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Research Article

In-vitro Antimicrobial Activity of *Urtica dioica* against Isolated Multidrug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* from surgical site

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Abstract

Background: A significant worldwide public health concern is the quick rise of multidrug-resistant (MDR) pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa*, especially when it comes to postoperative surgical site infections (SSIs). Antimicrobials derived from plants are being investigated more and more as alternatives to antibiotic resistance.

Methods: Ethanolic leaf extract's *in-vitro* antibacterial activity of *Urtica dioica* (stinging nettle) against MDR clinical isolates recovered from postoperative SSIs was assessed in this study. 6 *S. aureus* and 10 *P. aeruginosa* were among the 16 MDR isolates that were examined. Antimicrobial activity was measured using agar-well dilution and microbroth dilution method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were established.

Results: The *U. dioica* ethanolic extract demonstrated strong antimicrobial actions against both test species, with zone of inhibition that ranges 14.5 ± 0.5 to 29.5 ± 0.5 mm. MIC values for *S. aureus* and *P. aeruginosa* were 6.25 and 3.125 mg/mL. The comparable MBC results were 12.5 mg/mL and 6.25 mg/mL. A primarily bacteriostatic mode of action was indicated by the MIC to MBC ratios.

Conclusion: *Urtica dioica* has significant antibacterial activity against MDR microorganisms associated with SSIs. These findings imply that *U. dioica* possesses potential application in the production of plant-based antimicrobials. They also indicate how medicinal plants can be of use in addressing the issue of infections and drug resistance.

Keywords: Antimicrobial resistance; Herbal medicine; Multidrug-resistant pathogens; Surgical site infections; *Urtica dioica*

Introduction

The rise of antimicrobial resistance is among the greatest challenges confronting the field of medicine at present. This reduces the potency of antibiotics, which have been regarded as groundbreaking therapies in the treatment of infectious illnesses. Infections that arise from MDR pathogens like *S. aureus* and *P. aeruginosa* in hospitals are proving to be more difficult to manage, thus increasing mortality. These microorganisms are a major contributor to surgical site infections (SSIs), which severely impair postoperative recovery and lead to a greater incidence of complications following major surgical procedures, including exploratory laparotomy and abdominal surgeries [1, 2, 3].

AMR has transcended laboratory settings and clinical environments to become a global public health crisis. Its societal, economic and human impact is severe. An estimated 1.27 million deaths were directly caused by bacterial AMR, which also contributed to an additional 4.95 million deaths globally [4, 5]. Unless effective interventions are implemented, deaths related to AMR are expected to rise sharply by the middle of this century, with low- and middle-income nations shouldering a disproportionate share [6]. As a

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consequence of AMR, there are longer hospital stays, increased costs of treatment, and increased pressure on an already overburdened healthcare system, as well as increased mortality. The World Health Organization has verified the vital relevance of AMR through recent global health assessments, recognizing its severity and rating it as one of the top ten global health risks [7].

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In this sense, medicinal herbs can be an effective tool. Medicinal plant bioactive chemicals have demonstrated structural variety and multi-target biological effects, including antibacterial, wound-healing, and anti-inflammatory qualities [8]. Herbal treatments are readily available, cost-effective, and culturally acceptable, which makes them attractive choices for sustainable healthcare, especially where there are scarce resources [7, 9]. The inclusion of these plant-based treatments in the field of antimicrobial research is right in line with the global need for new ways to combat the problem of AMR without adding too much stress to the environment and the economy.
Because of its anti-inflammatory qualities, *Urtica dioica* is one of the most meticulously researched medicinal plants and has been used for centuries to treat wounds, inflammation, and infections. *Urtica dioica* has a high concentration of phenolic compounds and flavonoids, as well as different terpenoids and other secondary metabolites with demonstrated antibacterial and antioxidant properties, according to phytochemical studies [10]. The compounds have been demonstrated to impair the integrity of bacterial cell walls, interfere with membrane function, and impede growth; all of these effects bolster the plant's therapeutic effectiveness against potentially harmful bacteria [11, 12, 13].

