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LC-MS/MS analysis, antimicrobial and antioxidant potential of phenolic extracts derived from *Urtica dioica* leaves and roots

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Abstract: This research examined the antimicrobial and antioxidant properties of phenolic compounds from *Urtica dioïca*, a plant widely used in traditional medicine. The total polyphenol content in the leaves and roots were 5.37mg EAG/g and 1.21 mg EAG/g, respectively, while the flavonoid content was 0.40 mg QAE/g (leaves) and 0.19 mg QAE/g (roots). Using LC-MS/MS, 22 compounds were identified in the roots and 27 in the leaves, with gallic acid and 4-methylguaiacol found predominantly in both extracts. Unique to the leaf extract were Phenylethyl Ester and 3,5-Dimethoxyphenol. The extracts were tested against bacterial strains including *Escherichia coli, Klebsiella, Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa*, and *Proteus mirabilis*, all showing sensitivity. MIC values ranged from 0.07 to 0.15mg/ml and MBC values ranged from 0.7 to 0.30mg/ml, indicating primarily bactericidal activity. Additionally, anti-free radical tests using DPPH showed 80.54% inhibition for the leaf extract and 49.23% for the root extract. This study highlights the significant antimicrobial and antioxidant properties of *Urtica dioica*, supporting its traditional medicinal use and suggesting its potential in developing novel therapies.

Keywords: *Urtica dioica* - Phenolic extracts - phytochemical screening- antioxidant activity-antibacterial activity.

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INTRODUCTION

Urinary tract infections (UTIs) have emerged as a significant public health concern, affecting a substantial number of individuals, particularly women, who are at risk of recurrent infections and potential complications (Czajkowski *et al.*, 2021). Urinary tract infections arise when bacteria infiltrate the urinary tract, causing an inflammatory response in the urothelium (Ezugwu *et al*., 2021). In some cases, UTIs may lead to infection-induced lithiasis, characterised by the formation of solid, insoluble deposits within the excretory system due to bacterial urease activity (Wagenlehner *et al*., 2020). Hormonal changes are a primary factor in the increased prevalence of urinary tract infections among perimenopausal, postmenopausal and pregnant women (Czajkowski *et al.*, 2021). To prevent these infections, researchers have studied the potential of cranberries. Additionally, the risk factors associated with UTIs in pregnant women have been comprehensively reviewed, highlighting their increased susceptibility. Symptomatic urinary tract infection (UTI) during pregnancy is associated with preterm delivery (Baer *et al*., 2021). Moreover, current research reveals varying definitions of UTIs, indicating a need for standardised criteria in future studies (Tullus and Shaikh, 2020). The successful eradication of infections hinges on the administration of appropriate and sufficiently prolonged antibiotic therapy to ensure complete sterilisation of the urinary tract. The introduction of antibiotics has revolutionised the treatment of infectious diseases; however, the rise of antibiotic resistance poses a significant threat to global health. This challenge is underscored by reports of increasing resistance among microbial isolates from patients in various regions, emphasising the need for new strategies to manage UTIs in the era of antibiotic resistance (Chraibi *et al*., 2021).

Infections caused by antibiotic-resistant bacteria pose significant challenges and have emerged as a major public health concern (Tse Sum Bui *et al*., 2022). In response, there is growing interest in exploring naturally occurring substances with anti-infective properties. These substances, which include a diverse array of secondary metabolites, possess the ability to inhibit or impede bacterial growth and exert their effects in a targeted manner without adverse side effects. The rising challenge of infections from antibiotic-resistant bacteria in low- and middle-income countries underscores the urgency of developing new therapeutic strategies. Advances in

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biofunctionalization of materials and the use of functional nanomaterials in antimicrobial therapy offer promising perspectives for addressing antibiotic resistance.

Natural substances, including those derived from plants, represent a vast reservoir of bioactive molecules. These compounds often exhibit unique structures that are challenging and costly to synthesise artificially. The significance of bioactive compounds for human and planetary health is increasingly recognised, highlighting their potential in various applications. Additionally, researchers have turned attention to the biomedical applications of endophytic fungi, as these organisms produce a range of promising molecules (Perumal *et al.*, 2023).

The Algerian population traditionally uses a variety of aromatic plants for medicinal purposes (Ayari-Guentri *et al*., 2022). Our research specifically focused on *Urtica dioïca* L., commonly known as nettle, which is a native plant belonging to the Urticaceae family (Taheri *et al*., 2022). Nettle has a long history of traditional use in treating various health conditions.

Numerous studies have demonstrated that *Urtica dioïca* possesses a broad spectrum of pharmacological effects, including antiviral, antimicrobial, antioxidant, anti-inflammatory, and analgesic properties (Grauso *et al*., 2020; Taheri *et al*., 2022), among others. Nettle plant extracts are also employed as natural reducing agents in various steps of nanoparticle synthesis (Vardatsikos *et al*., 2013). While chemotherapy drugs can be effective, they may cause adverse side effects. In contrast, natural products like nettle exhibit a biological balance and fewer side effects due to their limited accumulation in the body. Additionally, nettle is used in treating various urinary, bladder and kidney diseases. The diverse activities of nettle can be attributed to its chemical composition, which possesses various properties and employs novel mechanisms of action against pathogenic microbes (Dhouibi *et al*., 2020).

