Journal of Anatolian Environmental and Animal Sciences
(*Anadolu Çevre ve Hayvancılık Bilimleri Dergisi*)
DOI: https://doi.org/10.35229/jaes.1669409

JAES
Year: 10, No: 5, 2025 (533-539)
AÇEH
Yıl: 10, Sayı: 5, 2025 (533-539)

ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Qualitative Phytochemical Screening, Total Phenolics Content, *In Vitro* Antioxidant Activity and Antibacterial Activities of *Arbutus unedo* L. Leaves

Ahmet BEYATLI[1,2]*

[1] *University of Health Sciences, Hamidiye Vocational School of Health Services, Department of Medicinal and Aromatic Plants, 34668, Istanbul, Türkiye*
[2] *School of Agriculture, Food and Ecosystem Sciences, Faculty of Science, Food Science and Nutrition, The University of Melbourne, Australia*

Received: *03.04.2025* ***Accepted:*** *05.08.2025* ***Published:*** *30.09.2025*

How to cite: **Beyatli, A. (2025).** Qualitative Phytochemical Screening, Total Phenolics Content, *In Vitro* Antioxidant Activity and Antibacterial Activities of *Arbutus unedo* L. Leaves. *J. Anatolian Env. and Anim. Sciences*, ***10***(5), 533-539. https://doi.org/10.35229/jaes.1669409

Atıf yapmak için: **Beyatli, A. (2025).** *Arbutus unedo* L. Yapraklarının Kalitatif Fitokimyasal Taraması, Toplam Fenolikler İçeriği, *İn Vitro* Antioksidan Aktivitesi ve Antibakteriyel Etkileri. *Anadolu Çev. ve Hay. Dergisi*, ***10***(5), 533-539. https://doi.org/10.35229/jaes.1669409

*: https://orcid.org/0000-0001-5225-6217

***Corresponding author's:**
Ahmet BEYATLI
University of Health Sciences, Hamidiye Vocational School of Health Services, Department of Medicinal and Aromatic Plants, 34668, Istanbul, Türkiye
✉: ahmet.beyatli@sbu.edu.tr

Abstract: Traditionally, the leaves of *Arbutus unedo* were used in Mediterranean folk medicine as an antiseptic for wound healing and skin infections, as well as for treating various other ailments**.** In this study phytochemical composition, phenolic content, and bioactivities were investigated. The phytochemical screening revealed the presence of multiple bioactive compounds, including flavonoids, tannins, terpenoids, steroids, and glycosides, distributed across different fractions. Quantitative analysis demonstrated that the ethanolic extract contained substantially greater amounts of both total phenolics and flavonoids compared to other extracts (738.43 ± 5.11 mg GAE/g and 98.14 ± 2.13 mg CE/g, respectively). *In vitro* antioxidant activity, assessed through DPPH, ABTS, and FRAP assays, the ethyl acetate fraction demonstrated the strongest antioxidant effects in every assay. Additionally, the ethyl acetate fraction exhibited the strongest antibacterial activity, showing the largest inhibition zones against *S. aureus* (15.25 ± 1.10 mm) and *P. aeruginosa* (17.75 ± 1.33 mm), along with the lowest MIC values (78–156 µg/mL). The ethanol extract exhibited moderate activity, while the hexane, chloroform, and aqueous fractions were less effective, with MICs >625 µg/mL for most strains. The results demonstrate that *A. unedo* leaf extracts exhibit significant antioxidant and antimicrobial properties, validating their traditional medicinal applications and suggesting their potential as a natural alternative for therapeutic or preservative purposes.

Keywords: Antibacterial activity, Antioxidant activity, *Arbutus unedo*, Phenolics, Phytochemical screening.

Arbutus unedo L. Yapraklarının Kalitatif Fitokimyasal Taraması, Toplam Fenolikler İçeriği, *İn Vitro* Antioksidan Aktivitesi ve Antibakteriyel Etkileri

***Sorumlu yazar:**
Ahmet BEYATLI
Sağlık Bilimleri Üniversitesi, Hamidiye Sağlık Hizmetleri Meslek Yüksekokulu, Tıbbi ve Aromatik Bitkiler Bölümü, 34668, İstanbul, Türkiye
✉: ahmet.beyatli@sbu.edu.tr