Research on *Urtica dioica's* capacity to stop the growth of MDR bacterial isolates from wounds from recent surgical procedures is still lacking, despite increasing proof of the plant's antimicrobial properties. The reference strains or non-clinical isolates used in a large number of recent studies may not be representative of the true resistance patterns present in clinical settings, like hospitals. Since current antibiotics frequently fail to treat MDR pathogens that cause SSIs, alternative treatment options are required. MDR pathogens are a major and critical public health issue.

This study looks at *Urtica dioica's* capacity *in vitro* antibacterial efficacy against MDR *S. aureus* and *P. aeruginosa* that were isolated from infection sources following surgery. The research will provide novel data on how these clinically relevant organisms can provide new potential uses for traditional herbal medicines as sustainable antimicrobial agents. The results contribute to improved practice for controlling infections and developing more affordable therapeutic strategies for combating antimicrobial resistant organisms.

Methodology

Plant Material Collection

The Manali region of Kullu District, Himachal Pradesh, India (Latitude: 32.239° N; Longitude: 77.1887° E; altitude: roughly 2,050 m above sea level) is where fresh *Urtica dioica* leaves were gathered. To guarantee the highest possible phytochemical content, the plant material was gathered during the proper growing season. Dr. Garima Bartariya, a botanist from IIMT University in Meerut, India, performed the botanical identification and authentication of U. dioica. For future use and confirmation, a voucher specimen of *U. dioica* (stinging nettle) was placed in the Herbarium of the Chaudhary Charan Singh (CCS) University Department of Botany in Meerut, India (Voucher No: Bot/PB/455).

The gathered leaves were properly washed with sterile distilled water to get rid of any remaining dust, surface impurities, and soil particles that were sticking to them. In order to preserve heat-sensitive bioactive compounds, after that, the leaves were left to dry at ambient temperature in the shade for 72 hours. Leaves were stored in sealed containers until they were required for extraction and antibacterial analysis after being thoroughly dried and processed into a sterilized electric grinder to create a fine powder.

Preparation of Extract

In sterile reagent bottles, 50 grams of Urtica dioica leaves powder were macerated 70% ethanol (v/v) in 500 mL at a 1:10 ratio (w/v). To improve solvent penetration for the extraction of bioactive chemicals, the mixture was tightly sealed, stirred occasionally, and let 72 hours to settle at room temperature. During the extraction procedure, the containers were kept in a cold, dark area to minimize phytochemical degradation. Adhering to the maceration, the extract was filtered with Whatman No. 1 filter to get rid of coarse plant debris. The filtrate was centrifuged at 3500 rpm for 15 minutes in order to further remove colloidal suspensions and tiny particles. The clear supernatant was concentrated under lower pressure

until the dried crude extract was produced after the solvent was allowed to evaporate for seven days at room temperature. By following the methods as described above, along with some minor modifications, the dry extract was accurately measured and stored in airtight containers and kept at a temperature range of 2-4 °C for further antibacterial assays [14]. The crude extract yield percentage was calculated using the formula below:

$$\% \text{ Yield (w/w)} = \frac{\text{wt. of dried extract (g)} \times 100}{(\text{wt. of dried plant material used (g)})}$$

Regarding ***U. dioica*** (leaves):

$$\% \text{ Yield (w/w)} = \frac{(6.3 \text{ g}) \times 100}{(50 \text{ g})} = 12.6 \% \text{ (w/w)}$$

