Journal of Research in Pharmacy

Research Article

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Comparative evaluation of the antioxidant, phenolic and antimicrobial activities of *Plantago lanceolata* L. leaf extracts from two regions of Türkiye: Giresun and Sakarya

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Received: 9 July 2026 / Accepted: 15 September 2026

ABSTRACT: *Plantago lanceolata* is a medicinal plant widely used in phytotherapeutic applications in Türkiye. This study evaluated the antioxidant, total phenolic, and antimicrobial activities of *Plantago lanceolata* leaf extracts collected from the Tirebolu (Giresun) and Adapazarı (Sakarya) districts. Ethanol, methanol, and aqueous extracts were prepared by ultrasound-assisted extraction. Antioxidant activity was determined using the CUPRAC and ABTS assays, total phenolic content by the Folin–Ciocalteu method, and antimicrobial activity by agar well diffusion and broth microdilution assays. Minimum bactericidal and fungicidal concentrations were also determined. The CUPRAC and ABTS antioxidant capacities (mmol TE/g dw) were 0.136–0.234 and 0.389–0.641, respectively, for the Giresun samples and 0.139–0.207 and 0.406–0.584, respectively, for the Sakarya samples. The total phenolic content (mg GAE/g dw) was 244.61–324.78 for the Giresun samples and 239.87–310.99 for the Sakarya samples. Among the extraction solvents, methanol extracts consistently showed the highest antioxidant capacity and phenolic content. The ethanol extracts formed inhibition zones against *Pseudomonas aeruginosa* (9.24 and 7.15 mm) and *Acinetobacter baumannii* (7.25 and 8.19 mm) for the Giresun and Sakarya samples, respectively. All extracts inhibited the tested microorganisms, with minimum inhibitory concentration values of 0.196–1.563 mg/mL. The ethanol extracts also showed bactericidal activity against all tested bacteria, with minimum bactericidal concentration values of 0.396–6.25 mg/mL and 0.781–1.563 mg/mL for the Giresun and Sakarya samples, respectively. These findings indicate that *Plantago lanceolata* is a promising natural source of antioxidant and phenolic compounds with notable antimicrobial activity, and that ultrasound-assisted extraction is an effective approach for recovering its bioactive constituents.

KEYWORDS: *Plantago lanceolata* L.; antioxidant activity; phenolic content; antimicrobial activity; UV-Vis spectroscopy.

1. INTRODUCTION

Phytotherapy refers to the use of medicinal plants or their various parts, including flowers, leaves, roots, bark, seeds, and fruits, either individually or in combination, for the prevention and treatment of diseases [1]. To ensure their safe and effective use, medicinal plants should be scientifically evaluated in terms of quality, efficacy, and safety. In recent years, consumer interest in natural and plant-derived products has increased substantially worldwide, driven by growing awareness of healthy lifestyles and the increasing demand for complementary and alternative therapeutic approaches. According to the World Health Organization (WHO), medicinal plants play a significant role in global healthcare, and a considerable proportion of the world's population regularly uses herbal products to maintain health or to support the treatment of various diseases. The wide range of pharmacological activities exhibited by plant-derived compounds, together with the fact that many therapeutic agents have originated from natural sources, has further stimulated scientific interest in medicinal plants. Consequently, medicinal plants have become the focus of extensive research not only because of their traditional uses but also owing to their potential applications in drug discovery, functional foods, and complementary medicine. In particular, the investigation of the bioactive constituents and biological activities of traditionally used medicinal plants is essential for elucidating their therapeutic

How to cite this article: Kurt-Cucu A, Domac B, Rayaman P, Erdogan G. Comparative evaluation of the antioxidant, phenolic and antimicrobial activities of Plantago lanceolata L. leaf extracts from two regions of Türkiye: Giresun and Sakarya. J Res Pharm. 2026; 30(5): 1241-1252.

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ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1991005

potential, supporting the development of natural health products, and facilitating the discovery of novel therapeutic agents [2].

Plantago lanceolata L. (*P. lanceolata*), commonly known as ribwort plantain, is one of the most important medicinal plant species used in phytotherapy. In Türkiye, it is widely distributed along roadsides, meadows, grasslands, and pastures and is traditionally known by several local names, including sinir otu, damar otu, sinirli yaprak, bağa yaprağı, and ateş yaprağı. The plant has been used for medicinal purposes since antiquity, with documented use dating back to the ancient Greek and Roman civilizations. Moreover, *P. lanceolata* and other species of the Plantago genus have been used in Traditional Chinese Medicine for approximately 3,000 years. The genus Plantago, belonging to the Plantaginaceae family, comprises perennial herbaceous species with a broad global distribution and is represented by approximately 21 species in Türkiye. Among these, *P. lanceolata* and *Plantago major* are the most widespread and extensively utilized medicinal species [3, 4]. Previous studies have demonstrated that *P. lanceolata* extracts possess a wide range of biological activities, including antioxidant, antiinflammatory, antibacterial, antispasmodic, viscoelastic, and rheological properties. In traditional medicine, the leaves are commonly used to promote wound healing, enhance immune function, and support the treatment of cancer as well as respiratory, gastrointestinal, and urogenital disorders. In addition, *P. lanceolata* is used in the form of herbal teas, syrups, and tablets to relieve pain, treat dermatological conditions, accelerate the healing of boils, and alleviate asthma symptoms [5, 6]. Beyond its medicinal applications, *P. lanceolata* has attracted considerable interest in pharmaceutical, cosmetic, food, agricultural, and environmental fields. It is incorporated into cosmetic formulations such as creams and lotions, used as a food additive, investigated as a natural pesticide, employed as a biomonitor of environmental heavy metal contamination, and explored for various agricultural applications. These diverse biological and industrial applications are largely attributed to the plant's rich phytochemical composition, which includes numerous bioactive compounds responsible for its pharmacological properties [2].

Oxidative stress results from the excessive production of reactive oxygen species (ROS), which can cause oxidative damage to essential biomolecules, including proteins, lipids, carbohydrates, enzymes, and DNA. DNA damage and oxidative alterations at the cellular level play a critical role in the pathogenesis of numerous chronic diseases, including cancer, cardiovascular diseases, diabetes mellitus, autoimmune disorders, and neurodegenerative diseases. Natural antioxidants, particularly phenolic compounds and flavonoids present in medicinal plants, can neutralize free radicals and thereby mitigate oxidative damage [7]. Consequently, the determination of the antioxidant capacity and phenolic content of medicinal plants is essential for evaluating their biological potential and supporting their therapeutic applications [8].