Öz: Geleneksel olarak *Arbutus unedo*'nun yaprakları Akdeniz halk hekimliğinde yara iyileşmesi ve cilt enfeksiyonları için antiseptik olarak ve ayrıca çeşitli diğer rahatsızlıkların tedavisinde kullanılmıştır. Bu çalışmada fitokimyasal bileşim, fenolik içerik ve biyoaktiviteler araştırılmıştır. Çeşitli fraksiyonların fitokimyasal analizinde, flavonoidler, tanenler, terpenoidler, steroidler ve glikozitler belirlenmiştir. Toplam fenolik içerik ve toplam flavonoid içeriği etanol ekstresinde önemli ölçüde daha yüksek (sırasıyla 738,43 ± 5,11 mg GAE/g ve 98,14 ± 2,13 mg CE/g) bulunmuştur. DPPH, ABTS ve FRAP analizleri ile değerlendirilen *in vitro* antioksidan aktivitede, etil asetat fraksiyonu tüm analizlerde en güçlü antioksidan etkiyi göstermiştir. Ayrıca, etil asetat fraksiyonu en güçlü antibakteriyel aktiviteyi göstermiştir ve *S. aureus*'a (15,25 ± 1,10 mm) ve *P. aeruginosa*'ya (17,75 ± 1,33 mm) karşı en büyük inhibisyon bölgelerini ve en düşük MİK değerlerini (78-156 µg/mL) belirtmiştir. Etanol ekstresi orta düzeyde aktivite gösterdi, hekzan, kloroform ve sulu fraksiyonlar ise daha az etki göstermiştir ve çoğu suşa karşı MİK'ler > 625 µg/mL olarak belirlenmiştir. Bulgular, *A. unedo* yapraklarının geleneksel ve gelecekteki doğal bir antimikrobiyal ve antioksidan ajan olarak potansiyelini vurgulamaktadır.

Anahtar Kelimeler: Antibakteriyel aktivite, Antioksidan aktivite, *Arbutus unedo*, Fenolikler, Fitokimyasal tarama.

INTRODUCTION

Contemporary scientific and public interest has increasingly focused on phytotherapeutic applications for disease management, health promotion, and preventive medicine. Botanical sources offer an economically viable alternative for novel drug development, characterized by reduced adverse effects compared to synthetic compounds. The historical use of vegetal materia medica dates to ancient healing traditions, where plant-derived substances formed

the foundation of therapeutic formulations. Modern pharmacognosy has demonstrated remarkable progress in isolating bioactive phytochemicals, substantially influencing contemporary therapeutic approaches (Chanda & Dave, 2009). Plant-derived extracts and phytochemicals demonstrate significant antioxidant properties, which hold considerable therapeutic importance. This is particularly relevant given that elevated oxidative stress has been identified as a key pathological mediator in the progression of numerous serious human diseases (Yoo et al., 2018; Beyatli et al., 2022).

Medicinal plants have re-emerged as critical resources for combating antimicrobial resistance and chronic diseases, particularly in Mediterranean ecosystems where biodiversity harbors untapped pharmacological potential (Miguel et al., 2014; Tastekin, & Çiftci, 2023; Morales, 2022). *Arbutus unedo* L. (Ericaceae), an evergreen shrub endemic to the Mediterranean Basin, exemplifies this dual role as both an ecological keystone species and a repository of bioactive compounds (Wahid et al., 2019; El Haouari et al., 2021). In Türkiye, *A. unedo* populations thrive in coastal and subcoastal forests, where its leaves and fruits have been traditionally utilized as antiseptics, diuretics, and anti-inflammatory agents (Koyu et al., 2019). Despite that *A. unedo* classified as "Least Concern" on the IUCN Red List, the species faces habitat fragmentation due to climate change and anthropogenic pressures, underscoring the urgency to document its phytochemical properties for sustainable utilization (IUCN, 2017).

The ecological and ethnopharmacological significance of *A. unedo* is well-documented in Mediterranean traditional medicine. Turkish populations have historically used leaf decoctions to manage hypertension, diabetes, and urinary infections, owing to their rich tannin, flavonoid, and polyphenol content (Baytop, 1999; Morales, 2022). Extensive studies in regions such as Morocco and Portugal confirm the plant's antioxidant and antimicrobial properties, primarily attributed to compounds like quercetin, gallic acid, and vitamins (El Haouari et al., 2021; Zitouni et al., 2021). However, despite its widespread distribution, phytochemical variability driven by climatic and edaphic factors remains insufficiently explored, particularly in Türkiye (Morales, 2022). Notably, northern microclimates like Yalova have not been thoroughly investigated for their influence on bioactive compound synthesis in *A. unedo*.

This study provides the first detailed fraction-based phytochemical analysis of *A. unedo* leaves from Yalova, Türkiye, addressing a critical gap in regional comparative research. By combining qualitative screening, total phenolics quantification, and in vitro bioactivity assays, we offer novel insights into how Yalova's unique biogeography may enhance the plant's pharmacological potential. Our findings not only bridge traditional ethnomedicinal knowledge with modern analytical validation but also contribute to the sustainable utilization of Mediterranean plant resources in evidence-based applications.

MATERIAL AND METHOD

Plant Material and Extraction: *A. unedo* leaves were harvested in June 2022 from the Armutlu District of Yalova, Türkiye (geographical coordinates: 40°31'35.7"N, 28°47'23.3"E). Taxonomic identification was confirmed by the Herbarium of Istanbul University's Faculty of Pharmacy, where a voucher specimen (ISTE-118582) was deposited (Figure 1).