Bacterial Isolation and Identification

MDR *S. aureus* and *P. aeruginosa* clinical isolates were obtained from patients with postoperative surgical site infections at the Lala Lajpat Rai Memorial (LLRM) Medical College and Hospital in Meerut, Uttar Pradesh, India. Sterile swabs were utilized to aseptically collect wound swab samples from infected surgical sites. The clinical samples were directly swabbed onto blood agar, cetrimide agar, mannitol salt agar, and MacConkey agar plates and then incubated at 37 °C for 18 to 24 hours. The growth of bacteria was checked based on their morphology, color, haemolytic activity on blood agar, and lactose fermentation on MacConkey agar. Further confirmation of the presumptive isolates was identified by the use of standard biochemical techniques as well as the Gram staining technique. The bacterial resistance patterns to several antimicrobial drugs were analysed using the Kirby-Bauer disk diffusion method [15]. Inhibitory zone diameters were evaluated, and breakpoints were interpreted in accordance with CLSI recommendations. Isolates that showed resistance to three or more antimicrobial drug classes were selected for further antibacterial agent susceptibility testing.

Using 16 ribosomal RNA gene sequencing for identification of MDR *Staphylococcus aureus* and *Pseudomonas aeruginosa*

For sequencing, MDR strains were sent to Biokart India Pvt. Ltd., a biological service organization located in Bengaluru, Karnataka, India. The 16S rRNA genes were amplified using polymerase chain reaction to identify the strains. To authenticate this result, a 1500 bp fragment of 16S rDNA was amplified using a high-fidelity PCR polymerase. ABI3130xl technology was utilized to perform the sequencing of the products in the forward and reverse directions. To identify the bacteria and their nearest relatives in the phylogenetic tree, the sequences of the products were analyzed. To prevent cross-contamination of the samples, stringent measures were taken. The necessary controls were included to ensure the data obtained were of the highest quality.

Furthermore, the study's 16S rRNA gene sequences were added to the National Center for Biotechnology Information's (NCBI) GenBank database. The submitted sequences accession numbers are as follows: *Staphylococcus aureus* isolates – GenBank Accession No: [PX023482 and PX024849], *Pseudomonas aeruginosa* isolates – GenBank Accession No: [PX458929 and PX480726].

Antimicrobial Assays

Agar Well Diffusion Assay

Antimicrobial assay of the ethanolic extract of *Urtica dioica* was established by the well diffusion technique against a panel of 16 multidrug-resistant (MDR) clinical isolates, including 10 MDR *P. aeruginosa* and 6 MDR *S. aureus*. This is one of the commonly employed techniques as a preliminary screen for assessing the antimicrobial potency of plant-based bioactive compounds. In brief, bacterial inocula were prepared by standardizing overnight culture to a turbidity of 1.5 x 10^8 CFU/mL is similar to 0.5 McFarland. In order to produce a consistent bacterial lawn, cotton swabs were employed to equally distribute standardized bacterial solutions in Mueller-Hinton Agar (MHA) plates. Aseptic wells with diameters of 6 mm were penetrated the agar with a sterilized cork-borer. To each well, 100 µL of crude ethanolic *Urtica dioica* extract was added at a 100 mg/mL concentration. The positive control contained 5 µg of ciprofloxacin, whereas the negative control contained 70% ethanol. For 18-24 hours the plates were incubated at 37 °C, with a 15-minute pre-incubation at room temperature. A measuring scale was applied to ascertain the inhibitory zone's diameter that developed surrounding each well following incubation. Every experiment was carried out in three times, and the mean inhibition zone diameter ± standard error of the mean (SEM) was used to express the results.

MIC and MBC determination using broth microdilution assay

The Clinical and Laboratory Standards Institute recommendations were followed in determining the MIC and MBC of the ethanolic leaf extract of *U. dioica* against MDR *S. aureus* and *P. aeruginosa* clinical isolates using the broth microdilution method [16].

MIC determination

MIC values were determined using a 96-well sterile round-bottom micro-titer plate. Mueller-Hinton broth (MHB) was employed to create two-fold dilutions of the ethanolic extract of U. dioica. Wells 2–11 were initially filled with 50 µL of MHB. Subsequently, after adding 100 µL of plant extract to well 11, 50 µL was

serially transferred from well 11 to well 2, to obtain a concentration range of 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.19, and 0.097 mg/mL. After the final transfer, 50 µL was discarded to maintain uniform volumes.