In line with this objective, our research aims to analyse both the aerial and underground parts of *Urtica dioica* to identify the polyphenols, flavonols and phenolic acids using high-performance liquid chromatography coupled with mass spectrometry (LC-MS/MS). We anticipate that the results will demonstrate *in vitro* an enhancement in the antioxidant and antimicrobial potency of the methanolic extract derived from the leaves and roots of *U. dioica*. By doing so, we seek to minimise reliance on synthetic chemicals and address the challenges posed by microbial resistance, which predominantly affects human health.

MATERIALS AND METHODS

Material

Preparation of nettle extract

The plant specimen for the study was collected from the Mostaganem region and their identification was verified by Professor Rabeh CHADLI, botanist at the University of Mostaganem. The extraction of solutes was conducted in the biochemistry laboratory attached to the faculty of sciences and technologies. To do this, the fresh leaves and roots of *U. dioica* were carefully dried in the shade, away from direct sunlight, at room temperature (17-19°C) for fifteen days. Once completely dried, they were finely powdered using an electric blender and stored in labeled airtight bottles. To prepare the extract, the dried leaves and roots were subjected to extraction with pure methanol. This process was carried out in a water bath at 60°C for the duration of 20 min. The solvent was then removed using a rotavapor at a temperature of 40°C (Sujith *et al.*, 2011). The extract obtained was preserved at 4°C to maintain its stability. To dilute the extract, the procedure outlined by (Valverde *et al*., 2023) was adopted. Specifically, the methanol extract was mixed with dimethyl sulfoxide (DMSO) to achieve a concentration of 2%, yielding a 30% stock solution.

Colorimetric determination of phenolic compounds

Determination of total phenolic compounds (TPC)

Colorimetry was conducted following the protocol described by (Adane *et al*., 2023) utilising the Folin-Ciocalteu reagent. Methanolic leaf extract (30 µl) was mixed with 2.5ml of a tenfold diluted Folin-Ciocalteu reagent in test tubes. After 3 minutes, 2 ml of a sodium carbonate solution (75 g/l) was added. The mixture was then kept in darkness at room temperature for 30 minutes and subsequently analysed spectrophotometrically at a wavelength of 725 nm. The findings were quantified as milligrams of gallic acid equivalent per gram of plant material (mg GAE/g MV) by extrapolating data from a gallic acid calibration curve.

Determination of total flavonoids (TFC)

The determination of flavonoid content followed the protocol outlined by Dowd and endorsed by (Utami *et al.*, 2024). One millilitre of extract was combined with 1 ml of 2% aluminum chloride, followed by stirring and incubation in darkness for 10 minutes. The absorbance was then measured at 415 nm. The flavonoid content was quantified as milligrams of quercetin equivalent per gram of plant material (mg EQ/g MV).

LC-MS/MS Analysis of U. dioica Methanol Extract

In the analysis of *Urtica dioica* phytochemicals, a precise LC-MS/MS method is employed. initially, a methanol extracts of the plant is prepared, diluted to 1000mg/L and filtered using a 0.2mm syringe filter. The analytical system comprises a UHPLC Nexera connected to an 8040 triple quadrupole mass spectrometer, incorporating a degasser, dual pumps, a column oven and an autosampler. The chromatographic separation occurs on a C18 column with specific dimensions and conditions, using a mobile phase composition that includes water, ammonium formate, and formic acid in different concentrations and methanol. A detailed gradient program controls the

percentage of solvent B in the mobile phase, crucial for the efficient elution of phytochemicals. The mass spectrometry analysis employs ESI in both positive and negative modes, with specific operational parameters for optimal ionisation. The MRM mode is crucial for the quantification process, ensuring the selection of characteristic transitions for each phytochemical. The method's robustness is validated through rigorous testing for linearity, accuracy, precision and detection limits, ensuring reliable performance. This comprehensive approach allows for the detailed profiling of up to 27 phytochemicals in *Urtica dioica* highlighting the plant's medicinal and nutritional significance. The optimised LC-MS/MS method stands out for its precision, sensitivity, and reliability in phytochemical analysis.

Preparation of test microorganisms and detection of mic and mbc by broth micro dilution test

To assess antimicrobial activity, this study employed standard strains from the American Type Culture Collection (ATCC) and clinical isolates from the hygiene laboratory of Hassi Mamache Mostaganem. The reference strains included *Escherichia coli* (ATCC 25922), *Klebsiella* (ATCC 60703), *Staphylococcus aureus* (ATCC 25923), *Candida albicans* (ATCC 10230), *Pseudomonas aeruginosa* (ATCC 27853) and *Proteus mirabilis* (ATCC 35695). Additionally, clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Pseudomonas aeruginosa* were included in the study. These isolates were sourced from the aforementioned hygiene laboratory to evaluate the efficacy of nettle extract against clinically relevant strains.

The minimum inhibitory concentration (MIC) of the extracts was determined to use the broth microdilution method, as described by (Pereira *et al*., 2023) and according to the Clinical and Laboratory Standards Institute (CLSI, 2018). A 96-well microplate was employed, where each well received 10 µl of a bacterial suspension with approximately $10^{\wedge 5}$ CFU/ml. Subsequently, 100µl of phenolic nettle extract, at concentrations ranging from 0.3 mg/ml to 0.009 mg/ml, was added to the respective wells. Negative controls contained only nutrient broth, removed positive controls included both nutrient broth and the bacterial inoculum. After adding the extracts and controls, the micro plate was incubated at 37°C for 24 hours. After incubation, 40µl of TTC (2,3,5-triphenyltetrazolium chloride) was added to each well and the plate was incubated for an additional 30 minutes at 37°C.

