilis* (MIC= 128 µg/mL) and *E. faecalis* (MIC= 1 µg/mL); LMD against *P. aeruginosa* (MIC= 128 µg/mL) and *S. aureus* (MIC= 64 µg/mL); and LMM against *P. aeruginosa* (MIC= 16 µg/mL), *S. aureus* (MIC= 512 µg/mL), and *E. faecalis* (MIC= 128 µg/mL). The LMS was not effective against any bacteria. Additionally, it was observed that the isolated LME1 was effective against *S. aureus* (MIC= 1024 µg/mL), and the isolated LME2 was effective against *E. coli* (MIC= 512 µg/mL) and *E. faecalis* (MIC= 128 µg/mL). No test material was found to be effective against *K. pneumoniae*. According to the antimicrobial activity results, LMH was the most effective against *E. faecalis* (MIC= 1 µg/mL). Then, LMM was found to be effective against *P. aeruginosa* (MIC= 16 µg/mL). The results are shown in Table 1.

Table 1. MIC Values of Test Substances (µg/mL)

	E. coli	*K. pneumonia*	*P. aeruginosa*	*P. mirabilis*	*S. aureus*	*E. faecalis*
LMS	-	-	-		-	-
LMH	-	-	-	128	-	1
LMD	-	-	128	-	64	-
LME	256	-	256	-	-	512
LMM	-	-	16	-	512	128
LME1	-	-	-	-	1024	-
LME2	512	-	-	-	-	128
Sulfomera zine (µg /mL)	1600	1600	1600	1600	6.25	3.12

MIC: Minimum inhibitory concentrations, **LMS:** Aqueous extract, **LMH:** n-Hexane extract, **LMD:** Dichloromethane extract, **LME:** Ethylacetate extract, **LMM:** Methanol extract, **LME1:** Apigenin-7-O-β-glucuronide, **LME2:** Myricetin 3-O-α-rhamnoside

Root extracts of *L. meyeri* have been found to show a higher antimicrobial effect than the aerial part, especially against *S. aureus* (Yurchyshyn *et al.* 2017). In the present study, it was observed that dichloromethane extract, methanol extract, and apigenin-7-O-β-glucuronide were effective against *S. aureus*. Antimicrobial activities of different

Limonium species are also recorded in the literature. Different fractions of the rhizome of the *L. brasiliense* species were tested. It has been shown to be effective against *Enterococcus faecium*, *S. aureus*, and *K. pneumoniae* (Blainski *et al*. 2017). Ethyl acetate extract of *L. pruinosum* showed activity against *E. coli* and *S. aureus* (Sultan *et al*. 2024). Methanol extracts of *L. effusum*, *L. lilacinum*, and *L. globuliferum* species were effective against many bacteria except *E. coli* (Avaz 2010). In the present study, ethyl acetate extract and myricetin 3-O-α-rhamnoside were effective against *E. coli*. In a conducted study, it was observed that the dichloromethane extract of *L. lopadusanum* was effective against *E. faecalis* (Gargiulo *et al.* 2024). In the present study, the ethyl acetate extract, methanol extract of *L. meyeri*, and myricetin 3-O-α-rhamnoside were found to be effective against *E. faecalis*. Moussaoui *et al*. (2010) reported that apigenin-7-O-β-glucuronide was moderately effective against *E. coli, S. aureous, P. mirabilis, Proteus vulgaris, Klebsiella oxytoca, K. pneumonia, Morganella morgani*, *Enterobacter* sp., *Streptococcus* sp., and *Seratia* sp. by the disk diffusion method. Additionally, they detected susceptibility only against *S. aureus* by the microdilution method. Aderogba *et al*. (2013) showed that myricetin 3-O-α-rhamnoside was effective against *E. coli, K. pneumonia*, and *S. aureus*. Myricetin 3-O-α-rhamnoside was found to be effective against *E. coli* and *E. faecalis* in this study.

Tyrosinase Enzyme Inhibition Results

In the research conducted with extracts, LMM, LME, LMS, LMD, LMH extracts showed tyrosinase inhibitory effects at 400 µg/mL concentration of 62.0%, 57.6%, 46.5%, 30.6%, 7.5%, respectively (Kojic acid, 94.75%). Then, the tyrosinase inhibitory effects of the compounds isolated from the ethyl acetate extract were investigated. LME1 and LME2 showed tyrosinase inhibitory activity (IC_{50} = 43.0 ± 0.9 and 27.7 ± 0.6 µg/mL, respectively). These results are shown in Table 2.

