

Antimicrobial activity of ethanolic and aqueous extracts of *Urtica dioica*, *Mentha piperita*, *Plantago lanceolata* and *Cynara scolymus* leaves

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Natural antimicrobial agents, particularly plant extracts from *Urtica dioica*, *Mentha piperita*, *Plantago lanceolata* and *Cynara scolymus*, have attracted considerable attention due to their broad biological activity driven by diverse bioactive compounds and synergistic effects. The efficacy of these extracts depends on plant species, chemical composition, extraction method, and solvent, emphasizing the need for systematic evaluation of ethanolic and aqueous extracts as potential alternatives to synthetic antimicrobials. The aim of this study was to determine and compare the antimicrobial activity of ethanolic and aqueous leaf extracts of *Urtica dioica*, *Mentha piperita*, *Plantago lanceolata* and *Cynara scolymus*. Reliability was ensured through triplicate testing and standardised methodology (well diffusion method, 100 mg/ml concentration), confirming that the results are representative and statistically valid. Findings indicate that ethanolic extracts demonstrated significantly stronger antimicrobial activity than aqueous extracts ($p < 0.05$), particularly against Gram-positive bacteria (*Staphylococcus epidermidis*, *Bacillus cereus*, *Enterococcus faecalis*) and the fungus *Candida albicans*, while Gram-negative bacteria (*Salmonella enteritidis*, *Yersinia enterocolitica*) were largely resistant except *Pseudomonas aeruginosa*. *Mentha piperita* and *P. lanceolata* ethanolic extracts exhibited the broadest and most potent antimicrobial spectrum, *U. dioica* and *C. scolymus* leaves showed weaker activity but remain valuable sources of bioactive compounds.

Keywords: *Urtica dioica*, *Mentha piperita*, *Plantago lanceolata*, *Cynara scolymus*, ethanolic extracts, aqueous extracts, antimicrobial activity

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INTRODUCTION

In recent decades, natural antimicrobial agents have attracted considerable attention as a promising area for the search for effective and safe alternatives to synthetic antimicrobial agents. Extracts from plants such as *Urtica dioica*, *Mentha piperita*, *Plantago lanceolata*, and *Cynara scolymus* have been extensively studied due to their broad spectrum of biological activity, which is associated with the diversity of bioactive compounds they contain and their synergistic interactions (Paulauskienė et al., 2021; Repajić et al., 2021). The quality and concentration of these bioactive components are influenced by various factors, including plant species, genotype, habitat, climatic conditions, and vegetative maturity stage (Jaiswal et al., 2023). As a result, differences in these parameters lead to significant differences in the phytochemical composition and, consequently, the biological efficacy of plant extracts (Errington et al., 2020; Grauso et al., 2020). *Urtica dioica* is one of the most extensively studied medicinal plants, notable for its high content of phenolic compounds, flavonoids, tannins, volatile compounds, fatty acids, polysaccharides, sterols, terpenes, proteins, vitamins (C, B, K), and minerals (calcium, iron, magnesium, phosphorus, potassium, sodium) (Abi Sleiman et al., 2024). These bioactive constituents have been associated with antioxidant, anti-inflammatory, hepatoprotective, and antimicrobial activities. The antimicrobial effects are largely attributed to the ability of flavonoids to disrupt microbial cell walls and membranes (Grauso et al., 2020). Empirical studies have demonstrated the activity of nettle extracts against *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella* spp., and *Candida albicans* (Harrison et al., 2022; Hashem et al., 2022). *Mentha piperita* is an aromatic and medicinal plant whose chemical composition and therapeutic properties are primarily determined by terpenoids, flavonoids and their glycosides, steroids, and phenolic acids present in its essential oils. The plant is also rich in minerals such as calcium, magnesium, zinc, manganese, iron, and phosphorus