Figure 1. Habit of *A. unedo* in its natural habitat (Armutlu, Yalova, Türkiye).

The air-dried leaf material was ground to a fine consistency using a mechanical grinder. Exactly 250 grams of this powder was then soaked in 80% ethanol (w/v) at room temperature (25±2°C) for three days with periodic agitation. The mixture was filtered through Whatman No. 1 filter paper, and the ethanolic solvent was removed using rotary evaporation at 40°C under vacuum. The obtained crude extract was redissolved in deionized water and systematically fractionated through solvent-solvent partitioning, employing hexane (non-polar), chloroform (intermediate polarity), and ethyl acetate (polar) in succession. This process generated four organic fractions (hexane-soluble, chloroform-soluble, ethyl acetate-soluble) along with a remaining water-soluble fraction (Hossain et al., 2014).

Phytochemical Screening: Preliminary qualitative screening of the ethanolic extract and solvent fractions was performed using standardized protocols (Tyler, 1993; Harborne, 1998). Tests targeted alkaloids (Mayer's reagent), flavonoids (Shinoda test), saponins (foam formation), terpenoids (Liebermann-Burchard reaction), steroids (Salkowski test), tannins (ferric chloride), and glycosides (Bornträger's test). Results were recorded as present (+) or absent (−) (El Haouari et al., 2021).

Determination of Total Phenolic Content (TPC): Quantified using the Folin-Ciocalteu (FC) assay, a redox-based method that measures electron transfer from phenolic antioxidants to phosphomolybdic/phosphotungstic acid complexes under alkaline conditions. Briefly, 2.5 µL of the extract was added to 195 µL of distilled water, followed by sequential addition of 2.5 µL of FC reagent and 50 µL of 7% sodium carbonate (Na_2CO_3) to adjust the pH to ~10. The reaction mixture was incubated in darkness for 30 minutes, and the resulting blue chromophore was measured spectrophotometrically at 765 nm. Results were standardized as gallic acid equivalents per gram of extract (mg GAE/g) using a calibration curve (Zhou et al., 2004; Morales, 2022).

Determination of Total Flavonoids Content (TFC): TFC was determined via aluminum chloride colorimetry as described by Dewanto et al. (2002) with modifications. Briefly, 250 µL of extract was reacted with 75 µL of 5% $NaNO_2$ (w/v) for 5 min, followed by addition of 150 µL of 10% $AlCl_3$ (w/v). The mixture was vortexed (30 sec), then alkalinized with 500 µL of 1 M NaOH. After 30 min dark incubation (25±1°C), absorbance was recorded at 510 nm. TFC was expressed as catechin equivalents (mg CE/g extract) based on a standard curve prepared with serial dilutions of (+)-catechin.

In Vitro Antioxidant Assays: The hydrogen atom donation capacity was evaluated using the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (1 mM in methanol). Test samples (100 µL) were reacted with 2.9 mL DPPH solution (final conc. 0.1 mM) under light-protected conditions (30 min, 25°C). Absorbance decay at 515 nm was quantified against a Trolox standard curve, with results expressed as Trolox equivalents per gram extract (mg TE/g) (Rumpf et al., 2023). The ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation was generated by oxidizing 7 mM ABTS with 2.45 mM $K_2S_2O_8$. The solution was reacted with samples (1:20 v/v) for 6 min. Electron/hydrogen transfer capacity was measured spectrophotometrically at 734 nm and calibrated against Trolox. Reducing power was assessed using the Fe^{3+}-TPTZ complex (300 mM acetate buffer pH 3.6, 10 mM TPTZ, 20 mM $FeCl_3 \cdot 6H_2O$). Samples were incubated with FRAP reagent (3 mL, 37°C, 10 min), and Fe^{2+} reduction was monitored at 593 nm. Data were normalized to a Trolox standard following Benzie & Strain (1996).

Antibacterial Activity: This research employed four standardized pathogenic strains, comprising two Gram-positive cocci (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 51299) and two Gram-negative bacilli (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853). Antimicrobial sensitivity was evaluated following the Clinical and Laboratory Standards Institute (CLSI, 2008) protocols. The agar well diffusion assay, performed on Mueller-Hinton agar, measured bacterial growth inhibition. Prior to testing, the bacterial strains were cultured overnight in nutrient broth at 37°C, adjusted to a 0.5 McFarland standard (1×10^8 CFU/mL). Wells (6 mm diameter) were punched into the agar using sterile cork borers, and 50 µL of each test extract was dispensed into them. After 24-hour incubation at 37°C, the zones of inhibition were measured (in triplicate), with data reported as mean ± SD. Controls included 5% DMSO (negative) and Gentamicin (1 mg/mL; positive).