Table 2. Tyrosinase Enzyme İnhibitory Effects of LME1 and LME2

Compound	IC_{50} (µg/mL)
LME1	43.0 ± 0.9
LME2	27.7 ± 0.6
Kojik asit	12.4 ± 0.4

IC_{50}: Half-maximal inhibitory concentration (mean ± SD of three experiments), **LME1:** Apigenin-7-O-β-glucuronide, **LME2:** Myricetin 3-O-α-rhamnoside

Tyrosinase enzyme inhibitory activities of various fractions of *L. tetragonum* were previously investigated, and 85% aqueous methanol and n-butanol extracts were found to be effective. In addition, isolated myricetin 3-galactoside (65%) and quercetin 3-O-β-galactopyrroloside (63%) were effective (Lee *et al.* 2017). The finding that methanol extract of *L. delicatulum* leaves and roots was effective supports the current study (Bakhouche *et al.* 2021). In related work, the ethanol extract of *L. spathulatum* leaves showed tyrosinase inhibitory (IC_{50} = 0.34 mg/mL) effect (Youssef *et al.* 2023). Additionally, ethyl acetate fractions of *L. effusum* and *L. sinuatum* and n-hexane fraction of *L. effusum* showed antityrosinase activity (IC_{50}= 245.56±3.6, 295.18±10.57 and 148.27±3.33 g/mL, respectively) (Baysal *et al.* 2021). In the present study, the main methanol extract (62.0%), and ethyl acetate subfraction (57.6%) of *L. meyeri* showed the highest percentage tyrosinase inhibitory effect. In addition, myricetin 3-O-α-rhamnoside

(27.7 ± 0.6 µg/mL) showed a higher tyrosinase inhibitory effect than apigenin-7-O-β-glucuronide (43.0 ± 0.9µg/mL). It is known that phenolic compounds and flavonoids are effective in inhibiting tyrosinase and melanin synthesis (Zolghadri *et al*. 2019). The high effects of methanol and ethyl acetate extracts may be due to their rich content of phenolic compounds and flavonoids.

CONCLUSIONS

1. Myricetin 3-O-α-rhamnoside and apigenin-7-O-β-glucuronide were isolated from *L. meyeri*. Various extracts and isolated compounds of *L. meyeri* exhibited antibacterial effects against different bacteria. Particularly, the n-hexane extract showed significant activity against *E. faecalis*.
2. Among the extracts, methanol and ethyl acetate extracts had a higher tyrosinase inhibitory effect, while both of the isolated compounds exhibited anti-tyrosinase activity.
3. It was observed that the some extracts and isolated compounds had tyrosinase inhibitory and antimicrobial effects. These findings may guide further research.

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REFERENCES CITED

Abbasoğlu, U. (1996). "The antimicrobial activity research methods," *FABAD Journal Pharmaceutical Science* 22, 111-118.

Aderogba, M. A., Ndhlala, A. R., Rengasamy, K. R. R., and Staden, J. V. (2013). "Antimicrobial and selected *in vitro* enzyme inhibitory effects of leaf extracts, flavonols and indole alkaloids isolated from *Croton menyharthii*," *Molecules* 18(10), 12633-12644. DOI: 10.3390/molecules181012633

Alghonaim, M. I., Alsalamah, S. A., Alsolami, A., and Abdel Ghany, T. M. (2023). "Characterization and efficiency of *Ganoderma lucidum* biomass as an antimicrobial and anticancer agent," *BioResources* 18(4), 8037-8061. DOI: 10.15376/biores.18.4.8037-8061

Al-Rajhi, A. M. H., Bakri, M. M., Qanash, H., Alzahrani, H. Y., Halawani, H., Algaydi, M. A., and Abdelghany, T. M. (2023). "Antimicrobial, antidiabetic, antioxidant, and anticoagulant activities of *Cupressus sempervirens in vitro* and *in silico*," *Molecules* 28(21), article 7402. DOI: 10.3390/molecules28217402

Álvarez-Martínez, F. J., Barrajón-Catalán, E., and Micol, V. (2020). "Tackling antibiotic resistance with compounds of natural origin: A comprehensive review," *Biomedicines* 8(10), article 405. DOI: 10.3390/biomedicines8100405

Avaz, S. (2010). *Antimicrobial Activities of Limonium Mill. Species Growing in Afyonkarahisar*, Master Thesis, Afyon Kocatepe University, Institute of Science, Afyon, Turkey.