Antimicrobials are used in the treatment protocols of various infectious diseases. However, the misuse and overuse of antimicrobial drugs has led to the emergence and spread of antibiotic resistant microorganisms [9, 10, 11]. Today, antimicrobial resistance is a global public health concerning all of the countries and this led the scientists to looking for alternative new compounds. It is known that plant extracts are known for possessing antimicrobial activitity and since the parmaceuticles derived from them have been shown to be effective against various pathogens many studies have been focused on searching of developing new compounds to cope with the infections. Folk medicine benefits from the medicinal plants during healing wounds and consequently it is important to investigate the new formation plant extracts possesing antimicrobial activity [9, 11].

P. lanceolata is rich in a diverse array of bioactive phytochemicals, including flavonoids (e.g., baicalein and luteolin), monoterpenoids (e.g., linalool), triterpenoids (e.g., oleanolic and ursolic acids), iridoid glycosides (e.g., aucubin), and various phenolic compounds. These phytochemicals have been reported to contribute significantly to the plant's biological activities, particularly its antioxidant and antiinflammatory properties [2, 12].

Several studies have investigated the total phenolic content (TPC) and antioxidant activity of extracts prepared from different parts of *P. lanceolata* using a variety of extraction solvents and analytical methods. TPC has been predominantly determined using the Folin–Ciocalteu assay [13, 14, 15, 16, 17, 18, 19, 20]. Antioxidant activity has been evaluated using several assays, including ORAC and FRAP for lyophilized ethanol (EtOH) extracts [13]; ABTS and CUPRAC for hexane, EtOH, and aqueous (A) extracts [21]; DPPH, CUPRAC, FRAP, and ABTS for A, methanol (MeOH), and EtOH extracts [14]; β-carotene bleaching and ferrous ion chelating assays for A extracts, together with the phosphomolybdenum assay for MeOH extracts [22]; ABTS, DPPH, and FRAP for A extracts [16] ABTS for distilled water, 96% EtOH, aqueous propylene glycol, and aqueous glycerol extracts [23]; DPPH, CUPRAC, and FRAP for EtOH extracts [17]; ORAC for EtOH

extracts [19]; ABTS and DPPH for lyophilized MeOH–water extracts [20]; DPPH for EtOH, MeOH, propan-1-ol, and propan-2-ol extracts [24]; and DPPH, ABTS, FRAP, and CUPRAC for EtOH extracts [25].

A review of the literature revealed several studies investigating the antimicrobial activity of *P. lanceolata* extracts. Kassaw et al. (2018) reported that EtOH leaf extracts of *P. lanceolata* produced an inhibition zone of 9.00 mm against *Pseudomonas aeruginosa*, with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 25 and 50 mg/mL, respectively [26]. In the same study, both the MIC and MBC values against *Staphylococcus aureus* and *Escherichia coli* were reported as 25 mg/mL. Yiğit et al. (2009) determined an MIC value of 5 mg/mL for the MeOH extract of *P. lanceolata* collected from Erzurum against *Candida albicans* [27]. In contrast, the corresponding MIC value obtained in the present study was 1.563 mg/mL for samples collected from both Giresun and Sakarya, indicating greater antifungal activity under the experimental conditions employed. Dessie et al. (2025) reported MIC values of 6.25 mg/mL for *S. aureus* and *Staphylococcus epidermidis*, 12.5 mg/mL for *E. coli*, and 25 mg/mL for *Klebsiella pneumoniae* using MeOH extracts of *P. lanceolata* [10]. The corresponding MBC values were 6.25 mg/mL for *S. aureus*, 50 mg/mL for *S. epidermidis* and *E. coli*, and 80 mg/mL for *K. pneumoniae*. Furthermore, Orhan et al. (2012) reported MIC and MBC values of 32 and 128 mg/mL, respectively, against *Acinetobacter baumannii* [11]. The observed differences among these studies may be attributed to several factors, including geographical origin, environmental and climatic conditions, extraction solvent, extraction procedure, phytochemical composition, and experimental methodology, all of which are known to influence the antimicrobial activity of medicinal plant extracts.

However, in Türkiye, the hexane, EtOH, and A maceration extracts of *P. lanceolata* collected from Ordu Province were evaluated only for their antioxidant activities using the CUPRAC and ABTS assays, whereas their TPC values were not determined [21]. In another study, ultrasound-assisted A extracts of *P. lanceolata* collected from İzmir Province were analyzed for antioxidant activity using the DPPH, CUPRAC, FRAP, and ABTS assays, and their TPC values were determined [14]. However, no comparison was made using different extraction solvents, and the antimicrobial activities of the extracts were not investigated in either study.

The present study investigated the antioxidant, total phenolic, and antimicrobial properties of EtOH, MeOH, and A leaf extracts of *P. lanceolata* L. collected from two geographically distinct regions of Türkiye, namely the Black Sea Region (Tirebolu, Giresun) and the Marmara Region (Adapazarı, Sakarya). The extracts were prepared using ultrasound-assisted extraction. Antioxidant activity was evaluated spectrophotometrically using the CUPRAC and ABTS assays, while TPC was determined by the Folin–Ciocalteu method. Antimicrobial activity was assessed using the agar well diffusion and broth microdilution methods to determine the MIC, followed by the determination of the MBC and minimum fungicidal concentration (MFC). Comparing the biological activities of *P. lanceolata* growing under different climatic and environmental conditions provides valuable information for assessing its potential as a natural source of bioactive compounds, evaluating the influence of geographical origin on plant quality, and comparing the findings with previously published data. Therefore, this study aimed to provide comprehensive scientific evidence regarding the antioxidant and antimicrobial potential of this medicinal and edible plant and to contribute to its phytotherapeutic applications.

2. RESULTS AND DISCUSSION

2.1. Total phenolic content results

The TPC of the *P. lanceolata* extracts is presented in Table 1 and Fig. 1. The highest TPC was observed in the MeOH extracts of samples collected from Giresun and Sakarya, with values of 324.78 ± 0.87 and 310.99 ± 0.43 mg GAE/g dw, respectively. In contrast, the lowest TPC was obtained from the EtOH extracts, with values of 244.61 ± 4.31 and 239.87 ± 4.31 mg GAE/g dw for the Giresun and Sakarya samples, respectively.

Table 1. Total phenolic content (TPC) of the dry weight

Extracts	TPC (mg GAE/g dw)
S-EtOH	239.87±4.31
S-MeOH	**310.99**±0.43
S-A	284.27±0.86
G-EtOH	244.61±4.31
G-MeOH	**324.78**±0.87
G-A	266.59±4.31

S: Sakarya.
G: Giresun.
EtOH: Ethanol.

MeOH: Methanol.
A: Aqueous.
TPC: Total phenolic content.
GAE: Gallic acid equivalent.