The broth dilution method, performed according to CLSI standards (CLSI, 2008), was used to determine the minimum inhibitory concentration (MIC). Initially, bacterial strains were grown on tryptic soy agar plates under aerobic conditions at 35°C for 24–48 hours. The resulting cultures were diluted in 0.85% sterile saline to achieve a turbidity of 0.5 McFarland (10^8 CFU/mL). For the assay, U-bottom 96-well microplates were prepared with: Negative controls (medium + extract only), positive controls (medium + bacteria only) and test wells containing extracts at concentrations from 5000 to 62.5 µg/mL. The final bacterial inoculum concentration was standardized to 1×10^5 CFU/mL in Mueller-Hinton Broth. Following 24-hour incubation at 35°C, bacterial growth inhibition was evaluated by spectrophotometric absorbance measurements at 620 nm.

Statistical Analysis: Data from triplicate experiments are expressed as mean ± SD (standard deviation). One-way ANOVA and Tukey's post-hoc test (SPSS v21.0, $p < 0.05$) were used for comparisons.

RESULTS AND DISCUSSIONS

Antibiotic resistance and shortages have become critical global health issues, affecting countries worldwide. Globally, approximately 1.3 million fatalities in 2019 are attributed to infections that are bacterial antimicrobial resistance (AMR), highlighting the severity of this worldwide crisis (Murray et al., 2022). The COVID-19 pandemic intensified antibiotic shortages by disrupting global pharmaceutical supply chains, exposing systemic vulnerabilities in drug manufacturing and distribution networks. A significant dependence on a limited number of manufacturers, particularly in China and India, has further intensified these shortages, with 76% of intermediaries and 59% of active pharmaceutical ingredient (API) producers concentrated in these two countries (Klimek et al., 2023). Such shortages frequently result in excessive reliance on last-resort or specialized antibiotics, raising the likelihood of antimicrobial resistance and complicating infection treatment (Pandey et al., 2025). Given these challenges, medicinal plants offer promising alternatives due to their complex chemical profiles, which exhibit multifaceted mechanisms of action against pathogens. Plant-based

antimicrobials are less likely to induce resistance due to their ability to target multiple pathways simultaneously.

The solvent-solvent fractionation approach used in this study provides a more refined analysis of bioactive compounds compared to studies relying solely on crude extracts (Hernandez et al., 1999; Ertürk, 2006). This method allows for selective enrichment of different phytochemical classes based on polarity, enabling better correlation between specific fractions and bioactivities. Similar fractionation strategies have been employed in recent phytochemical studies (Endalew et al., 2024; Haruni et al., 2025), demonstrating improved resolution of active components. This aligns with current trends in phytochemical research emphasizing targeted bioactivity-guided fractionation. Phytochemical analysis of *A. unedo* leaf extracts confirmed the existence of multiple bioactive compounds, including flavonoids, tannins, terpenoids, steroids, and glycosides, across different fractions. Alkaloids and saponins were absent across all tested fractions (Table 1). Flavonoids were particularly abundant in the ethyl acetate fraction, consistent with previous studies that identified these compounds as major contributors to the plant's bioactivity (El Haouari et al., 2021). Tannins were present in both polar and aqueous fractions, known for their ability to bind bacterial proteins and disrupt cellular membranes (Huang et al., 2024).

The presence of terpenoids and steroids suggests additional mechanisms of antimicrobial activity, such as inhibition of bacterial enzyme systems or interference with membrane integrity. These findings align with earlier studies on Mediterranean plants that emphasize their rich phytochemical profiles as key contributors to therapeutic efficacy (Koyu et al., 2019; Radulovic et al., 2013).

As shown in Table 2, the ethanol extract had the maximum TPC (738.43 ± 5.11 mg GAE/g), exceeding both the ethyl acetate and chloroform fractions. Additionally, TFC (98.14 ± 2.13 mg CE/g) was notably higher than other fractions. These findings align with previous reports that phenolics and flavonoids are concentrated in polar solvents due to their hydrophilic nature (Morales, 2022). Zitouni et al. (2021) observed lower TPC and TFC (30.98 mg GAE/g and 5.20 mg Rutin/g, respectively) in Moroccan samples versus our samples, suggesting geographic variability in bioactive accumulation. These disparities underscore the influence of regional environmental factors on phytochemical profiles. As antioxidants, phenolics work by providing hydrogen atoms or electrons to scavenge and deactivate free radicals.

Table 1. Chemical constituent analysis of *A. unedo* extract and fractions.

Phytochemicals	Ethanol Extract	Hexane Fraction	Chloroform Fraction	Ethyl acetate Fraction	Aqueous Fraction
Alkaloids	–	–	–	–	–
Flavonoids	+	–	+	+	+
Saponins	–	–	–	–	–
Terpenoids	+	+	+	+	–
Steroids	+	+	+	–	–
Tannins	+	–	+	+	+
Glycosides	+	–	+	+	+

(−) absent; (+) present.