(Abbas et al., 2022; Hudz et al., 2023). *Mentha piperita* exhibits its antioxidant, anti-inflammatory, antiallergic, antiviral, antidiabetic, and antimicrobial activities (Chakraborty et al., 2022; Tafrihi et al., 2021). Antimicrobial studies highlight the efficacy of *Mentha piperita* extracts against *K. pneumoniae*, *E. coli*, *S. aureus* and *C. albicans* (Gariya et al., 2020). *Plantago lanceolata* is recognised for its substantial content of phenolic compounds, iridoid glycosides, and triterpenoid acids. Phenolic compounds contribute to its significant antioxidant potential, while iridoids are primarily responsible for its anti-inflammatory activity (Abate et al., 2022). Experimental studies have demonstrated the antimicrobial activity of *P. lanceolata* extracts against *E. coli*, *S. boydii*, *K. pneumoniae* and *C. albicans*, which varies depending on the solvent and extract concentration (Pol et al., 2021; Abate et al., 2022). *Cynara scolymus* is widely used for its hepatoprotective, antihypercholesterolemic, and antioxidant effects, largely attributed to flavonoids, caffeoylquinic acids, and other phenolic compounds (Sharma et al., 2021; Porro et al., 2024). Antimicrobial studies have shown that *C. scolymus* extracts exhibit activity against *S. aureus*, *E. coli*, *Salmonella* spp., *P. aeruginosa*, and *B. cereus* (Mohamed et al., 2021). The biological activity of different plant extracts is determined not only by their chemical composition but also by the extraction method and the solvent employed. The polarity of the solvent influences the spectrum of compounds obtained: polar solvents, such as water and ethanol, enable the extraction of polar bioactive compounds; however, their efficacy may vary due to differences in solubility, stability, or antibacterial properties. Given the increasing prevalence of antibiotic-resistant microorganisms and the urgent need for effective natural alternatives, it is important to systematically evaluate the antimicrobial activity of ethanol and aqueous extracts from *U. dioica*, *M. piperita*, *P. lanceolata*, and *C. scolymus*. The bioactive properties of these plants could expand the spectrum of natural antibacterial agents and provide additional opportunities for their application, especially considering that

comprehensive studies on their antimicrobial activity have not yet been conducted in Lithuania. Further research may reveal new potential applications for these plants.

The aim of this study was to determine and compare the antimicrobial activity of ethanol and aqueous leaf extracts of *Urtica dioica*, *Mentha piperita*, *Plantago lanceolata* and *Cynara scolymus* leaves.

MATERIALS AND METHODS

A quantitative research strategy was selected to evaluate antimicrobial activity. Data were collected through a microbiological laboratory study assessing the antimicrobial effects of aqueous and ethanolic leaf extracts of *U. dioica*, *M. piperita*, *P. lanceolata*, and *C. scolymus* leaves against twelve microbial strains with distinct characteristics (*S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *S. epidermidis* ATCC 23624, *C. albicans* ATCC 10231, *B. cereus* ATCC 11778, *K. pneumoniae* ATCC 13883, *Y. enterocolitica* ATCC 23715, *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *S. enteritidis* ATCC 13076, *B. subtilis* ATCC 6633, *P. mirabilis* ATCC 6633). *Urtica dioica* and *M. piperita* leaves were purchased from X pharmacy, while *C. scolymus* and *P. lanceolata* leaves were obtained from X herbal store. The study was repeated three times. Antimicrobial activity of ethanolic and aqueous extracts of *U.*, *M. piperita*, *P. lanceolata* and *C. scolymus* leaves against the 12 reference microbial strains was evaluated using a microbiological laboratory protocol, recording three inhibition zones per test, which were subsequently averaged by plant type, extract type, and microorganism species.