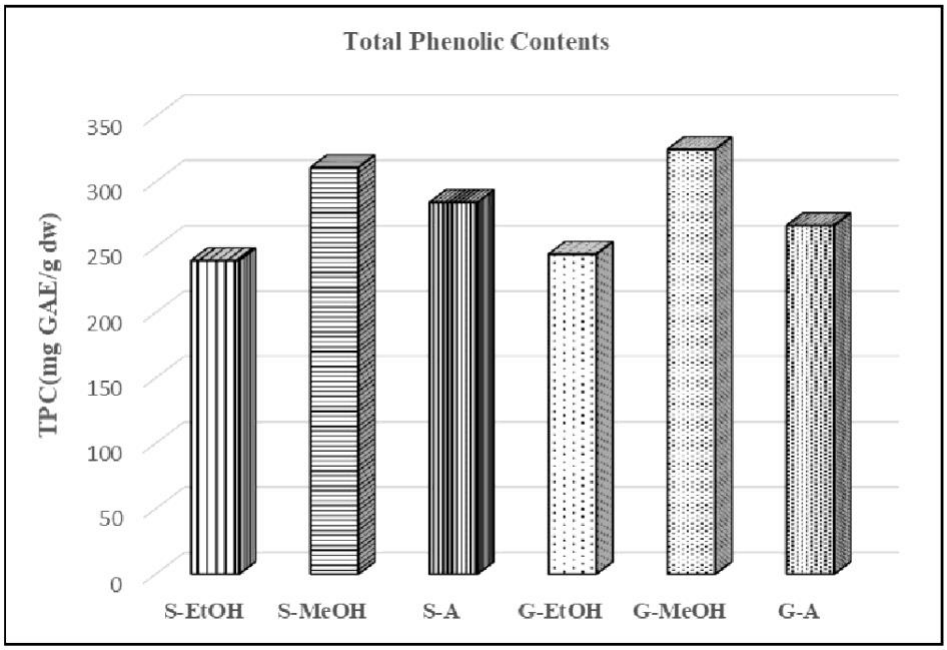

Figure 1. Total phenolic content (TPC) of *Plantago lanceolata* samples collected from Sakarya (S) and Giresun (G) extracted with ethanol (EtOH), methanol (MeOH), and aqueous (A). Results are expressed as mg gallic acid equivalents per g dry weight (mg GAE / g dw)

Overall, the TPC values obtained in the present study were generally higher than those reported in previous studies using comparable extraction solvents. Nevertheless, direct comparisons should be interpreted with caution because of differences in extraction methods, plant materials, analytical protocols, and the units used to express TPC values. Dalar and Konczak (2013) reported a TPC of 80.4 ± 1.8 mg GAE/g dw in infusion samples [13]. Similarly, Akgün et al. (2022) reported TPC values of 30.25 ± 2.00, 61.10 ± 5.50, and 49.81 ± 4.30 mg GAE/g dw for A, MeOH, and EtOH extracts, respectively [14]. Kaygısız (2021) determined the TPC of the MeOH extract of commercially available *P. lanceolata* as 11.73 ± 0.84 g GAE/kg dw [15]. Ziarno et al. (2021) reported a TPC of 41.84 ± 0.20 mg GAE/g dw for A extracts [16], whereas Lukova et al. (2018) found TPC values ranging from 39.26 ± 1.55 to 41.75 ± 2.26 mg GAE/g dw [17]. Using Soxhlet extraction with ethanol as the extraction solvent, Mazzutti et al. (2017) reported a TPC of 65.5 ± 0.9 mg GAE/g extract [18]. More recently, Laanet et al. (2024) reported TPC values ranging from 45.9 to 55.5 mg GAE/g [19], while Sanna et al. (2022) reported TPC values between 65.2 and 240.3 g GAE/kg dw for lyophilized methanol–water extracts [20].

2.2. Antioxidant activity results

The antioxidant activities of the *P. lanceolata* extracts are presented in Table 2 and Fig. 2. According to the CUPRAC assay, the highest antioxidant activity was observed in the MeOH extract of the Giresun samples, with a value of 0.234 ± 0.001 mmol TE/g dw, whereas the lowest activity was recorded in the EtOH extract from the same region (0.136 ± 0.002 mmol TE/g dw). Similarly, the ABTS assay showed the highest antioxidant activity in the MeOH extract of the Giresun samples (0.641 ± 0.003 mmol TE/g dw), while the lowest value was obtained for the corresponding EtOH extract (0.389 ± 0.008 mmol TE/g dw).

Table 2. Antioxidant activity results of the dry weight (n=3)

Extracts	CUPRAC (mmol TE/g dw)	ABTS (mmol TE/g dw)
S-EtOH	0.139±0.003^{d}	0.406±0.008^{c}
S-MeOH	0.207±0.001^{b}	0.578±0.009^{b}
S-A	0.169±0.002^{c}	0.584±0.006^{b}
G-EtOH	0.136±0.002^{d}	0.389±0.008^{c}
G-MeOH	0.234±0.001^{a}	0.641±0.003^{a}
G-A	0.160±0.003^{c}	0.540±0.008^{b}

Values sharing the same letter are not significantly different ($p > 0.05$), whereas values with different letters differ significantly ($p < 0.05$).
S: Sakarya.
G: Giresun.
EtOH: Ethanol.
MeOH: Methanol.
A: Aqueous.
TE: Trolox equivalent.

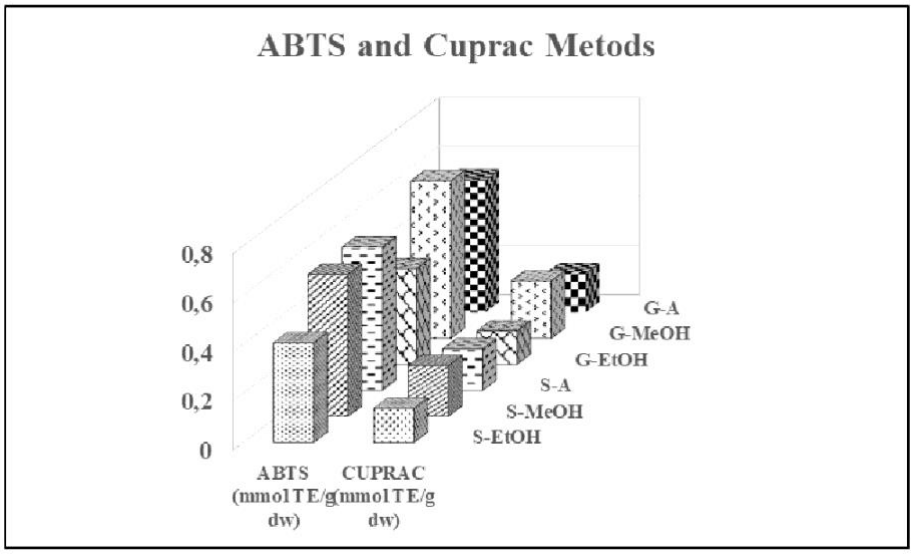

Figure 2. Comparison of the antioxidant capacities of *Plantago lanceolata* collected from Sakarya (S) and Giresun (G), as determined by the ABTS and CUPRAC assays for ethanol (EtOH), methanol (MeOH), and aqueous (A) extracts. Results are expressed as mmol TE / g dw