The minimum bactericidal concentration (MBC) was defined as the concentration resulting in no more than 0.01% surviving bacteria. To determine the MBC, dilutions from the MIC test were streaked into 5 cm-long grooves on Müller-Hinton agar using a 2µl calibrated loop. The agar plates were then incubated for 24 hours. Following incubation, bacterial growth was assessed and compared to the MIC findings. The MBC was identified as the lowest concentration of the nettle extract that allowed no visible bacterial growth or only minimal growth, equivalent to or less than 0.01% of the initial inoculum.

Total antioxidant capacity

DPPH radical scavenging activity

The antioxidant activity of the methanol extracts from nettle leaves and root was assessed using the DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical assay, a widely accepted method for evaluating antioxidant potential (Gulcin and Alwasel, 2023; Zeghib *et al*., 2017). The scavenging of DPPH free radicals by the extracts was quantified using UV-Vis spectroscopy at 517 nm (Isrul *et al*., 2024; Molyneux, 2004). The following procedure was applied: One millilitre of the ethanolic extract, at varying concentrations, was combined with 3ml of a 0.1 mM/l DPPH solution in ethanol. This mixture was incubated in darkness at room temperature for 20 minutes to promote the reaction between the extract and the DPPH radicals. After the incubation, the absorbance at 517 nm was measured against a negative control to determine the reduction of DPPH radicals.

PI% = [(Abs control - Abs test)/Abs control] x 100

STATISTICAL ANALYSIS

The study employed MINITAB® 19 statistical software for analysing total phenolic and flavonoid compounds. ANOVA was utilised with a significance level (α) set at 0.05. Additionally, both Tukey test were employed to compare variances, considering differences as statistically significant when $p<0.05$.

RESULTS

Extraction efficiency

The yields of various extracts were calculated by determining the ratio of the amount of extracted plant compounds to the mass of plant material used. For the phenolic extracts obtained from the dry leaves of *Urtica dioica,* the yield was 8.75%. For the extraction of phenolic compounds from the root portion, the yield was 28.6%.

Determination of phenolic compounds

The polyphenol and flavonoid content of prepared extracts is determined spectrophotometrically using the linear regression equation of the standard curve plotted with gallic acid and quercetin calibration line. Results are expressed as mg GAE/g MS and mg EQ/g MS (table 1).

Analysis and determination of phenolic compounds by LC-MS/MS (Bouhalla et al., 2024)

Compounds identified in the root extract of *Urtica dioïca* include Gallic Acid, Catechol and Phloroglucinol, detected in moderate quantities and known for their robust antioxidant activities. 4-Hydroxybenzoic Acid was found

in significant amounts and is recognized for its antimicrobial properties. Homovanillic Acid and 4-Acetocatechol, although present in smaller quantities, contribute to the plant's antioxidant profile. Pyrogallol and Resorcinol were also identified, suggesting potential antimicrobial and antioxidant activities. Syringic Acid, p-Coumaric Acid, Caffeic Acid, Ferulic Acid and Chlorogenic Acid are noteworthy for their association with various health benefits, including anti-inflammatory and neuroprotective effects. Additionally, 4-Methylcatechol, Sinapic Acid and Syringol were detected in varying quantities, contributing to the overall antioxidant capcity of the root extract, as detailed in (table 2, fig. 1).

Similar compounds were found in the leaf extract albeit at different concentrations compared to the root extract. Notably, the leaf extract displays a broader range of polyphenolic compounds, suggesting a higher antioxidant potential. Unique compounds such as trans-cinnamic acid and resveratrol, known for their anti-inflammatory and cardio-protective benefits, were also identified in the leaves. Phenylethyl Ester and 3,5-Dimethoxyphenol, exclusive to the leaf extract, indicate specific roles in plant defense mechanisms and potential contributions to the plant's medicinal properties, as shown in (table 2 and fig. 2).

Determination of the minimum inhibitory and bactericidal concentration

The determination of inhibition parameters, such as the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC), more active phrasing. It enable the confirmation, quantification, and comparison of the antimicrobial activities of different extracts, as well as the characterisation of the effects exerted by an extract on specific microorganisms. In the experimental setup, a 96-well micro plate is prepared with wells containing various microorganisms, *Urtica dioica* extract and nutrient broth. The use of the TTC (2-3-5-triphenyl-2H-tetrazolium chloride) method has proven effective due to its visual clarity, facilitated by the dye's fluorescence. The TTC dye is nontoxic to bacterial cells and active voice change to pink and fluorescence when an oxidoreductase reaction occurs, indicating the reduction of oxygen and the production of acid. Pink wells indicate microbial growth, while green wells signify inhibition. Measurements are typically taken after 18 hours of interaction between the bacteria and the extract (Laktib and others, 2024).