Table 2 Total polyphenolic content (TPC and TFC) and free radical scavenging and reducing power potential of *A. unedo* extract and fractions.

Extracts	TPC (mg GAE/g)	TFC (mg CE/g)	DPPH (mg TE/g)	ABTS (mg TE/g)	FRAP (mg TE/g)
Ethanol Extract	738.43 ±5.11^{a}	98.14 ± 2.13^{a}	429.21±5.52^{b}	97.32±4.11^{b}	343.83±3.13^{ab}
Hexane Fraction	295.27 ±3.36^{c}	56.28 ± 1.46^{c}	342.24±2.94^{d}	71.53±1.63^{c}	109.94±1.31^{d}
Chloroform Fraction	301.60 ±2.926^{c}	60.82 ± 1.73^{c}	434.51±4.53^{b}	82.94±2.11^{bc}	250.19±5.14^{c}
Ethyl acetate Fraction	688.81 ±4.46^{b}	91.72 ± 1.67^{b}	475.37±3.86^{a}	116.75±1.63^{a}	364.23±2.31^{a}
Water Fraction	204.28 ±2.86^{d}	48.88± 1.52^{d}	358.49±5.61^{c}	101.24±2.38^{ab}	271.58±3.82^{bc}

Means within a column followed by different letters are significantly different ($p < 0.05$).

The antioxidant potential of *A. unedo* extracts was evaluated using DPPH, ABTS radical scavenging assays, and FRAP assays. Among the extracts tested, the ethyl acetate fraction exhibited the highest antioxidant activity across all methods (DPPH 475.37±3.86; ABTS 116.75±1.63; FRAP 364.23±2.31mg Trolox/g) (Table 2). Samples from Portugal showed (DPPH 110.67 µmol TE/g; ABTS 122.16 µmol TE/g; FRAP 151.94 µmol TE/g) which is similar to our study. (Di Lorenzo et al., 2025).

These findings align with Khanum et al. (2013), who reported that ethyl acetate fractions of *Argyrolobium roseum* exhibited superior ABTS activity, attributed to high flavonoid concentrations. Similarly, El Halim et al. (2021) found that polar fractions (ethanol and ethyl acetate) of *Aquilaria malaccensis* had stronger DPPH, ABTS and FRAP activity scavenging than non-polar fractions, supporting our results. However, Chua et al. (2015) observed higher DPPH and FRAP activity in chloroform fractions of *Citrus hystrix*, contrasting with our data, possibly due to differences in bioactive compound solubility.

This superior antioxidant activity of ethyl acetate fraction can be attributed to its high phenolic and flavonoid content. The ethanol extract also demonstrated notable

antioxidant activity but was less effective than the ethyl acetate fraction. The probable mechanism underlying this activity involves phenolic hydroxyl groups donating electrons to stabilize free radicals or chelating metal ions that catalyze oxidative reactions (Benzie & Strain, 1996). These mechanisms suggest that *A. unedo* leaf extracts could serve as natural antioxidants for pharmaceutical or nutraceutical applications.

The antibacterial efficacy of *A. unedo* extracts was assessed against two Gram-positive (*S. aureus* and *E. faecalis*) and two Gram-negative (*E. coli* and *P. aeruginosa*) bacterial strains using agar well diffusion and broth dilution methods. The ethyl acetate fraction exhibited the largest inhibition zones against *S. aureus* (15.25±1.10) and *P. aeruginosa* (17.75±1.33), followed by the ethanol extract (Table 3). The hexane and chloroform fractions showed moderate activity, while aqueous fraction was less effective.

The MICs of *A. unedo* leaf extracts and fractions against the used bacterial strains were determined to evaluate their antibacterial efficacy (Table 4). The ethyl acetate fraction exhibited the strongest antibacterial activity, with MIC values of 156 µg/mL against *S. aureus* and 78 µg/mL against *P. aeruginosa*. The ethanol extract also showed moderate activity, particularly against *E. coli* (156 µg/mL) and *P. aeruginosa* (625 µg/mL). In contrast, the hexane, chloroform, and aqueous fractions demonstrated weaker antibacterial effects, with MIC values exceeding 625 µg/mL for most strains.

Table 3. Antibacterial activity of *A. unedo* extract and fractions.