Compared with previous studies employing comparable extraction solvents, the antioxidant activities obtained in the present study were generally higher. Akgün et al. (2022) reported CUPRAC values of 82.5 ± 3.50, 179.5 ± 7.50, and 177.5 ± 6.50 µmol TE/g and ABTS values of 37.00 ± 4.50, 64.79 ± 5.25, and 64.05 ± 6.50 µmol TE/g for A, MeOH, and EtOH extracts, respectively [14]. Ziarno et al. (2021) reported an ABTS value of 0.150 ± 0.003 mmol TE/g dw for A extracts [16], whereas Lukova et al. (2018) reported CUPRAC values ranging from 55.23 ± 1.59 to 56.89 ± 1.13 µmol TE/g dw for ethanol extracts [17]. In addition, Sanna et al. (2022) reported ABTS values ranging from 8.7 to 25.8 mmol TE/100 g dw for lyophilized methanol–water extracts [20].

Overall, the antioxidant activities of the *P. lanceolata* extracts obtained from both Giresun and Sakarya were generally higher than those reported in previous studies using comparable extraction solvents. Nevertheless, direct comparisons should be interpreted with caution because of differences in extraction procedures, plant materials, analytical conditions, and the units used to express antioxidant activity.

2.3. Antimicrobial activity results

The antimicrobial activities of the *P. lanceolata* extracts collected from Giresun are presented in Table 3. In the agar well diffusion assay, only the ethanol extract exhibited inhibitory activity, producing inhibition zones of 9.24 mm against *P. aeruginosa* and 7.25 mm against *A. baumannii*. No inhibition zones were observed for the methanol or aqueous extracts.

According to the broth microdilution assay, all extracts exhibited antimicrobial activity against the tested microorganisms, with MIC values ranging from 0.196 to 1.563 mg/mL. Among the tested extracts, the EtOH extract showed the strongest antimicrobial activity, exhibiting the lowest MIC values against *P. aeruginosa*, *A. baumannii*, *S. aureus*, *S. epidermidis*, and *K. pneumoniae*. It also demonstrated antifungal activity against *C. albicans*, with an MIC value of 1.563 mg/mL. The MeOH extract displayed a similar antimicrobial profile, followed by the A extract, which showed the highest antifungal activity against *C. albicans* (ATCC 90028), with an MIC value of 0.781 mg/mL.

The EtOH extract also exhibited bactericidal activity against all bacterial strains tested, with MBC values ranging from 0.396 to 6.25 mg/mL. Similarly, the MeOH extract showed bactericidal activity against all bacteria except *E. coli* and exhibited fungicidal activity against *C. albicans* (ATCC 90028), with an MBC value of 3.125 mg/mL. The A extract demonstrated bactericidal activity against *S. epidermidis* among the Gram-positive bacteria and against *A. baumannii* and *E. coli* among the Gram-negative bacteria.

Table 3. Antimicrobial activity of *Plantago lanceolata* collected from Giresun

Microorganism	Ethanol Extract			Methanol Extract			Aqueous Extract		
	ZD (mm)	MIC (mg/mL)	MBC/MFC (mg/mL)	ZD (mm)	MIC (mg/mL)	MBC/MFC (mg/mL)	ZD (mm)	MIC (mg/mL)	MBC/MFC (mg/mL)
***S. aureus* ATCC 29213**	0	0.396	1.563	0	0.781	3.125	0	0.781	>6.25
***S. epidermidis* ATCC 11228**	0	0.396	1.563	0	0.396	3.125	0	0.781	3.125
***P. aeruginosa* ATCC 27853**	9.24±0,07	0.196	6.25	0	0.196	1.563	0	0.196	>6.25
***A. baumannii* ATCC 19606**	7.25±0,01	0.196	0.396	0	0.196	0.396	0	0.396	1.563
***E. coli* ATCC 25922**	0	0.781	1.563	0	0.396	>6.25	0	1.563	1.563
***K. pneumoniae* ATCC 4352**	0	0.396	1.563	0	0.396	1.563	0	0.781	>6.25
***C. albicans* ATCC 90028**	0	1.563	>6.25	0	1.563	3.125	0	0.781	>6.25

ZD: Zone diameter.
MIC: Minimum Inhibitory Concentration.
MBC: Minimum Bactericidal Concentration.
MFC: Minimum Fungicidal Concentration.

The antimicrobial activities of the *P. lanceolata* extracts collected from Sakarya are presented in Table 4. In the agar well diffusion assay, the ethanol extract exhibited inhibitory activity against *P. aeruginosa* and *A. baumannii*, producing inhibition zones of 7.15 and 8.19 mm, respectively. In addition, the MeOH extract inhibited the growth of *A. baumannii*, with an inhibition zone of 8.23 mm. No inhibition zones were observed for the remaining extracts.

According to the broth microdilution assay, all extracts exhibited antimicrobial activity against the tested microorganisms, with MIC values ranging from 0.196 to 1.563 mg/mL. Among the tested extracts, the EtOH extract showed the strongest antimicrobial activity, followed by the MeOH extract. Compared with the Giresun extracts (Table 3), all Sakarya extracts exhibited identical antifungal activity against *C. albicans*, with an MIC value of 1.563 mg/mL.

The EtOH extract also exhibited bactericidal activity against all bacterial strains tested, with MBC values ranging from 0.781 to 1.563 mg/mL. Similarly, the MeOH extract showed bactericidal activity against all bacteria except *E. coli* and exhibited fungicidal activity against *C. albicans* (ATCC 90028), with an MBC value of 3.125 mg/mL. The A extract demonstrated bactericidal activity against *S. epidermidis* among the Gram-positive bacteria and against *P. aeruginosa* and *E. coli* among the Gram-negative bacteria.