The phenolic extract from both the leaves and roots of *U. dioica* demonstrates significant antimicrobial activity, with similar effects observed across different microorganisms. The MIC values range from 0.3 to 0.15 mg/ml for all tested microorganisms. Notably, the leaf extract exhibits a particularly low MIC value against *Staphylococcus aureus* (clinical strain), which is known for its antibiotic resistance. These promising results suggest that the phenolic extract of *U. dioica* leaves may be effective in treating infections caused by *Staphylococcus aureus*, including those resistant to conventional antibiotics (table 03, figure. 3, 4).

To assess whether the extracts are bactericidal or bacteriostatic, a comparison is made between the MIC and MBC values for the tested strains. According to (Gasu *et al*., 2018; Ngwanguong *et al.*, 2023), the ratio of MBC to MIC is used for classification: an MBC/MIC ratio ≤2 indicates that the extract is bactericidal; an MBC/MIC ratio ≥4 suggests that the extract is bacteriostatic.

Assessment of the antioxidant activity

The DPPH assay is valued for its rapid adaptability to various samples and sensitivity to detect active ingredients at low concentrations. It is widely used to screen the antiradical activities of plant extracts, as noted by (Gulcin and Alwasel, 2023). The method involves the reduction of a DPPH alcoholic solution in the presence of an antioxidant that donates a hydrogen atom or an electron, leading to the formation of the non-radical DPPH-h (Parcheta *et al*., 2021). Our study demonstrated significantly higher free radical inhibition with the methanol extracts of *Urtica dioica* leaves and roots, achieving 80.54% and 49.23% inhibition respectively at a concentration of 5 mg/ml

Calculation of the inhibitory concentration IC_{50}

The IC_{50} value represents the concentration at which the antioxidant activity reaches 50% and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals are 50% removed (Caminiti *et al.*, 2024). (Lukman *et al*., 2024; Prakash *et al*., 2007) suggest that a lower IC_{50} value corresponds to greater antioxidant activity. In this study, the phenolic extracts obtained from leaves exhibited an IC_{50} value of 2.53 mg/ml, indicating a greater DPPH radical scavenging capacity compared to the phenolic extracts from roots, which had an IC_{50} value of 5.37mg/ml. These findings are visually represented in fig. 5, illustrating the disparity in antioxidant activity between the two types of extracts.

DISCUSSION

The results of this study are comparable to the yield of 10.94% reported by Meryem *et al.* (2020). However, Zekovic *et al*. (2017) documented lower extraction yields for polyphenols from *Urtica dioica* leaves, achieving 3.66% with 96% ethanol and 1.50% with ethyl acetate as the extraction solvent. Furthermore, Rathaur *et al*. (2023) observed even lower extraction yields, recording 0.10% with petroleum ether and 9% with a hydroalcoholic solvent, significantly lower than the yields reported in this study. These variations in yields likely result from differences in biotic conditions and the harvesting periods of the plant material.

Table 1: Total polyphenols and flavonoids content for the methanol extracts of the *U. dioica.*

Extract		Total phenolic content (mg GAE/g)	Total flavonoid content (mg QAE/g)	
Leaves	5,37	Abs=0,0013 [AG] + 0,1478	0,40	Abs=0,0129 [Que] - 0,0085
Roots	1,21	R^2 =0,9054	0,19	R^2 = 0,9992

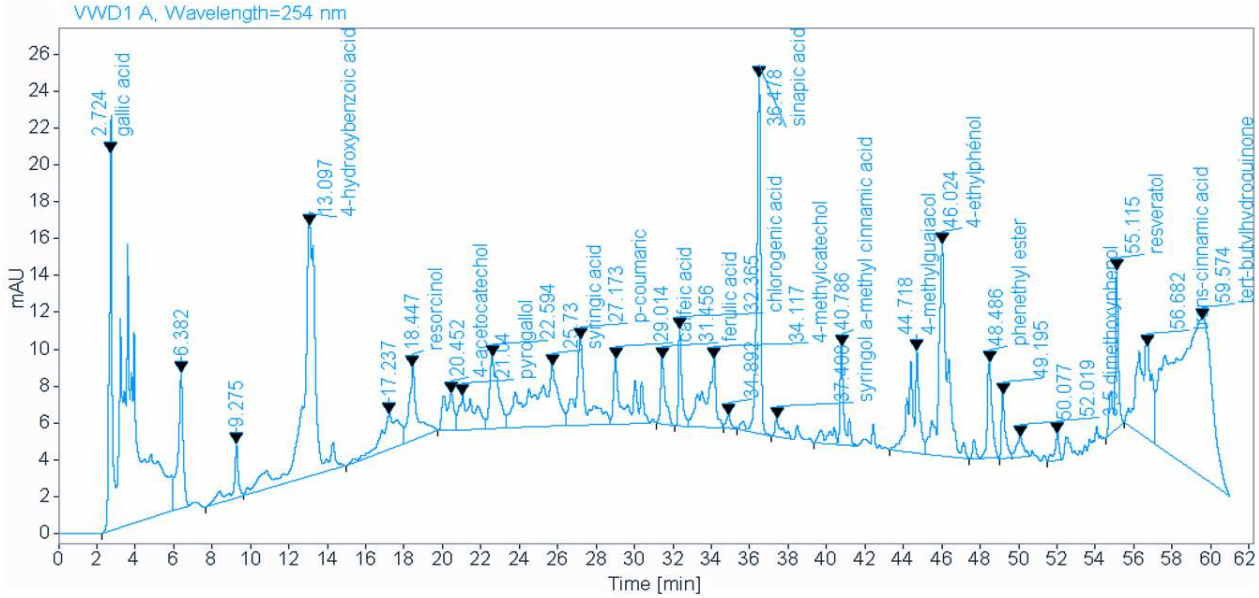


Fig. 1: Analysis of the phenolic composition of *U. dioica* roots via LC-MS/MS.