Microorganisms	Zone of Inhibition (mm)					
	Ethanol Extract (100 mg/mL)	Hexane Fraction (100 mg/mL)	Chloroform Fraction (100 mg/mL)	Ethyl acetate Fraction (100 mg/mL)	Aqueous Fraction (100 mg/mL)	Gentamicin (1 mg/mL)
S. aureus	13.50±0.57^{bc}	11.75±1.27^{cd}	10.00±1.00^{d}	15.25±1.10^{b}	9.00±1.65^{d}	23.00±1.57^{a}
E. faecalis	12.00±1.09^{b}	10.25±1.65^{c}	9.25±1.15^{c}	12.50±1.23^{b}	9.25±1.15^{c}	16.25±1.15^{a}
E. coli	11.75±1.15^{c}	11.00±0.57^{c}	10.25±0.57^{c}	14.50±0.78^{b}	8.25± 2.64^{d}	18.5±2.98^{a}
P. aeruginosa	16.50±0.59^{bc}	15.25±2.23^{c}	16.00± 1.22^{bc}	17.75±1.33^{b}	10.00±1.48^{d}	21.00±1.53^{a}

Table 4. MIC values of *A. unedo* extract and fractions against different microbial strains

Microorganisms	Tested Plant Extracts (MIC µg/mL)					
	Ethanol Extract	Hexane Fraction	Chloroform Fraction	Ethyl acetate Fraction	Water Fraction	Gentamicin
S. aureus	1250	2500	2500	156	2500	39
E. faecalis	1250	625	1250	625	2500	156
E. coli	156	625	2500	625	2500	39
P. aeruginosa	625	625	2500	78	2500	78

The superior efficacy of the ethyl acetate fraction aligns with findings from Mariem et al. (2014), who reported that ethyl acetate fractions of *Retama raetam* exhibited stronger antibacterial activity against *S. aureus* compared to other fractions, likely due to higher concentrations of phenolic compounds. Similarly, Ibrahim et al. (2017) demonstrated that ethyl acetate extracts of medicinal plants had enhanced antimicrobial effects, supporting the results of this study. However, our findings contrast with Nikkon et al. (2003), who found that chloroform fractions of *Moringa oleifera* were more effective against *S. aureus* (MIC = 32 µg/mL), suggesting that bioactive constituents vary significantly among plant species. Gentamicin, the positive control, displayed significantly lower MIC values (39–156 µg/mL), confirming its potency as a conventional antibiotic. However, the ethyl acetate fraction's comparable efficacy (78 µg/mL for *P. aeruginosa*) suggests its potential as a natural alternative.

Phenolic constituents in *A. unedo* extracts appear to exert antimicrobial activity potentially through membrane disruption or inhibition of key enzymes in bacterial cell wall formation and DNA synthesis. Flavonoids may further enhance antibacterial activity by forming complexes with bacterial proteins or interfering with quorum sensing pathways (Xie et al., 2015; Radulovic et al., 2013).

The ethyl acetate fraction of *A. unedo* leaves emerged as the most bioactive extract in this investigation, displaying remarkable antioxidant and antimicrobial properties that correspond with its high polyphenolic content, suggesting significant medicinal potential. The dual antioxidant-antibacterial properties observed suggest potential synergistic effects that could enhance therapeutic efficacy while minimizing resistance development risks. For instance, phenolics may weaken bacterial defenses against oxidative stress while directly targeting cellular structures or processes. Future research should aim to characterize isolated phytochemicals from *A. unedo* leaves in Armutlu region, employing sophisticated techniques to elucidate their exact pharmacological mechanisms.

CONCLUSION

The research shows that *A. unedo* leaf extracts could be valuable natural antioxidants and antimicrobial agents. The ethyl acetate fraction consistently outperformed other extracts due to its high phenolic

content, demonstrating significant antioxidant activity through free radical scavenging mechanisms and antibacterial efficacy against pathogens such as *S. aureus*. These findings support its potential for developing safe therapeutic agents against oxidative stress-related diseases and bacterial infections.

REFERENCES

Baytop, T. (1999). *Curing with plants in Turkey, in the past and today (Türkiye 'de bitkiler ile tedavi, geçmişte ve bugün)*. Nobel Medical Books, Capa, İstanbul, 299p.

Benzie, I.F.F., & Strain, J.J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry*, ***239***(1), 70-76. DOI: 10.1006/abio.1996.0292

Beyatli, A., Peker, E.G.G., Gül, N., & Cevher, Ş.C. (2022). Morin (2′, 3, 4′, 5, 7-Pentahydroxyflavon) antioxidant effect in streptozotocin-ınduced diabetic rat brain and heart tissues. *Journal of Anatolian Environmental and Animal Sciences*, ***7***(3), 257-262. DOI: 10.35229/jaes.1103000

Chanda, S., & Dave, R. (2009). In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research*, ***3***(13), 981-996.

Chua, K.W., Sia, C.M., Akowuah, G.A., Samuagam, L., & Yim, H.S. (2015). Antioxidative properties and HPLC profile of chloroform fraction from ethanolic extract of the peel of *Citrus hystrix*. *Chiang Mai Journal of Science*, ***42***, 173-184.