A standardized bacterial suspension (50 µL) adjusted to 0.09–0.1 optical density at 600 nm (1×10^6 CFU/mL) were filled with wells 1–11. Well 1 used as a +ve control, containing bacteria in MHB, while Well 12 contained sterile MHB and acted as the -ve control. Plates were then incubated at 37 °C for 24 hours. After this, 10 µL of resazurin dye was then filled with each well to provide a colorimetric method of viability. After an additional 30 minutes to 2 hours of incubation, plates were checked for color changes. Growth of bacteria was demonstrated by a shift from purple to pink or colorless, while growth inhibition was indicated by wells that maintained their purple hue. The lowest extract concentration was known as the MIC that induced the lack of change in color and totally inhibited bacterial growth. MIC values were given as mean concentrations, and each assay was run in triplicate.

MBC Determination

To ascertain the MBC, aliquots (10–20 µL) from wells that matched the MIC and higher concentrations that showed no discernible growth were subcultured onto nutrient agar plates. Bacterial growth has been determined throughout the incubation period of 24-hour at 37 °C. Lower concentration of *U. dioica* extract that stopped any observable colonies from forming was known as the MBC, which indicates 99.9% decreases in the original bacterial inoculum.

Ethical Approval

Before sampling, the landowner gave permission for collection. Both local regulations and ethical guidelines for plant sample were followed during the collection process. For sampling on private property, obtain the landowner's consent letter.

Statistical Analysis

For every experiment, the results are shown as mean ± standard error of the mean (SEM) was carried out in triplicate. Using analysis of variance (ANOVA), group differences were statistically evaluated. When appropriate, one-way ANOVA was utilized to assess overall differences in experimental results, and two-way ANOVA was employed to examine how bacterial species and extract concentrations affected zones of inhibition, MIC, and MBC values. In every analysis, statistical significance was established using a p-value of < 0.05.

Results

Agar-Well Diffusion Technique

Agar-well diffusion technique were used to quantify *U. dioica's* antibacterial activity. 70% ethanolic leaf extract was examined against 16 MDR clinical isolates, including 6 S. aureus isolates and 10 P. aeruginosa isolates recovered from SSIs following surgery.

Antimicrobial Activity against *Staphylococcus aureus*

6 isolates of MDR *S. aureus* were used to test antimicrobial efficacy of a 70% ethanolic leaf extracts from *U. dioica* utilizing the well diffusion assay. The inhibitory zone ranges from 14.5 ± 0.5 to 29.5 ± 0.5 mm, the *U. dioica* extract demonstrated consistent and noticeable inhibitory activity against every isolate tested.

To confirm the resistant phenotype of all 6 clinical isolates of *S. aureus*, the standard antibiotic ciprofloxacin (5 µg) showed significantly lower activity, having inhibition zones between 00 ± 00 to 16.6 ± 0.4 mm. The negative control (70% ethanol) showed no inhibition.

The inhibitory effects of *U. dioica* extract and ciprofloxacin against MDR *S. aureus* isolates did not differ statistically significantly, according to one-way ANOVA statistical analysis ($F = 1.83$; $p = 0.189$). These results show that *U. dioica's* antibacterial activity against resistant *S. aureus* strains is similar to that of ciprofloxacin (Fig. 1), underscoring its potential as a plant-based substitute for treating MDR Gram-positive infections.