Table 4. Antimicrobial activity of *Plantago lanceolata* collected from Sakarya

Microorganism	Ethanol Extract			Methanol Extract			Aqueous Extract		
	ZD (mm)	MIC (mg/mL)	MBC/MFC (mg/mL)	ZD (mm)	MIC (mg/mL)	MBC/MFC (mg/mL)	ZD (mm)	MIC (mg/mL)	MBC/MFC (mg/mL)
***S. aureus* ATCC 29213**	0	0.781	1.563	0	1.563	1.563	0	0.781	>6.25
***S. epidermidis* ATCC 11228**	0	0.396	1.563	0	0.396	3.125	0	0.781	3.125
***P. aeruginosa* ATCC 27853**	7.15±0,07	0.196	1.563	0	0.196	3.125	0	0.196	3.125
***A. baumannii* ATCC 19606**	8.19±0,01	0.196	0.781	8.23±0,07	0.196	0.781	0	0.781	>6.25
***E. coli* ATCC 25922**	0	0.396	1.563	0	0.781	>6.25	0	1.563	3.125
***K. pneumoniae* ATCC 4352**	0	0.396	1.563	0	0.396	1.563	0	0.781	>6.25
***C. albicans* ATCC 90028**	0	1.563	>6.25	0	1.563	3.125	0	1.563	>6.25

ZD: Zone diameter.
MIC: Minimum Inhibitory Concentration.
MBC: Minimum Bactericidal Concentration.
MFC: Minimum Fungicidal Concentration.

3. CONCLUSION

Türkiye possesses a rich diversity of medicinal and aromatic plants owing to its varied climatic and ecological conditions. Among these, *P. lanceolata* L. is an important medicinal and edible species that has long been used in traditional medicine. In the present study, the TPC, antioxidant capacity, and antimicrobial activity of EtOH, MeOH, and A leaf extracts of *P. lanceolata* collected from Giresun and Sakarya were comparatively evaluated.

The results demonstrated that both extraction solvent and geographical origin significantly influenced the recovery of phenolic compounds and the antioxidant capacity of the extracts. Among the tested solvents, MeOH proved to be the most effective for extracting phenolic antioxidants, yielding the highest TPC as well as the greatest antioxidant activity in both the CUPRAC and ABTS assays. In particular, the MeOH extract of the Giresun samples exhibited the highest TPC (324.78 ± 0.87 mg GAE/g dw) and the strongest antioxidant activity (0.234 ± 0.001 mmol TE/g dw by the CUPRAC assay and 0.641 ± 0.003 mmol TE/g dw by the ABTS assay). In contrast, EtOH extracts showed the lowest antioxidant activity, whereas A extracts exhibited intermediate antioxidant potential.

The similar trends observed in the CUPRAC and ABTS assays indicate good agreement between the two analytical methods and further suggest that phenolic compounds contribute substantially to the antioxidant capacity of *P. lanceolata*. Moreover, the generally higher TPC values and antioxidant activities observed in the Giresun samples compared with those from Sakarya highlight the potential influence of geographical origin and environmental conditions on the phytochemical composition of this species.

The antimicrobial assays demonstrated that *P. lanceolata* extracts collected from both regions exhibited broad-spectrum antimicrobial activity against all tested bacterial strains and *C. albicans*. Among the extracts, the EtOH extract showed the strongest antimicrobial and bactericidal activities, whereas the MeOH and A extracts also exhibited considerable inhibitory effects against the tested microorganisms. These findings suggest that *P. lanceolata* represents a promising natural source of antimicrobial compounds with potential applications in phytotherapeutic formulations and natural preservative systems.

Overall, the findings of this study demonstrate that *P. lanceolata* leaves are a rich source of phenolic compounds with remarkable antioxidant and antimicrobial properties. These results support the potential use of this species as a natural antioxidant source for functional foods, phytotherapeutic products, and other value-added applications. Nevertheless, further studies are required to isolate and characterize the individual bioactive constituents responsible for these biological activities, elucidate their mechanisms of action, and confirm their efficacy and safety through appropriate in vivo investigations before clinical or industrial applications can be considered.

4. MATERIALS AND METHODS

4.1. Chemicals

The following analytical-grade chemicals were used in this study: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), neocuproine (2,9-dimethyl-1,10-phenanthroline), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), potassium persulfate ($K_2S_2O_8$), sodium carbonate (Na_2CO_3), gallic acid ($C_7H_6O_5$), methanol (99.9%), ethanol (99%), copper(II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$), potassium dihydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), ammonium acetate (CH_3COONH_4), and acetic acid (CH_3COOH), all purchased from Merck (Darmstadt, Germany). Folin–Ciocalteu reagent (FCR) [$3H_2O \cdot P_2O_5 \cdot 13WO_3 \cdot 5MoO_3 \cdot 10H_2O$] was obtained from Sigma-Aldrich (Steinheim, Germany). All chemicals and reagents used were of analytical grade.

4.2. Instrumentation

The instruments used in this study included a UV–Vis spectrophotometer (Shimadzu UV-2550, Japan), a laboratory oven (Memmert UM 400, Germany), an ultrapure water purification system (NUVE ND 4, Türkiye), an analytical balance (Kern ABJ 220-4NM, Germany), an ultrasonic bath (Bandelin Sonorex, Germany), a vortex mixer (IKA MS2 Minishaker, Germany), a mechanical grinder, and an autoclave (Hirayama HV-85L, Japan).

4.3. Plant Material and Extraction

P. lanceolata L. samples were collected from two different provinces of Türkiye, namely Tirebolu (Giresun Province) and Adapazarı (Sakarya Province), during July and August 2022. The collected plant materials were shade-dried at room temperature, protected from direct sunlight, and stored under appropriate conditions until further processing. Botanical identification was performed by Dr. İsmail Şenkardeş, Department of Pharmaceutical Botany, Faculty of Pharmacy, Marmara University, Türkiye. After taxonomic identification, the leaves were separated from the remaining plant parts, ground into a homogeneous fine powder using a mechanical grinder, and stored in amber glass bottles at room temperature until analysis.

The extraction procedure was carried out according to the method described by Yılmaz et al. (2019), with minor modifications, and was performed in triplicate [28]. Briefly, 0.5 g of powdered leaf sample was transferred into a stoppered Erlenmeyer flask, and 20 mL of MeOH was added. Ultrasound-assisted extraction was performed at 40 °C for 1 h. The extract was subsequently filtered through Macherey-Nagel Blue Ribbon filter paper (Macherey-Nagel, Germany), after which the plant residue was re-extracted with an additional 20 mL of MeOH under identical conditions. The same extraction procedure was applied using EtOH and distilled water as extraction solvents. The resulting extracts were filtered and stored in amber glass bottles at 4 °C until analysis. All analytical results were expressed on a dry weight (dw) basis.

4.4. Antioxidant Activity

4.4.1. ABTS assay

The ABTS radical scavenging activity was determined using a modified method based on those described by Re et al. (1999) and Rice-Evans and Miller (1994) [29, 30]. An ABTS solution (7.0 mM) was prepared and mixed with potassium persulfate ($K_2S_2O_8$) to obtain a final concentration of 2.45 mM. The reaction mixture was kept in the dark at room temperature for 16 h to allow the formation of the $ABTS^{\bullet+}$ radical cation. The resulting $ABTS^{\bullet+}$ solution was diluted 1:50 (v/v) with EtOH, and its absorbance was adjusted to approximately 0.700 at 734 nm.