CLSI. (2008). *Performance standards for antimicrobial susceptibility testing.* (Clinical and Laboratory Standards Institute 28th ed. CLSI supplement M100). Wayne, PA.

Dewanto, V., Wu, X., Adom, K.K., & Liu, R.H. (2002). Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. *Journal of Agricultural and Food Chemistry*, ***50***(10), 3010-3014. DOI: 10.1021/jf0115589

Di Lorenzo, R., Ferraro, M. G., Carrera, C., Iazzetti, F., Chinchilla, N., Maisto, M., Aliaño-González, M.J., Piccolo, V., Romano, A., Ricci, L., Medronho, B., Marzocchi, A., Piccolo, M., Tenore, G.C., Irace, C., & Laneri, S. (2025). Valorization of *Arbutus unedo* L. Pomace: Exploring the recovery of bioactive phenolic compounds from distillation by-products. *Antioxidants*, ***14***(3), 278. DOI: 10.3390/antiox14030278

Endalew, S.A., Taddese, M.G., & Muhammed, M. (2024). Evaluation of antioxidant and antibacterial properties of dehydrocostus lactone isolated from *Echinops kebericho* root. *Health Science Reports*, ***7***(3), e1990. DOI: 10.1002/hsr2.1990

El Haouari, M., Assem, N., Changan, S., Kumar, M., Daştan, S.D., Rajkovic, J., Taheri, Y., & Sharifi-Rad, J. (2021). An insight into phytochemical, pharmacological, and nutritional properties of *Arbutus unedo* L. from Morocco. *Evidence-Based Complementary and Alternative Medicine*, ***2021***(1). DOI: 10.1155/2021/1794621

Ertürk, Ö. (2006). Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. *Biologia*, ***61***(3), 275-278. DOI: 10.2478/s11756-006-0050-8

Halim, A.P., Wijayanti, N., Hidayati, L., & Nuringtyas, T.R. (2022). Antioxidant activity evaluation of agarwood *Aquilaria malaccensis* Lamk. leaves extract using DPPH, FRAP and ABTS assays. 7th *International Conference on Biological Science* (pp. 18-25). Atlantis Press.

Harborne, A.J. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. Springer Science & Business Media.

Haruni, M.J., Parvin, M.S., Munira, S., & Islam, M.E. (2025). Phytochemical profile and evaluation of the antioxidant, anticancer, and antimicrobial potential of *Artocarpus lacucha* bark extracts: In vitro and ın silico studies. *Journal of Food Biochemistry*, ***2025***(1), 8016598. DOI: 10.1155/jfbc/8016598

Hernandez, M.M., Heraso, C., Villarreal, M.L., Vargas-Arispuro, I., & Aranda, E. (1999). Biological activities of crude plant extracts from *Vitex trifolia* L.(Verbenaceae). *Journal of Ethnopharmacology*, ***67***(1), 37-44. DOI: 10.1016/S0378-8741(99)00041-0

Hossain, M.A., Al-Hdhrami, S.S., Weli, A.M., Al-Riyami, Q., & Al-Sabahi, J.N. (2014). Isolation, fractionation and identification of chemical constituents from the leaves crude extracts of *Mentha piperita* L grown in Sultanate of Oman. *Asian Pacific Journal of Tropical Biomedicine*, ***4***, S368-S372. DOI: 10.12980/APJTB.4.2014C1051

Huang, J., Zaynab, M., Sharif, Y., Khan, J., Al-Yahyai, R., Sadder, M., … & Li, S. (2024). Tannins as antimicrobial agents: Understanding toxic effects on pathogens. *Toxicon*, ***247***, 107812. DOI: 10.1016/j.toxicon.2024.107812

Ibrahim, A.M., Lawal, B., Abubakar, A.N., Tsado, N.A., Kontagora, G.N., Gboke, J.A., & Berinyuy, E.B. (2017). Antimicrobial and free radical scavenging potentials of N-hexane and ethyl acetate fractions of Phyllanthus Fraternus. *Nigerian Journal of Basic and Applied Sciences*, ***25***(2), 6-11.

IUCN. (2017). IUCN Red List of Threatened Species: *Arbutus unedo*. Retrieved March 28, 2025, from https://www.iucnredlist.org/species/202930/68076133

Khanum, R., Jahangir, M., Abbasi, M.A., Mazhar, F., Kausar, S., Riaz, T., & Ajaib, M. (2013). Phytochemical screening and antioxidant evaluations of different fractions of *Argyrolobium*

roseum. *Asian Journal of Chemistry*, ***25***(13), 7485-7489.

Klimek, P., Dervic, E., Friesenbichler, K., Gerschberger, M., & Yang, L. (2023). *The anatomy of the current antibiotics shortage*. Vienna, Austria: Supply Chain Intelligence Institute. Retrieved from https://papers.ssrn.com/sol3/papers.cfm?abstract_id=4451526

Koyu, H., Koyu, E.B., Demir, S., & Baykan, Ş. (2019). *Arbutus unedo* L.(Kocayemiş). *Türk Farmakope Dergisi*, ***4***(3), 29-51.