Preparation of extracts. Aqueous plant leaf extracts were prepared by infusion following the recommendations indicated on the plant packaging. The required amounts were 3.5 g of *U. dioica*, 1.5 g of *M. piperita* leaves, and 2 g each of *P. lanceolata* and *C. scolymus* leaves. These amounts were poured into 200 mL of boiling water, and extraction proceeded for 20 min. Each extract was filtered through a sterile filter into sterile flasks and filled up to the original sterile water volume before filtration, then

sealed. Tinctures were prepared by maceration at a 10:100 ratio, in which 10 g of plant leaves were poured with 100 mL of 70% ethanol. Extracts were stored in glass containers sealed with sterile glass stoppers and left to macerate for 24 h in a dark, sun-protected place. After 24 h, each extract was filtered through a sterile filter into sterile flasks. The filtrates were then topped up with 70% ethanol to restore the initial volume, maintaining a uniform extract concentration. A rotary evaporator was used to remove the solvent and concentrate the extracts. As evaporator parameters depend on the specific solvent, the Buchi 20/40/60 rule was applied (University of Wollongong, 2010), which specifies a water bath temperature of 60°C, condenser water temperature of approximately 20°C, and vacuum set to achieve a boiling temperature of 40°C for the solvent. Each filtered plant extract was placed in the rotary evaporator flask, secured with a plastic clamp, and the water bath temperature set to 60°C. The flask was submerged in the water bath, the vacuum pump activated, and pressure adjusted according to the solvent: 175 mbar for ethanol extracts and 72 mbar for aqueous extracts. The flask was rotated at 200 rpm, and condenser water was turned on. During evaporation, solvents boiled off and condensed into the receiving flask, with extracts concentrated to a constant volume.

Preparation of extract concentrations. For this study, an extract concentration of 100 mg/ml was selected. Before evaporation, the empty rotary evaporator flask was weighed; after evaporation, the flask with the extract residue was weighed again. The initial flask weight was subtracted, and the required amount of 10% dimethyl sulfoxide (DMSO) to achieve a 100 mg/ml extract concentration was calculated proportionally. Once the solvent had evaporated, the extract residue was dissolved in 10% DMSO. Previous studies have shown that DMSO lacks antimicrobial properties and is suitable for use as a negative control (Rahmi et al., 2020; Saral et al., 2019).

Preparation of reference microbial cultures. A total of six Gram-positive bacteria (*S. aureus* ATCC 25923, *S. epidermidis* ATCC

23624, *B. cereus* ATCC 11778, *E. faecalis* ATCC 29212, *B. subtilis* ATCC 6633, *P. mirabilis* ATCC 6633), five Gram-negative bacteria (*P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 13883, *Y. enterocolitica* ATCC 23715, *E. coli* ATCC 25922, *S. enteritidis* ATCC 13076), and one fungal species (*C. albicans* ATCC 10231) were selected (Biometrija, Vilnius, Lithuania). These microorganisms were chosen due to their distinct structural characteristics and their frequent occurrence in the environment and the human body. Microbial suspensions were prepared from pure cultures using physiological saline (0.9% NaCl). The turbidity of the suspensions was measured using a McFarland standard indicator and adjusted to 0.5 (corresponding to a bacterial concentration of 1.5×10^8 CFU/ml). Culture media were prepared according to the manufacturer's instructions. Mueller–Hinton agar (Mueller-Hinton agar (CM 0337) Oxoid Ltd, Basingstoke, Hampshire, England) was boiled and dispensed into Petri dishes (40 ml per plate). The media were allowed to solidify, after which four wells with a diameter of 6 mm were made in each plate. A sterile swab was immersed in the microbial suspension and pressed against the wall of the test tube to remove excess liquid. The agar surface was then uniformly inoculated using the swab. Subsequently, 0.1 ml of different plant extracts was added to each well. A 10% DMSO solution was used as a negative control. Finally, the Petri dishes containing the extracts and inoculated microorganisms were incubated at 36°C for 24 h. After incubation, the diameters of the inhibition and sterile zones formed around each well were measured using a ruler.

RESULTS AND DISCUSSION

The antimicrobial activity of aqueous (A) and ethanolic (E) extracts of *U. dioica*, *M. piperita*, *P. lanceolata*, and *C. scolymus* leaves was evaluated against a panel of gram-positive and gram-negative bacteria as well as *C. albicans*. The results, expressed as mean inhibition zone diameters (mm) \pm standard deviation, are presented in the Table.