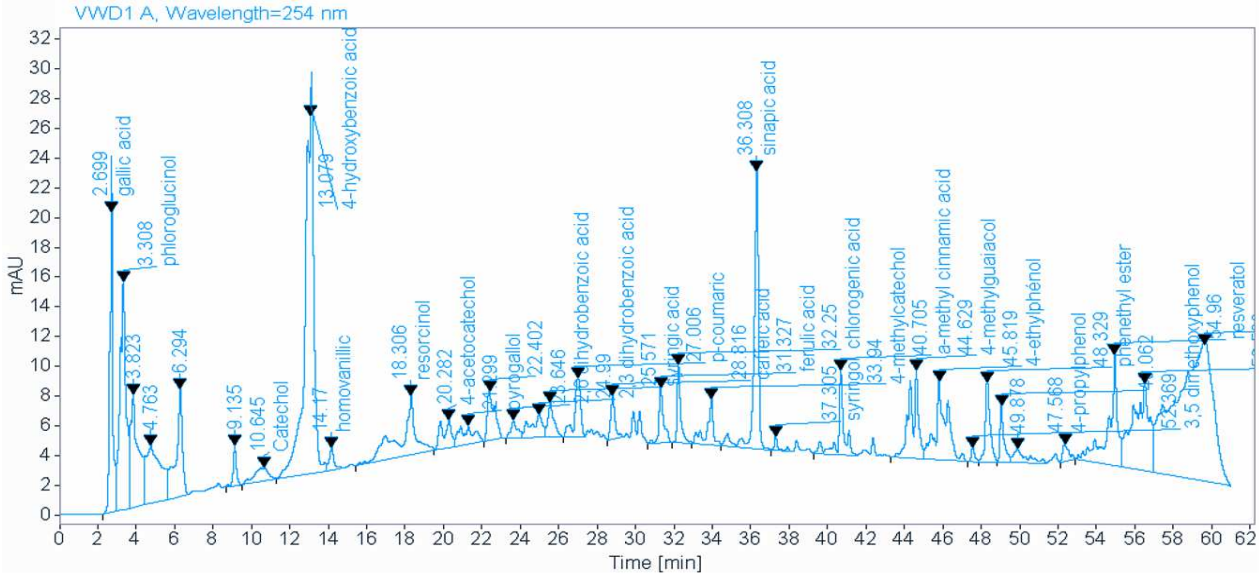


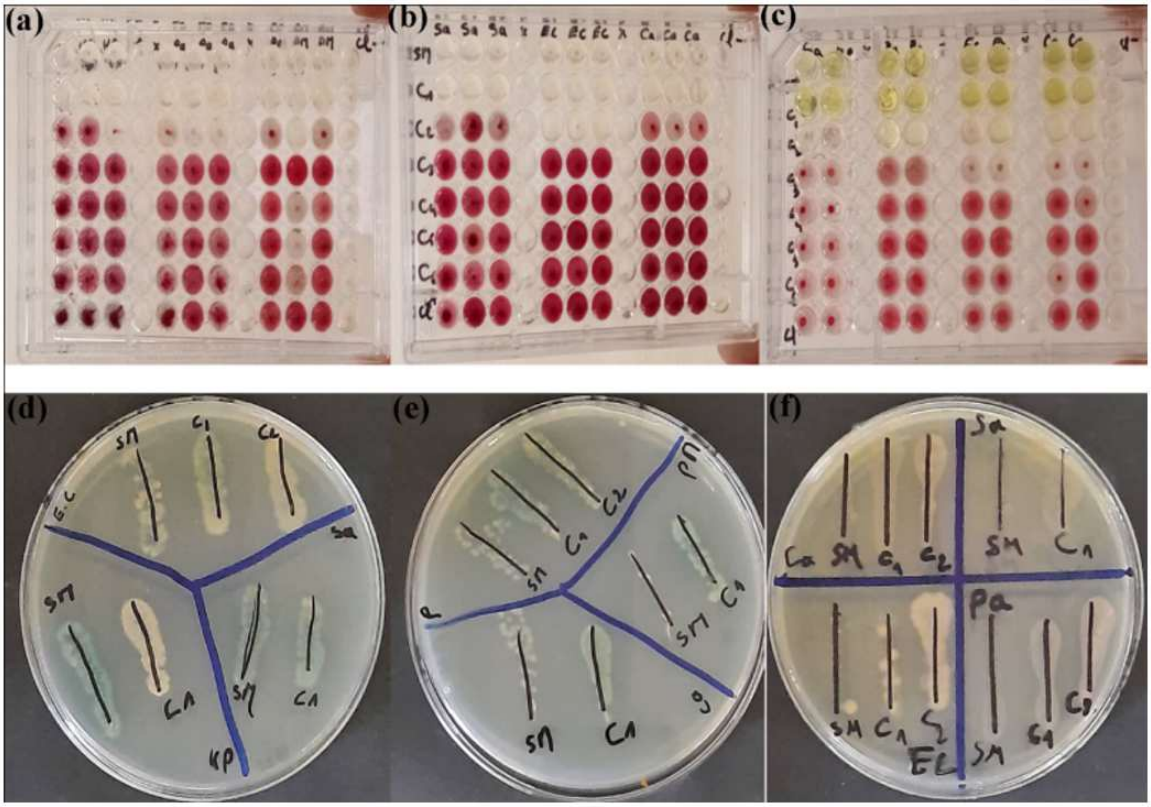
Fig. 2: Chromatographic analysis of compounds present in *U. dioica* leaves by LC-MS/MS