Markovinović, A.B., Karačonji, I.B., Jurica, K., Lasić, D., Babojelić, M.S., Duralija, B., Žlabur, J.S., Putnik, P., & Kovačević, D.B. (2022). Strawberry tree fruits and leaves (*Arbutus unedo* L.) as raw material for sustainable functional food processing: A review. *Horticulturae*, ***8***(10), 881. DOI: 10.3390/horticulturae8100881

Mariem, S., Hanen, F., Inès, J., Mejdi, S., & Riadh, K. (2014). Phenolic profile, biological activities and fraction analysis of the medicinal halophyte *Retama raetam*. *South African Journal of Botany*, ***94***, 114-121. DOI: 10.1016/j.sajb.2014.06.010

Miguel, M.G., Faleiro, M.L., Guerreiro, A.C., & Antunes, M.D. (2014). *Arbutus unedo* L.: Chemical and biological properties. *Molecules*, ***19***(10), 15799-15823. DOI: 10.3390/molecules191015799

Morales, D. (2022). Use of strawberry tree (*Arbutus unedo*) as a source of functional fractions with biological activities. *Foods*, ***11***(23), 3838. DOI: 10.3390/foods11233838

Murray, C.J.L., Ikuta, K.S., Sharara, F., Swetschinski, L., Aguilar, G.R., Gray, A., …& Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet*, ***399***(10325), 629-655. DOI: 10.1016/S0140-6736(21)02724-0

Nikkon, F., Saud, Z.A., Rahman, M.H. & Haque, Md. E. (2003). In vitro antimicrobial activity of the compound isolated from chloroform extract of *Moringa oleifera* Lam. *Pakistan Journal of Biological Sciences*, ***6***(22), 1888-1890.

Pandey, A.K., Cohn, J., Nampoothiri, V., Gadde, U., Ghataure, A., Kakkar, A. K., … & Charani, E. (2025). A systematic review of antibiotic drug shortages and the strategies employed for managing these shortages. *Clinical Microbiology and Infection*, ***31***(3), 345-353. DOI: 10.1016/j.cmi.2024.09.023

Radulovic, N.S., Blagojevic, P.D., Stojanovic-Radic, Z.Z. & Stojanovic, N.M. (2013). Antimicrobial plant metabolites: Structural diversity and mechanism of action. *Current Medicinal Chemistry*, ***20***(7), 932-952.

Rumpf, J., Burger, R. & Schulze, M. (2023). Statistical evaluation of DPPH, ABTS, FRAP, and Folin-Ciocalteu assays to assess the antioxidant capacity of lignins. *International Journal of Biological Macromolecules*, ***233***, 123470. DOI: 10.1016/j.ijbiomac.2023.123470

Tastekin, B., & Çiftci, G. (2023). Antioxidant capacity and antibacterial potential of rosehip (Rosa canina) fruits grown. *Journal of Anatolian Environmental and Animal Sciences*, ***8***(1), 103-109. DOI: 10.35229/jaes.1240877

Tyler, V.E. (1993). *Phytomedicines in Western Europe: Potential impact on herbal medicine in the United States*. ACS Publications.

Wahid, N., Faida, R., Aabdousse, J., Boulli, A., & Bouda, S. (2019). Ethnobotanical uses and distribution status of Arbutus (*Arbutus unedo* L.) in Morocco. *Ethnobotany Research and Applications*, ***18***, 1-12. DOI: 10.32859/era.18.30

Xie, Y., Yang, W., Tang, F., Chen, X., & Ren, L. (2015). Antibacterial activities of flavonoids: Structure-activity relationship and mechanism. *Current Medicinal Chemistry*, ***22***(1), 132-149. DOI: 10.2174/0929867321666140916113443

Yoo, S., Kim, K., Nam, H., & Lee, D. (2018). Discovering health benefits of phytochemicals with integrated analysis of the molecular network, chemical properties and ethnopharmacological evidence. *Nutrients*, ***10***(8), 1042. DOI: 10.3390/nu10081042

Zhou, K., Su, L., & Yu, L. (2004). Phytochemicals and antioxidant properties in wheat bran. *Journal of Agricultural and Food Chemistry*, ***52***(20), 6108-6114. DOI: 10.1021/jf049214g

Zitouni, H., Hssaini, L.H., Ouaabou, R., Viuda-Martos, M., Hernandez, F., Ercisli, S., … & Hanine, H. (2021). Functionnal and Technological Properties of Five Strawberry (*Arbutus Unedo* L.) Fruit as Bioactive Ingredients in Functional Foods. *International Journal of Food Properties*, ***24***(1), 380-399. DOI: 10.1080/10942912.2021.1883058