Overall, ethanolic extracts exhibited stronger antimicrobial activity than aqueous extracts, forming larger inhibition zones against most tested microorganisms. This trend confirms that ethanol is a more efficient solvent for extracting antimicrobial phytochemicals such as phenolic compounds, flavonoids and terpenoids. Among all tested plants, *M. piperita* ethanolic extract demonstrated the broadest antimicrobial spectrum and the highest inhibitory activity, serving as a reference for statistical comparison. *Urtica dioica* showed limited antimicrobial activity. The aqueous extract inhibited only *B. cereus* (7.6 ± 0.6 mm) and *P. aeruginosa* (15.0 ± 6.2 mm), while the ethanolic extract was active against *B. cereus* (9.3 ± 0.6 mm), *P. aeruginosa* (17.3 ± 6.7 mm) and *E. faecalis* (11.0 ± 0.0 mm). No inhibitory effect was observed against *S. epidermidis*, *E. coli*, *S. aureus*, *C. albicans*, or enteric pathogens. When compared with *Mentha piperita*, *Urtica dioica* aqueous and ethanolic extracts generally produced significantly smaller inhibition zones ($p < 0.05$), highlighting their weaker antimicrobial potential. These findings partially agree with previous studies reporting the antimicrobial properties of nettle; however, the lack of activity against *S. aureus* and *E. coli* suggests that extraction conditions, plant material variability or compound concentration may strongly influence antimicrobial outcomes (Harrison et al., 2022; Hashem et al., 2022). However, in the present study, nettle extracts did not exhibit inhibitory activity against *S. aureus* or *E. coli*, which may be attributed to differences in extraction conditions, extract concentration, and the timing of plant material collection (Paulauskienė et al., 2021). Previous studies emphasise that the antimicrobial activity of nettle is largely determined by the content of flavonoids and phenolic compounds, which varies depending on the plant's vegetative stage (Repajić et al., 2021; Grauso et al., 2020). *Plantago lanceolata* extracts exhibited moderate to strong antimicrobial activity, particularly in ethanolic form. The ethanolic extract showed very strong inhibition against *E. faecalis* (27.0 ± 0.0 mm), significantly exceeding most other plant extracts and

Table. Comparison of the antimicrobial activity of aqueous and ethanolic extracts of *U. dioica*, *M. piperita*, *P. lanceolata* and *C. scolymus* leaves against microorganisms, expressed as the mean diameter of inhibition zones, mm

Microorganism	<i>U. dioica</i>		<i>P. lanceolata</i>		<i>C. scolymus</i>		<i>M. piperita</i>	
	A	E	A	E	A	E	A	E
Inhibition zone diameter ± standard deviation (mm) from three independent experiments								
<i>S. epidermidis</i>	–	–	12.0 ± 2.0	12.0 ± 2.0*	11.7 ± 1.5	13.0 ± 2.9*	12.3 ± 1.5	24.0 ± 1.0
<i>E. coli</i>	–	–	9.3 ± 1.2	11.0 ± 1.7	–	–	10.3 ± 2.1	12.0 ± 3.5
<i>P. mirabilis</i>	–	–	–	12.3 ± 1.2	–	–	9.7 ± 0.6	13.0 ± 0.0
<i>S. aureus</i>	–	–	9.3 ± 1.2**	11.3 ± 1.5*	10.0 ± 1.7	–	9.7 ± 0.6	12.3 ± 2.3
<i>B. cereus</i>	7.6 ± 0.6**	9.3 ± 0.6*	8.0 ± 1.0**	10.7 ± 0.6*	9.3 ± 0.6**	11.3 ± 1.5*	10.3 ± 0.6	14.3 ± 1.2
<i>P. aeruginosa</i>	15.0 ± 6.2	17.3 ± 6.7	11.6 ± 1.2	13.6 ± 2.3	10.0 ± 1.0	11.0 ± 3.6	16.0 ± 0.0	18.0 ± 5.2
<i>C. albicans</i>	–	–	–	20.0 ± 0.0	–	14.3 ± 0.6*	–	20.7 ± 1.2
<i>Y. enterocolitica</i>	–	–	–	10.0 ± 0.0	–	–	–	11.0 ± 0.0
<i>S. enteritidis</i>	–	–	–	9.3 ± 0.6	–	–	–	10.3 ± 0.6
<i>E. faecalis</i>	–	11.0 ± 0.0*	–	27.0 ± 0.0*	–	24.3 ± 0.6*	–	21.7 ± 0.6
<i>K. pneumoniae</i>	–	–	–	–	–	–	–	–
<i>B. subtilis</i>	–	–	–	–	–	–	–	–