Bakhouche, I., Aliat, T., Boubellouta, T., Gali, L., Şen, A., and Bellik, Y. (2021). "Phenolic contents and *in vitro* antioxidant, anti-tyrosinase, and anti-inflammatory effects of leaves and roots extracts of the halophyte *Limonium delicatulum*," *South African Journal of Botany* 139(3), 42-49. DOI: 10.1016/j.sajb.2021.01.030

Baysal, I., Ekizoglu, M., Ertas, A., Temiz, B., Agalar, H.G., Yabanoglu-Ciftci, S., Temel, H., Ucar, G., and Turkmenoglu, F.P. (2021). "Identification of phenolic compounds by LC-MS/MS and evaluation of bioactive properties of two edible halophytes: *Limonium effusum* and *L. sinuatum*," *Molecules* 26, article 4040. DOI: 10.3390/molecules26134040

Blainski, A., Gionco, B., Oliveira, A. G., Andrade, G., Scarminio, I. S., Silva, D. B., Lopes, N. P., and Mello, J. C. P. (2017). "Antibacterial activity of *Limonium brasiliense* (Baicuru) against multidrug-resistant bacteria using a statistical mixture design," *Journal of Ethnopharmacology* 198(12), 313-323. DOI: 10.1016/j.jep.2017.01.013

Chen, J., Teng, J., Ma, L., Tong, H., Ren, B., Wang, L., and Li, W. (2017). "Flavonoids isolated from the flowers of *Limonium bicolor* and their *in vitro* antitumor evaluation," *Pharmacognosy Magazine* 13(50), 222-225. DOI: 10.4103/0973-1296.204566

Chen, S., Wang, X., Cheng, Y., Gao, H., and Chen, X. (2023). "A review of classification, biosynthesis, biological activities and potential applications of flavonoids," *Molecules* 28, article 4982. DOI: 10.3390/molecules28134982

CLSI M100-S27 (2017). "Performance standards for antimicrobial susceptibility testing. Twenty-seventh informational supplement," CLSI, Wayne, PA, USA.

Das, G., Patra, J. K., Kang, S. S., and Shin, H. S. (2022). "Pharmaceutical importance of some promising plant species with special reference to the ısolation and extraction of bioactive compounds: A review," *Current Pharmaceutical Biotechnology* 23(1), 15-29. DOI: 10.2174/1389201022666210122125854

Dias, M. C., Pinto, D. C. G. A., and Silva, A. M. S. (2021). "Plant flavonoids: Chemical characteristics and biological activity," *Molecules* 26, article 5377. DOI: 10.3390/molecules26175377

Doğan, M., Akaydın, G., and Erdal, J. (2020). "Phytogeography, ecology and conservation of the genus *Limonium* (Plumbaginaceae) in Turkey and a taxonomic revision," *Plant Systematics and Evolution* 306, article 89. DOI: 10.1007/s00606-020-01706-3

Gadetskaya, A. V., Mohamed, S. M., Tarawneh, A. H., Mohamed, N. M., Ma, G., Ponomarev, B. N., Zhusupova, G. E., Cantrell, C. L., Cutler, S. J., and Ross, S. A. (2017). "Phytochemical characterization and biological activity of secondary metabolites from three *Limonium* species," *Medicinal Chemistry Research* 26(11), 2743-2750. DOI: 10.1007/s00044-017-1973-z

Gargiulo, E., Roscetto, E., Galdiero, U., Surico, G., Catania, M. R., Evidente, A., and Taglialatela-Scafati, O. (2024). "Antibacterial metabolites produced by *Limonium lopadusanum*, an endemic plant of Lampedusa Island," *Biomolecules* 14, article 134. DOI: 10.3390/biom14010134

Hassan, M., Shahzadi, S., and Kloczkowski, A. (2023). "Tyrosinase ınhibitors naturally present in plants and synthetic modifications of these natural products as anti-

melanogenic agents: A review," *Molecules* 28, article 378. DOI: 10.3390/molecules28010378