The phenolic content of *Urtica dioica* leaves, as presented in table 1, is consistent with prior findings. (Milovanovic *et al*., 2023) reported a phenolic content of 5.38mg GAE/g for plants cultivated in the Nitra region of Portugal, corroborated by (Wambui *et al*., 2024), who observed a similar content of 6.26±0.276mg GAE/g. Conversely, the total flavonoid content of the methanol: dichloromethane extract from *U. dioica* leaves was determined to be 1.76±0.315mg QAE/g, which contrasts with lower values reported by (Begić and others, 2020) ranging from 0.0081 to 0.0180 mg QAE/g for methanolic extracts. (Karima *et al*., 2022) further detailed the levels of polyphenols and flavonoids in *U. dioica* leaves as 26.02 mg GAE/g and 86.43mg EAq/g, respectively. The polyphenolic content of plants shows qualitative and quantitative variations due to several factors: Genetic variations, environmental conditions, and cultivation techniques, The genetic makeup of the plant, timing and stage of harvesting, and its developmental stage (Karima *et al.*, 2022) and the methodologies employed for extraction and quantification, which significantly influence the assessment of total polyphenol content

Table 2: Concentrations of phenolic compounds detected in *U. dioica* leaf and root extracts in ppm

Name of compound	Root	Leaf
Gallicacid	105,44	105,44
Phloroglucinol	14,23	14,23
Catechol	1 ,19	1,19
4-hydroxybenzoic acid	48,01	48,01
Homovanillic	0,53	0,53
Resorcinol	2,79	2,79
4-acetocatechol	0,84	0,84
Pyrogallol	1,05	1,05
2,6 dihydrobenzoicacid	1,79	1,79
2,3 dihydrobenzoicacid	5,60	5,60
Syringicacid	1,66	1,66
p-coumaric	7,16	7,16
Caffeicacid	1,31	1,31
Ferulicacid	30,13	30,13
Chlorogenicacid	0,67	0,67
4-methylcatechol	0,77	0,77
Sinapicacid	1,56	1,56
Syringol	0,77	0,77
a-methylcinnamicacid	16,15	16,15
4-ethylphénol	17,61	17,61
4-propylphenol	0,33	0,33
phenethyl ester	ND	9,07
3,5 dimethorypnenot	ND	0,15
Resveratol	ND	0,76
trans-cinnamicacid	ND	1,79
Tert-butylhydroqrinon	ND	70,62

ND. Not determined



(a), (b), (d), (e): reference strains. (c), (f): clinical strains. Pa: *Pseudomonas aeruginosa*, Ec: *Escherichia coli*, Sa: *Staphylococcus aureus* Ca: *candidaalbicans,* KP: *klebsiellapneumoniae,* PM: *Proteus mirabilis*, CL+ :test positif C: concentration (SM:0,3/C1: 0,15/C2: 0.075/C3 :0,037/C4 :0,018/ C5 :0.09 C6 :0,045mg/ml), CL- :test negative