A calibration curve was constructed using Trolox standard solutions prepared from a 1 mM stock solution. Briefly, 2.00 mL of the $ABTS^{\bullet+}$ solution was mixed with an appropriate volume of Trolox standard solution, and the final volume was adjusted to 3.00 mL with EtOH. After vortex mixing, the reaction mixtures were incubated in the dark at room temperature for 6 min, and the absorbance was measured at 734 nm. The reference solution consisted of 2.00 mL of $ABTS^{\bullet+}$ solution and 1.00 mL of EtOH.

The percentage of ABTS radical scavenging activity was calculated using the following equation:

$$\text{ABTS radical scavenging activity (\%)} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 is the absorbance of the reference solution and A_1 is the absorbance of the Trolox standard or plant extract. The calibration curve was constructed by plotting the percentage inhibition against the Trolox concentration. Plant extracts were analyzed using the same procedure, and their antioxidant capacities were calculated from the calibration curve and expressed as mmol Trolox equivalents (TE)/g dry weight (dw). All measurements were performed in triplicate, and the results are presented as the mean ± standard deviation.

4.4.2. CUPRAC Assay

The cupric ion reducing antioxidant capacity (CUPRAC) assay was performed according to the method described by Apak et al. (2004) [31]. The reaction mixture consisted of 1.00 mL of $CuCl_2$ solution, 1.00 mL of neocuproine (Nc) solution, 1.00 mL of ammonium acetate (CH_3COONH_4) buffer, an appropriate volume of either the Trolox standard solution or plant extract, and ultrapure water to adjust the final volume. After vortex mixing, the reaction mixtures were incubated in the dark at room temperature for 30 min to allow the reduction of the Cu(II)–Nc complex to the Cu(I)–Nc chelate by antioxidant compounds. The absorbance was then measured at 450 nm against a reagent blank.

A calibration curve was constructed using Trolox standard solutions at different concentrations. The antioxidant capacities of the plant extracts were determined under the same experimental conditions and calculated from the calibration curve. The results were expressed as mmol Trolox equivalents (TE)/g dry weight (dw). All measurements were performed in triplicate, and the results are presented as the mean ± standard deviation.

4.4.3. Folin–Ciocalteu Assay

The TPC was determined using the Folin–Ciocalteu method as described by Albayrak et al. (2010) and Magalhães et al. (2008) [32, 33]. Prior to use, the Folin–Ciocalteu reagent was diluted with ultrapure water at a ratio of 1:3 (v/v). A calibration curve was constructed using gallic acid standard solutions of different concentrations prepared from a stock solution in MeOH.

For the analysis, an appropriate volume of either the gallic acid standard solution or plant extract was mixed with ultrapure water and 0.100 mL of the diluted Folin–Ciocalteu reagent. After 3 min, 3.00 mL of 2% (w/v) sodium carbonate (Na_2CO_3) solution was added, and the reaction mixture was incubated in the dark at room temperature for 2 h. The absorbance of the resulting blue-colored solution was measured at 760 nm against a reagent blank.

The TPC of the plant extracts was calculated from the gallic acid calibration curve and expressed as mg gallic acid equivalents (GAE)/g dry weight (dw). All measurements were performed in triplicate, and the results are presented as the mean ± standard deviation.

4.5. Antimicrobial Activity

The antimicrobial activities of the *P. lanceolata* extracts were evaluated using the agar well diffusion and broth microdilution methods. The MIC, MBC, and MFC values of the extracts were determined according to standard microbiological procedures. The test microorganisms included *S. aureus* (ATCC 29213), *S. epidermidis* (ATCC 12228), *P. aeruginosa* (ATCC 27853), *A. baumannii* (ATCC 19606), *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 4352), and the yeast *C. albicans* (ATCC 90028). MHA, CAMHB, tryptic soy agar (TSA), Sabouraud dextrose agar (SDA), and RPMI-1640 medium were used for the antimicrobial assays. Meropenem and amphotericin B were used as the positive control agents for antibacterial and antifungal assays, respectively.

4.5.1. Agar Well Diffusion Assay

The antimicrobial activity of the *P. lanceolata* extracts was initially evaluated using the agar well diffusion method. Bacterial suspensions were prepared from colonies grown on TSA, whereas the yeast suspension was prepared from colonies grown on SDA. The bacterial suspensions were adjusted to approximately 1×10^8 CFU/mL and the yeast suspension to 1×10^6 CFU/mL according to the 0.5 McFarland turbidity standard. The standardized suspensions were evenly spread onto MHA plates for bacteria and SDA plates for yeast under aseptic conditions using sterile cotton swabs. Wells of 5 mm diameter were then punched into the agar using a sterile cork borer.

Subsequently, 50 µL of each plant extract prepared in EtOH, MeOH, and distilled water was added to the respective wells. Meropenem (10 µg/well) and amphotericin B (100 µg/well) were used as positive controls for antibacterial and antifungal assays, respectively, whereas EtOH, MeOH, distilled water, and PSS served as negative controls. The inoculated bacterial plates were incubated at 37 °C for 18–24 h, whereas the

yeast plates were incubated at 35 °C for 24–48 h. After incubation, the diameters of the inhibition zones were measured and expressed in millimeters (mm). All experiments were performed in triplicate [9, 34, 35, 36].

4.5.2. Determination of the Minimum Inhibitory Concentration (MIC) for Bacteria

The MICs of the plant extracts against the bacterial strains were determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) guidelines. Bacterial inocula were prepared from fresh cultures, adjusted to the 0.5 McFarland turbidity standard, and diluted to obtain a final inoculum concentration of 5×10^5 CFU/mL. Briefly, 100 µL of CAMHB was dispensed into each well of a sterile U-bottom 96-well microtiter plate. Subsequently, 100 µL of each plant extract (25 mg/mL) was added to the first well, and two-fold serial dilutions were prepared. Thereafter, 5 µL of the bacterial suspension was added to each well, and the plates were incubated at 37 °C for 24 h. Following incubation, the lowest concentration of the extract showing no visible bacterial growth was recorded as the MIC. CAMHB, EtOH, MeOH, water, and meropenem were included as growth, solvent, and positive controls, respectively [9, 37].