Hwang, I. W., and Chung, S. K. (2018). "Isolation and identification of myricitrin, an antioxidant flavonoid, from daebong persimmon peel," *Preventive Nutrition and Food Science* 23(4), 341-346. DOI: 10.3746/pnf.2018.23.4.341

Kamil Hussain, M., Saquib, M., and Faheem Khan, M. (2019). "Techniques for extraction, ısolation, and standardization of bio-active compounds from medicinal plants," in: *Natural Bio-active Compounds*, M. Swamy and M. Akhtar (eds.), Springer, Singapore. DOI: 10.1007/978-981-13-7205-6_8

Kandil, F. E., Ahmed, K. M., Hussieny, H. A., and Soliman, A. M. (2000). "A new flavonoid from *Limonium axillare*," *Archiv der Pharmazie - Chemistry in Life Sciences* 333(8), 275-277. DOI: 10.1002/1521-4184(20008)333:8

Karaoglan, E. S., Hancı, H., Koca, M., and Kazaz, C. (2023). "Some bioactivities of isolated apigenin-7-O-glucoside and luteolin-7-O-glucoside," *Applied Science* 13, article 1503. DOI: 10.3390/app13031503

Katlego, E. M., Abdalla, M. A., Leonard, C. M., Eloff, J. N., and McGaw, L. J. (2020). "Inhibitory effect of *Newtonia* extracts and myricetin-3-O-rhamnoside (myricitrin) on bacterial biofilm formation," *BMC Complementary Medicine and Therapies* 20(1), article 358. DOI: 10.1186/s12906-020-03139-4

Kozhamkulova, Z. A., Radwan, M. M., Zhusupova, G. E., Abilov, Z. Z., Rahadilova, S. N., and Ross, S. A. (2010). "Gmelinoside I, a new flavonol glycoside from *Limonium gmelinii*," *Natural Product Communications* 5(7), 1061-1062. DOI: 10.1177/1934578X1000500715

Lee, S. G., Karadeniz, F., Seo, Y., and Kong, C. S. (2017). "Anti-melanogenic effects of flavonoid glycosides from *Limonium tetragonum* (Thunb.) Bullock *via* inhibition of tyrosinase and tyrosinase-related proteins," *Molecules* 22(9), article 1480. DOI: 10.3390/molecules22091480

Lin, L. C., and Chou, C. J. (2000). "Flavonoids and phenolics from *Limonium sinense*," *Planta Medica* 66(4), 382-383. DOI: 10.1055/s-2000-8547

Lin, L. C., Kuo, Y. C., and Chou, C. J. (2000). "Anti-herpes simplex virus type-1 flavonoids and a new flavanone from the root of *Limonium sinense*," *Planta Medica* 66(4), 333-336. DOI: 10.1055/s-2000-8540

Lin, J. H., Lin, Y. T., Huang, Y. J., Wen, K. C., Chen, R. M., Ueng, T. H., and Liao, C. H. (2001). "Isolation and cytotoxicity of flavonoids from *Daphnis genkwae* Flos," *Journal of Food and Drug Analysis* 9, 6-11. DOI: 10.38212/2224-6614.2810

Ma, Q., Zhang, X. M., Jiang, J. G., and Zhu, W. (2017). "Apigenin-7-O-β-D-glucuronide inhibits modified low-density lipoprotein uptake and foam cell formation in macrophages," *Journal of Functional Foods* 35, 615-621. DOI: 10.1016/j.jff.2017.06.026

Masuda, T., Yamashita, D., Takeda, Y., and Yonemori, S. (2005). "Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from *Garcinia subelliptica*," *Bioscience, Biotechnology, and Biochemistry* 69(1), 197-201. DOI: 10.1271/bbb.69.197

Moussaoui, F., Zellagui, A., Segueni, N., Touil, A., and Rhouati, S. (2010). "Flavonoid constituents from algerian *Launaea resedifolia* (O.K.) and their antimicrobial activity," *Records of Natural Products* 4(1), 91-95.