Fig. 3: MIC and MBC result of phenolic extract of leaves on the strains tested

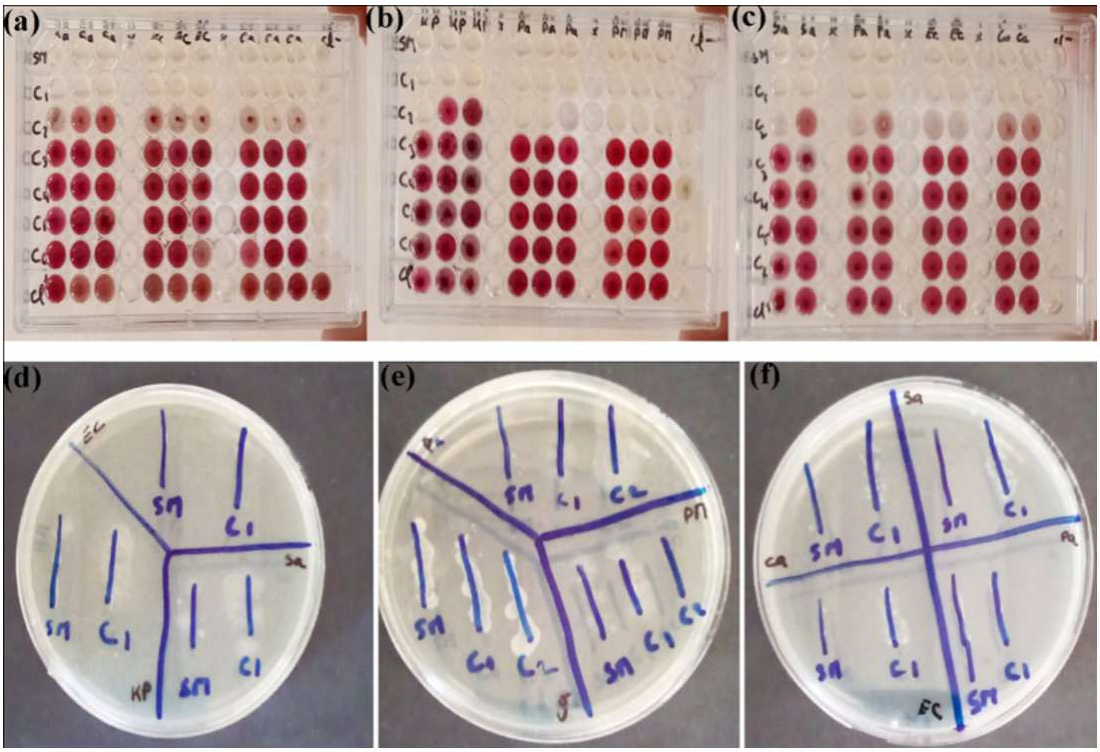


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Fig. 4: MIC and MBC result of phenolic extract of roots on the strains tested

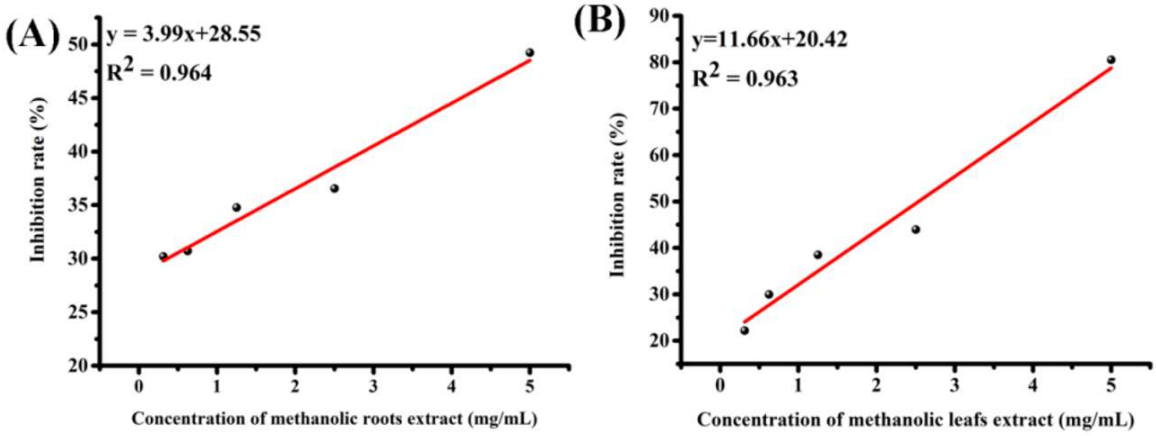

Fig. 5: % DPPH inhibition as a function of roots and leaves extract concentrations

(Duran *et al.*, 2022). These elements highlight the complexity involved in analysing and interpreting the polyphenolic content of plants.

This study utilized Liquid Chromatography-Mass Spectrometry (LC-MS) to analyze the polyphenolic compounds present in methanolic extracts of *U. dioica* (stinging nettle) from both root and leaf sources. Polyphenols are vital due to their medicinal and nutritional benefits, particularly their antioxidant, anti-inflammatory, and antimicrobial properties. Research by Uğur and Güzel (2023) highlights the richpolyphenolic content of *U. dioica*, identifying key compounds such as gallic acid and catechol, which align with our findings. While our data supports the antioxidant capacity and enzyme inhibition effects noted in previous studies, it does not quantitatively explore these aspects. The consistency of identified compounds across various studies reinforces the recognized antioxidant potential of *U. dioica*. Koraqi *et al*. (2023) focused on optimized extraction methods that may yield higher concentrations of specific compounds compared to our standard methanolic extraction. Despite these differences, the presence of significant polyphenols like caffeic acid and

chlorogenic acid confirms their status as robust markers of *U. dioica* phytochemical profile. Brahmi-Chendouh *et al*. (2021) provided a comprehensive list of polyphenols identified through HR-MS/MS techniques, including compounds not quantified in our analysis. Their detection of resveratrol and trans-cinnamic acid, also found in our leaf extracts, highlights compositional differences between plant parts and emphasizes the diverse polyphenolic landscape of *U. dioica*. Further studies by García *et al.* (2021), Jeszka-Skowron *et al.* (2022) and Repajić *et al*. (2021) corroborate our measured concentrations of polyphenols such as gallic acid and caffeic acid, confirming the robustness of our results. These findings underscore the influence of extraction methods, plant parts, and environmental conditions on phytochemical profiles, emphasizing the need for standardized methodologies for comparative analysis. The detection of specific compounds like resveratrol and trans-cinnamic acid in leaf extracts suggests avenues for further research into their unique biological activities and therapeutic applications. Overall, our results align with existing literature, affirming the strong polyphenolic composition of *U. dioica* and its potential applications in antioxidants, antimicrobial activities, and enzyme inhibition.