A – aqueous extract; E – ethanolic extract

□ Bactericidal activity ■ Bacteriostatic activity

* $p < 0.05$, a statistically significant difference in the mean inhibitory zones was determined by comparing the ethanolic extracts of various leaves with the ethanolic extract of *Mentha piperita* leaves.

** $p < 0.05$, a statistically significant difference in the mean inhibitory zones was determined by comparing the aqueous extracts of various leaves with the aqueous extract of *Mentha piperita* leaves.

comparable to peppermint ethanolic extract ($p < 0.05$). Strong antifungal activity was also observed against *C. albicans* (20.0 ± 0.0 mm). Additionally, *P. lanceolata* extracts inhibited *S. epidermidis*, *E. coli*, *P. mirabilis*, *S. aureus*, *B. cereus*, *P. aeruginosa*, *Y. enterocolitica* and *S. enteritidis*, indicating a broad antimicrobial spectrum. Aqueous extracts were consistently less effective than ethanolic ones, though they still demonstrated measurable activity against several gram-positive and gram-negative bacteria. When comparing the obtained results with the literature, other authors have reported that *P. lanceolata* extracts exhibit antimicrobial activity against *E. coli*, *S. aureus*, and *P. mirabilis* (Pol et al., 2021; Abate et al., 2022). However, Alemu et al. (2014) indicated that in their study, the highest antimicrobial effect against *C. albicans* was observed using an aqueous *P. lanceolata* extract. In contrast to these findings, the present microbiological study demon-

strated that the ethanolic *P. lanceolata* extract exhibited strong antimicrobial activity against *C. albicans*, with an average inhibition zone of 20 mm, while the aqueous extract showed no inhibitory effect (Alemu et al., 2014). These discrepancies may be attributed to differences in the extraction method, solvent properties, and the concentration of bioactive compounds, which collectively influence the antimicrobial potential of the extracts (Pol et al., 2021; Abate et al., 2022). *Cynara scolymus* extracts showed selective and moderate antimicrobial activity. The ethanolic extract demonstrated strong inhibition against *E. faecalis* (24.3 ± 0.6 mm), while the aqueous extract exhibited notable antifungal activity against *C. albicans* (14.3 ± 0.6 mm). Activity against *S. epidermidis*, *S. aureus*, *B. cereus*, and *P. aeruginosa* was present but weaker compared to peppermint and plantain extracts. No inhibitory effect was detected against *E. coli*, *P. mirabilis*, *Y. enterocolitica*, or *S. enteritidis*,

suggesting limited effectiveness against certain gram-negative bacteria. Statistically significant differences ($p < 0.05$) compared with peppermint extracts further confirm the comparatively lower antimicrobial potency of artichoke leaves. These findings are consistent with the literature, which describes artichoke (*C. scolymus*) as containing phenolic compounds and quercetin that confer antibacterial activity (Sharma et al., 2021; Porro et al., 2024; Mohamed et al., 2021). Phenolic compounds and caffeoylquinic acids can disrupt bacterial cell walls, while flavonoids may interfere with protein functions. *Mentha piperita* extracts exhibited the strongest and most consistent antimicrobial activity among all tested plants. The ethanolic extract produced the largest inhibition zones against *S. epidermidis* (24.0 ± 1.0 mm), *B. cereus* (14.3 ± 1.2 mm), *P. aeruginosa* (18.0 ± 5.2 mm), *C. albicans* (20.7 ± 1.2 mm) and *E. faecalis* (21.7 ± 0.6 mm). Activity was also observed against enteric pathogens (*Y. enterocolitica* and *S. enteritidis*), which were largely resistant to other plant extracts. Aqueous *M. piperita* extracts were also active, particularly against *P. aeruginosa* (16.0 ± 0.0 mm) and *S. epidermidis* (12.3 ± 1.5 mm), confirming peppermint's high intrinsic antimicrobial potential. Due to this consistent efficacy, *M. piperita* ethanolic and aqueous extracts were used as reference standards for statistical comparisons in this study. The results of other studies partially align with those of the present study, as *S. aureus*, *E. coli*, *E. faecalis* and *C. albicans* were found to be sensitive to *M. piperita*. Differences arise regarding the susceptibility of *B. subtilis* and *K. pneumoniae*, as some authors report that these microorganisms are significantly sensitive to *M. piperita* extracts (Gariya et al., 2020; Tafrihi et al., 2021).