4.5.3. Determination of the Minimum Inhibitory Concentration (MIC) for Yeast

The MIC against *C. albicans* (ATCC 90028) was determined according to the CLSI guidelines. *C. albicans* was cultured on SDA and incubated at 37 °C for 48 h. Subsequently, 100 µL of RPMI-1640 medium was dispensed into each well of a sterile U-bottom 96-well microtiter plate. The plant extracts were serially diluted in RPMI-1640 medium. A yeast suspension was prepared from fresh SDA cultures, adjusted to the 0.5 McFarland turbidity standard (1×10^6–5×10^6 cells/mL), and further diluted with RPMI-1640 medium to obtain a final inoculum concentration of 5×10^2–2.5×10^3 cells/mL. Subsequently, 100 µL of the yeast suspension was added to each well containing the serially diluted extracts. The microplates were incubated at 37 °C for 24–48 h. The plates were evaluated after 24 h and re-examined after 48 h to confirm fungal growth. The lowest concentration of the extract showing no visible yeast growth was recorded as the MIC. RPMI-1640, EtOH, MeOH, water, and amphotericin B were used as the growth, solvent, and positive controls, respectively [38, 39].

4.5.4. Determination of the Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The MBC and MFC values were determined from the wells showing no visible microbial growth in the MIC assay. Briefly, a 5 µL aliquot from each well without visible growth was streaked onto MHA plates for bacteria and SDA plates for yeast. The bacterial plates were incubated at 37 °C for 24 h, whereas the yeast plates were incubated at 37 °C for 48 h. The lowest concentration of the extract at which no microbial growth was observed on the agar plates was recorded as the MBC for bacteria or the MFC for yeast [39, 40].

4.6. Statistical analysis

Statistical analyses were performed using two-way ANOVA followed by Tukey's multiple comparison test. Differences were considered significant at $p<0.05$. All analyses were carried out using triplicate measurements (n=3).

Acknowledgements: This work was supported by the TÜBİTAK 2209-A Undergraduate Research Projects Support Program (Project No. 1919B012205353).

Author contributions: Concept – A.K.C., G.E.; Design – A.K.C., G.E.; Supervision – A.K.C., G.E.; Resources – A.K.C., B. D., P. R., G.E.; Materials – A.K.C., P. R., G.E.; Data Collection and/or Processing – A.K.C., B. D., P. R., G.E.; Analysis and/or Interpretation – A.K.C., P. R., G.E.; Literature Search –A.K.C., B. D., P. R., G.E.; Writing – A.K.C., B. D., P. R., G.E.; Critical Reviews – A.K.C., P. R., G.E.

Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] Baytop T. 1984. Türkiye'de bitkiler ile tedavi (geçmişte ve bugün). İstanbul Üniversitesi Eczacılık Fakültesi Yayınları No: 40, İstanbul, 20: 20-23.

[2] Abate L, Bachheti RK, Tadesse MG, Bachheti A. Ethnobotanical Uses, Chemical Constituents, and Application of *Plantago lanceolata* L. J Chem. 2022; 2022: 1532031. https://doi.org/10.1155/2022/1532031

[3] Fons F, Gargadennec A, Gueiffier A, Roussel JL, Andary C. Effects of cinnamic acid on polyphenol production in *P. lanceolata*. Phytochem. 1998; 49: 697-702. https://doi.org/10.1016/S0031-9422(98)00210-6

[4] Harput US, Genc Y, Saracoglu I. Cytotoxic and antioxidative activities of *Plantago lagopus* L. and characterization of its bioactive compounds. Food Chem Toxicol. 2012; 50: 1554-1559. https://doi.org/10.1016/j.fct.2012.01.019

[5] Davis PH. Flora of Turkey and the East Aegean Islands. Edinburgh University Press Edinburgh. 1982; 7: 513-4.

[6] Bussmann RW, Batsatsashvili K, Kikvidze Z. *Plantago lanceolata* L. *Plantago major* L. Plantaginaceae. In: Batsatsashvili K, Kikvidze Z, Bussmann R, (editörler) Ethnobotany of the Mountain Regions of Central Asia and Altai. Ethnobotany of Mountain Regions. Springer, Cham. 2020, s. 585–593.

[7] Aslankoç R, Demirci D, İnan Ü, Yıldız M, Öztürk A, Çetin M, Savran EŞ, Yılmaz B. The Role of Antioxidant enzymes in oxidative stress- superoxide dismutase (Sod), catalase (Cat) and glutathione peroxidase (Gpx). Med J SDU. 2019; 26(3): 362-369. https://doi.org/10.17343/sdutfd.566969

[8] Wootton-Beard PC, Ryan L. Improving public health? The role of antioxidant-rich fruit and vegetable beverages. Food Res Int. 2011; 44: 3135-3148. https://doi.org/10.1016/j.foodres.2011.09.015

[9] Rayaman P, Şafak S, Rayaman E, Şenkardeş İ, Taşkın T. Investigation of Antimicrobial, Antibiofilm and Antiurease Activities of *Teucrium polium* L. Extracts. Turk J Agric Food Sci Technol. 2025; 13(s2): 3598-3603. https://doi.org/10.24925/turjaf.v13is2.3598-3603.8226

[10] Dessie Y, Amsalu N, Fassil A, Liyew M. Antibacterial Potential of Selected Traditional Medicinal Plants for Wound Healing in Sekela District, Northwestern Ethiopia. J Herbs Spices Med Plants. 2025; 31(2): 113–130. https://doi.org/10.1080/10496475.2024.2439299

[11] Orhan DD, Özçelik B, Hoşbaş S, Vural, M. Assessment of antioxidant, antibacterial, antimycobacterial, and antifungal activities of some plants used as folk remedies in Turkey against dermatophytes and yeast-like fungi. Turk J Biol. 2012; 36 (6): 672-686. https://doi.org/10.3906/biy-1203-33

[12] Beara IN, Lesjak MM, Orcic DZ, Simin ND, Cetojevic-Simin DD, Bozin BN, Mimica-Dukic NM. Comparative analysis of phenolic profile, antioxidant, anti-inflammatory and cytotoxic activity of two closely-related Plantain species: *Plantago altissima* L. and *Plantago lanceolata* L. LWT- Food Sci Technol. 2012; 47: 64-70. https://doi.org/10.1016/j.lwt.2012.01.001

[13] Dalar A, Konczak I. Phenolic contents, antioxidant capacities and inhibitory activities against key metabolic syndrome relevant enzymes of herbal teas from Eastern Anatolia. Ind Crops Prod. 2013; 44: 383-390. https://doi.org/10.1016/j.indcrop.2012.11.037

[14] Akgün B, Genç M, Genç S, Güzelsoy NA, Tosunoglu H, Hamzaoğlu M, Deniz A, Kılınç A, Gökçay RZ, Arıcı M, Tosunoglu A. Impact of Aqueous Extracts of Turkish Wild Edible Plants on Acrylamide Formation in Potato Crisps. J Food Qual Hazards Control. 2022; 9: 64-77. http://jfqhc.ssu.ac.ir/article-1-955-en.html

[15] Kaygısız, A. Yüksek Lisans tezi. Sinirli otu, civanperçemi ve biberiye ekstraktlarının antioksidan aktivitelerinin belirlenmesi. İstanbul Sabahattin Zaim Üniversitesi. İstanbul, Türkiye, 2021.