The phenolic extract of *U. dioica* leaves and roots is rich in hydroxycinnamic acids, such as ferulic and cinnamic acids, which have demonstrated broad-spectrum antibacterial activity against both gram-negative and Gram-positive bacteria, including strains such as *E. coli* NCTC 10418, *Staphylococcus aureus* NCTC 10788, and *Enterococcus hirae* NCTC 13383 (Malheiro *et al*., 2019). The presence of flavonoids like rutin has also been documented (Mueed and others, 2023), along with naringenin, which exhibits antibacterial effects against *S. aureus*, *E. typhi* and *E. coli* (Agus Suryawan *et al*., 2017). These compounds contribute significantly to the antimicrobial potential observed in this study. The bactericidal effect of *U. dioica*'s phenolic extract on *Staphylococcus aureus* was highlighted in research by (Du *et al*., 2024), with the hydroalcoholic extract at a concentration of 100mg/ml completely inhibiting the growth of Gram-positive bacteria isolated from urinary tract infections. Similar MIC values (0.39-0.78mg/mL) for nettle extracts were reported by (Sterniša *et al*., 2020), while higher values (6.25-50mg/mL) were noted in the study by (Rajput *et al*., 2019). These findings further underscore the broad-spectrum antimicrobial activity of *U. dioica* leaf extracts and suggest its potential as a natural antimicrobial agent. Comparing results across studies can be challenging due to variations in products, methods, and reporting styles. For instance (Harrison *et al.*, 2022) found that while *U. dioica* extracts did not exhibit significant bactericidal activity, they were effective in inhibiting the expression of virulent bacterial phenotypes, particularly biofilm formation. Biofilms provide a protective environment that enhances bacterial resistance to antimicrobial agents. By inhibiting biofilm formation, *U. dioica* extracts may indirectly reduce bacterial defences and enhance the efficacy of antimicrobial treatments. This approach, as suggested by (Belmamoun *et al*., 2022), targets the biofilm's protective capabilities, potentially offering therapeutic benefits in preventing and managing bacterial infections. Further research is necessary to elucidate the specific mechanisms by which *U. dioica* extracts inhibit biofilm formation and to fully understand their therapeutic potential and applications. Given that different molecules have varying structures and chemical compositions, polyphenols can demonstrate a wide range of antimicrobial effects, including the permeabilization and destabilization of the plasma membrane, as well as the inhibition of extracellular enzymes. Furthermore, these mechanisms of action are distinct from those of conventional antibiotics, potentially allowing plant phenolics to be effective against drug-resistant pathogens (Takó *et al.,* 2020).

In research conducted by (Karima *et al.*, 2022), the aqueous extract exhibited only a 5% inhibition of DPPH radicals, whereas the methanolic extract achieved up to 19% inhibition. In contrast, our study demonstrated significantly higher free radical inhibition with the methanol extracts of *U. dioica* leaves and roots, achieving 80.54% and 49.23% inhibition respectively at a concentration of 5 mg/ml. These results align with those reported by (Flórez *et al.*, 2022) who noted inhibition rates of 75% and 76.4% for methanolic and ethanolic extracts of *U. dioica* leaves, respectively, highlighting the potent antioxidant activity of extracts obtained through organic solvent-based maceration. Similarly (Albayrak *et al.*, 2012) reported a 40% inhibition rate for *U. dioica* leaf extract at a concentration of 2mg/ml, underscoring the efficacy of these extracts in radical scavenging activities.

(Belmamoun *et al*., 2023) reported that the methanolic extract of *U. dioica* demonstrated potent antioxidant activity with an IC_{50} value of 4.69mg/ml. (Wafa *et al.*, 2022) found that the Soxhlet extraction method using ethanol produced the most effective radical scavenging activity, achieving an IC_{50} of 0.343 ± 0.012 mg/ml. Similarly, (Rolta *et al.*, 2020) evaluated the antioxidant activities of both ethanolic and aqueous extracts from *U. dioica* leaves, reporting IC_{50} values of 245.65µg/ml and 142.94µg/ml, respectively.

The methanol extract of *U. dioica* leaves demonstrates superior scavenging capacity for $DPPH^{\cdot}$ radicals compared to the root extracts. This differential activity in antioxidant power is likely due to the higher concentration of phenolic compounds in the leaves. (Shen *et al*., 2022) suggest that plants with high antioxidant activities typically contain abundant phenolic groups, supporting the observed differences in radical scavenging effectiveness between the leaf and root extracts of *U. dioica*.

CONCLUSION

This study explored the biological activities of methanolic extracts from *Urtica dioica*, a plant valued for its medicinal and economic significance within the Urticaceae family. The extraction yields were substantial, at 8.75% for the leaves and 28.60% for the roots. All tested bacterial strains demonstrated sensitivity to the extracts, with minimum inhibitory concentrations (MICs) ranging from 0.07 to 0.15mg/ml. Furthermore, the extracts exhibited strong bactericidal activity, achieving a minimum bactericidal concentration (MBC) of 0.3 mg/ml against most tested microorganisms, thereby confirming their potential to effectively kill bacteria. These antimicrobial properties can be attributed to the phenolic compounds present in the plant. In addition, both leaf and root extracts showed significant antioxidant activities, inhibiting 80.54% and 49.23% of free radicals respectively, as measured by the DPPH assay. In conclusion, the summarized studies highlight the importance of polyphenolic extracts from *U. dioica* as promising antimicrobial and antioxidant compounds. They also provide alternatives for the ecological application of certain pharmaceutical products and for tackling resistance to synthetic antibiotics.

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Conflict of interest
There is no conflict of interest.

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