CONCLUSIONS

Ethanolic extracts demonstrated significantly stronger antimicrobial activity than aqueous extracts, particularly against gram-positive bacteria (*S. epidermidis*, *B. cereus*, *E. faecalis*) and the fungus *C. albicans*, while gram-negative

bacteria (*S. enteritidis*, *Y. enterocolitica*) were largely resistant, except *P. aeruginosa*. *Mentha piperita* and *P. lanceolata* ethanolic extracts exhibited the broadest and most potent antimicrobial spectrum, whereas *U. dioica* and artichoke *C. scolymus* showed weaker activity but remain valuable sources of bioactive compounds.

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ETANOLINIŲ IR VANDENINIŲ DIDŽIOSIOS DILGĖLĖS, PIPIRMĖTĖS, GYSLOČIO IR AR- TIŠOKO LAPŲ EKSTRAKTŲ ANTIMIKROBI- NIS AKTYVUMAS

Santrauka

Natūralūs antimikrobiniai agentai, ypač augalų ekstraktai iš *Urtica dioica*, *Mentha piperita*, *Plantago lanceolata* ir *Cynara scolymus*, sulaukia daug dėmesio dėl plačios biologinės veiklos, kurią lemia įvairūs bioaktyvūs junginiai ir jų sinerginis poveikis. Šių ekstraktų efektyvumas priklauso nuo augalo rūšies, cheminės sudėties, ekstrahavimo metodo ir tirpiklio, todėl būtina sistemingai įvertinti etanolinių ir vandeninių ekstraktų antimikrobinį aktyvumą kaip potencialią alternatyvą sintetinėms medžiagoms. Tyrimo tikslas – nustatyti ir palyginti *Urtica dioica*, *Mentha piperita*, *Plantago lanceolata* ir *Cynara scolymus* lapų etanolinių ir vandeninių ekstraktų antimikrobinį aktyvumą. Tyrimo patikimumą užtikrino

trys pakartojimai ($N = 288$ mėginiai) ir standartizuota metodika (šulinėlio difuzijos metodas, mg/ml koncentracija), todėl gauti rezultatai yra reprezentatyvūs ir statistiškai pagrįsti. Etanoliniai tirčių augalų ekstraktai pasižymėjo reikšmingai stipresniu antimikrobinium aktyvumu nei vandeniniai ($p < 0,05$) prieš gramteigiamas bakterijas (*S. epidermidis*, *B. cereus*, *E. faecalis*) ir grybus *C. albicans*, o gramneigiamos bakterijos (*S. enteritidis*, *Y. enterocolitica*) buvo labiausiai atsparios, išskyrus *P. aeruginosa*. Pipirmėtės (*Mentha piperita*) ir siauralapio gysločio (*Plantago lanceolata*) etanolinių ekstraktų antimikrobinis spektras buvo plačiausias ir stipriausias, o dilgėlės (*Urtica dioica*) ir artišoko (*Cynara scolymus*) ekstraktai turėjo silpnesnį poveikį, tačiau išlieka vertingi bioaktyvių junginių šaltiniai.

Reikšminiai žodžiai: *Urtica dioica*, *Mentha piperita*, *Plantago lanceolata*, *Cynara scolymus*, etanoliniai ekstraktai, vandeniniai ekstraktai, antimikrobinis aktyvumas