Özgen, R., Karaoğlan, E.S., Sevindik, H.G., Hancı, H., and Kazaz, C. (2024). "Antimicrobial, antioxidant, and antityrosinase activities of *Morina persica* L. and its isolated compounds," *Molecules* 29, article 3017. DOI: 10.3390/molecules29133017

Parvez, S., Kang, M., Hwan-Suck, C., and Hyunsu, B. (2007). "Naturally occurring tyrosinase inhibitors: Mechanism and applications in skin health, cosmetics and agriculture industries," *Phytotherapy Research* 21(9), 805-816. DOI: 10.1002/ptr.2184

Qanash, H., El Fadl, S. R. A., Binsaleh, N. K., Aljahdali, L. A., Altayar, M. A., Khalel, A. F., Alsalamah, S. A., Alghonaim, M. I., and Abdelghany, T. M. (2024). "Ecofriendly extraction approach of *Moringa peregrina* biomass and their biological activities *in vitro*," *Biomass Conversion and Biorefnery*, DOI: 10.1007/s13399-024-05916-4

Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B. S., Babalghith, A. O., Alshahrani, M. Y., Islam, S., and Islam, R. (2022). "Flavonoids a bioactive compound from medicinal plants and ıts therapeutic applications," *BioMed Research International*, article ID 5445291. DOI: 10.1155/2022/5445291

Sami, M. (2022). *Pharmacognostic Studies on Limonium meyeri (Boiss) Kuntze*, Master's Thesis, Ataturk University Institute of Health Sciences, Erzurum, Turkey.

Selim, S., Alruwaili, Y. S., Ejaz, H., Abdalla, A. E., Almuhayawi, M. S., Nagshabandi, M. K., Tarabulsi, M. K., Al Jaouni, S. K., Bazuhair, M. A., and Abdelghany, T. M. (2024). "Estimation and action mechanisms of cinnamon bark *via* oxidative enzymes and ultrastructures as antimicrobial, anti-biofilm, antioxidant, anti-diabetic, and anticancer agents," *BioResources* 19(4), 7019-7041. DOI: 10.15376/biores.19.4.7019-7041

Silva, N. C. C., and Fernandes, J. A. (2010). "Biological properties of medicinal plants: A review of their antimicrobial activity," *Journal of Venomous Animals and Toxins* 16(3), 402-413. DOI: 10.1590/S1678-91992010000300006

Sultan, M. H., Bedair, R., Ragab, O. G, Abd-ELShafy, E., Mahfouz, A.Y., and Daigham, G.E. (2024). "Biological activities and ecological aspects of *Limonium pruinosum* (L.) collected from Wadi Hof Eastern Desert, Egypt, as a promising attempt for potential medical applications," *Biomass Conversion and Biorefinery* 14, 23887-23907. DOI: 10.1007/s13399-023-04385-5

Temel, M., and Ünver, M. C. (2012). "The germination features of endemic *Limonium lilacinum* (Boiss. et Bal.) Wagenitz (Plumbaginaceae)," *AKU J. Sci.* 011006, 49-52.

Ye, G., and Huang, C. (2006). "Flavonoids of *Limonium aureum*," *Chemistry of Natural Compounds* 42(2), 232-234. DOI: 10.1007/s10600-006-0089-3

Youssef, S., Custódio, L., Rodrigues, M. J., Pereira, C. G., Calhelha, R. C., Jeko, J., Zoltán, C., and Hamed, K. B. (2023). "Harnessing the bioactive potential of *Limonium spathulatum* (Desf.) Kuntze: Insights into enzyme ınhibition and phytochemical profile," *Plants* 12, article 3391. DOI: 10.3390/plants12193391

Yurchyshyn, O. I., Rusko, G. V., and Kutsyk, R. V. (2017). "Antimicrobial effect of south Ukrainian sea lavender *Limonium meyeri* (Boiss.) O. Kuntze and *Limonium hypanicum* Klok. with especial emphasizing against *Staphylococci* and Propionibacteria," *The Pharma Innovation Journal* 6(10), 380-384.

Zolghadri, S., Bahrami, A., Hassan Khan, M. T., Munoz-Munoz, J., Garcia-Molina, F., Garcia-Canovas, F., and Saboury, A. A. (2019). "A comprehensive review on tyrosinase inhibitors," *Journal of Enzyme Inhibition and Medicinal Chemistry* 34(1), 279-309. DOI: 10.1080/14756366.2018.1545767

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