[16] Ziarno M, Kozłowska M, Ścibisz I, Kowalczyk M, Pawelec S, Stochmal A, Szleszyński B. The effect of selected herbal extracts on lactic acid bacteria activity. Appl Sci. 2021; 11(9): 3898. https://doi.org/10.3390/app11093898

[17] Lukova P, Karcheva-Bahchevanska D, Mollova D, Nikolova M, Mladenov R, Iliev I. Study of prebiotic potential and antioxidant activity in Plantago spp. leaves after enzymatic hydrolysis with hemicellulase and xylanase. Eng Life Sci. 2018; 18(11), 831-839. https://doi.org/10.1002/elsc.201800071

[18] Mazzutti S, Riehl CA, Ibañez E, Ferreira SR. Green-based methods to obtain bioactive extracts from *Plantago major* and *Plantago lanceolata*. J Supercrit Fluids. 2017; 119: 211-220. https://doi.org/10.1016/j.supflu.2016.09.018

[19] Laanet PR, Bragina O, Jõul P, Vaher M. *Plantago major* and *Plantago lanceolata* Exhibit Antioxidant and *Borrelia burgdorferi* Inhibiting Activities. Int J Mol Sci. 2024; 25(13): 7112. https://doi.org/10.3390/ijms25137112

[20] Sanna F, Piluzza G, Campesi G, Molinu MG, Re GA, Sulas L. Antioxidant Contents in a Mediterranean Population of *Plantago lanceolata* L. Exploited for Quarry Reclamation Interventions. Plants 2022; 11: 791. https://doi.org/10.3390/plants11060791

[21] Kurt BZ, Gazioğlu I, Sevgi E, Sönmez F. Anticholinesterase, antioxidant, antiaflatoxigenic activities of ten edible wild plants from Ordu area, Turkey. Iran J Pharm Res. 2018; 17(3): 1047-1056. PMID: 30127827; PMCID: PMC6094434

[22] Bahadori MB, Sarikurkcu C, Kocak MS, Calapoglu M, Uren MC, Ceylan O. *Plantago lanceolata* as a source of health-beneficial phytochemicals: Phenolics profile and antioxidant capacity. Food Biosci. 2020; 34: 100536. https://doi.org/10.1016/j.fbio.2020.100536

[23] Nizioł-Łukaszewska Z, Gaweł-Bęben K, Rybczyńska-Tkaczyk K, Jakubczyk A, Karaś M, Bujak T. Biochemical properties, UV-protecting and fibroblast growth-stimulating activity of *Plantago lanceolata* L. extracts. Ind Crops Prod. 2019; 138: 111453. https://doi.org/10.1016/j.indcrop.2019.06.016

[24] Gajewska S, Siemak J, Bilska J, Nowak A, Klimowicz A. Effect of storage on the antioxidant properties of *Plantago lanceolata* L. and *Plantago major* L. alcoholic extracts. Pomeranian J Life Sci. 2021; 67(4): 52-56. https://doi.org/10.21164/pomjlifesci.759

[25] Işık M, Sümer E. Phytochemical Compositions and Bioactive Properties of *Plantago lanceolata* L.: Anti-oxidant, Anti-diabetic, and Anti-cholinergic Potentials. J Oleo Sci. 2025; 74(8): 721-728. https://doi.org/10.5650/jos.ess25104

[26] Kassaw E, Yohannes T, Bizualem E. In vitro antibacterial activity of *Plantago lanceolata* against some selected standard pathogenic bacterial. Int J Biotechnol. 2018; 7: 44-50. https://doi.org/10.3389/fmicb.2026.1815167

[27] Yiğit D, Yiğit N, Özgen U. An investigation on the anticandidal activity of some traditional medicinal plants in Turkey. Mycoses. 2009; 52: 135-140. https://doi.org/10.1111/j.1439-0507.2008.01552.x

[28] Yılmaz DÇ, Özdoğan O, Bulut G, Seyhan SA. İki kekik türünün (Thymbra spicata var. spicata ve Origanum onites) antioksidan aktivitelerinin karşılaştırılması. IJEASED. 2019; 1(2): 296-306. https://izlik.org/JA98YB33GS

[29] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999; 26(9-10): 1231-1237. https://doi.org/10.1016/s0891-5849(98)00315-3

[30] Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. In Methods in enzymology. Academic Press. 1994, s.279-293.

[31] Apak R, Güçlü K, Özyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J Agric Food Chem. 2004; 52(26): 7970-7981. https://doi.org/10.1021/jf048741x

[32] Albayrak S, Sağdıç O, Aksoy A. Bitkisel ürünlerin ve gıdaların antioksidan kapasitelerinin belirlenmesinde kullanılan yöntemler. Erciyes Üniv Fen Bilim Enst Derg. 2010; 26(4): 401-409.

[33] Magalhães LM, Segundo MA, Reis S, Lima JL. Methodological aspects about in vitro evaluation of antioxidant properties. Anal Chim Acta. 2008; 613(1), 1-19. https://doi.org/10.1016/j.aca.2008.02.047

[34] Perez C, Pauli M, Bazerque P. An antibiotic assay by the agar-well diffusion method. Acta Biol Med Exp. 1990; 15: 113-115.

[35] Rex JH, Pfaller MA, Rinaldi MG, Polak A, Galgiani JN. Antifungal susceptibility testing. Clin Microbiol Rev. 1993; 6(4): 367-381.

[36] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016; 6(2): 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

[37] Clinical and Laboratory Standards Institute (CLSI), Performance Standarts for Antimicrobial Susceptibility Testing. 30th Ed, January CLSI supplement M100 Wayne, PA: Clinical and Laboratory Standarts Institue; 2020. https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf

[38] Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antifungal Susceptibility Testing of Yeasts, 3rd ed.; CLSI Supplement M27M44S.; Wayne, PA, USA, 2022.

[39] Yilmaz B, Deniz NG, Sayil C, Ozakpinar OB, Gurboga M, Sekerler T, Rayaman P, Rayaman E, Salihi EC. Synthesis, Molecular Docking, and Biological Studies of New Naphthalene-1,4-Dione-Linked Phenylpiperazine and Thioether. Chem Biodivers. 2025; 22(11): e00774. https://doi.org/10.1002/cbdv.202500774

[40] Kebede B, Shibeshi W. In vitro antibacterial and antifungal activities of extracts and fractions of leaves of Ricinus communis Linn against selected pathogens. Vet Med Sci. 2022; 8(4): 1802-1815. https://doi.org/10.1002/vms3.772