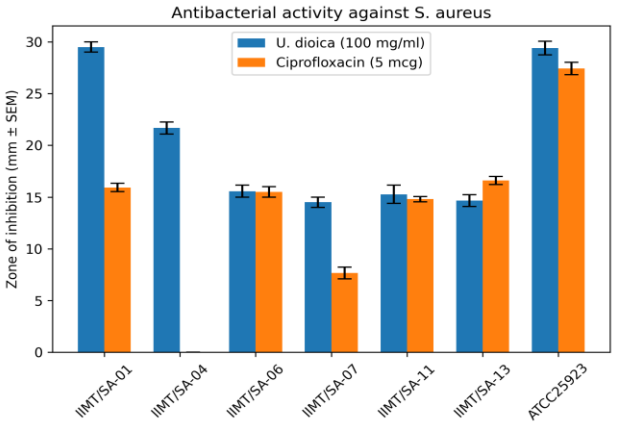

Fig. 1 The inhibition zone diameter (mm ± SEM) of *U. dioica* and ciprofloxacin (positive control) against MDR *Staphylococcus aureus* strains is compared in a bar graph

A one-way ANOVA test was employed for statistical analysis. No statistically significant differences were found ($p = 0.189$).

Antimicrobial Activity against *Pseudomonas aeruginosa*

10 MDR *P. aeruginosa* clinical strains were used to examined the antimicrobial efficacy of 70% ethanolic leaf extract from *U. dioica* using the well diffusion technique. The inhibitory regions range from 17.5 ± 0.5 mm to 24.7 ± 0.36 mm, the extract demonstrated distinct and reliable inhibitory effects against every isolate that was tested. On the other hand, resistance was found in most isolates, and the standard antibiotic ciprofloxacin (5 µg) demonstrated decreased efficacy, with inhibition zones range from 00 ± 00 mm to 26.6 ± 0.36 mm. There was no inhibitory effect from the negative control (70% ethanol).

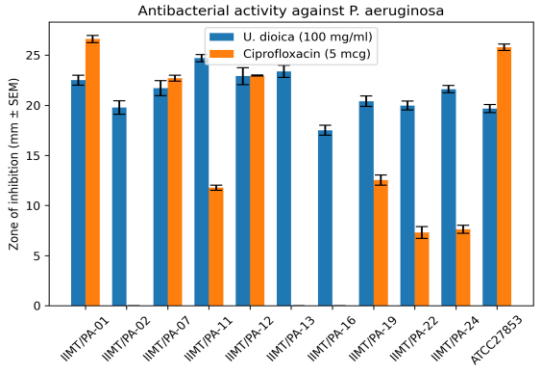

Fig. 2 Mean inhibition zone diameter (mm ± SEM) of MDR *P. aeruginosa* strains against *U. dioica* and ciprofloxacin (positive control) is displayed in a bar graph

There is no variability in dataset – all groups have identical values. If every value is identical, the test show no significant differences (p = 1.000).

Activity against *Pseudomonas aeruginosa*

10 clinical isolates of MDR *P. aeruginosa* showed strong antibacterial activity with exposure to a 70% ethanolic *U. dioica* leaf extract. For every tested isolate, the extract consistently demonstrated MIC of 3.125 mg/mL and MBC of 6.25 mg/mL. With MIC of 3.125 mg/mL and MBC of 6.25 mg/mL, the reference strain *P. aeruginosa* ATCC 27853 showed a similar susceptibility pattern (Table 2).

The tested isolates' antibacterial activity did not differ statistically significantly (p = 0.0895), suggesting Significant differences between treatments were found by statistical analysis using the test one-way ANOVA (p < 0.0059).

The extract from *U. dioica* exhibited superior antibacterial action against isolates of MDR *P. aeruginosa*, according to a one-way ANOVA statistical analysis that showed a significant difference in inhibitory activity between ciprofloxacin and *U. dioica* (F = 6.11; p = 0.0059) (Fig. 2). These results demonstrate *U. dioica's* potential as a plant-derived antimicrobial candidate against resistant Gram-negative pathogens linked to surgical site infections.

Assessment of MIC and MBC

Activity against *Staphylococcus aureus*

A 70% ethanolic leaf extract of *U. dioica* consistently suppressed all 6 clinical isolates of MDR *S. aureus*. The extract revealed MIC of 6.25 mg/mL and an MBC of 12.5 mg/mL for each isolate. With MBC of 12.5 mg/mL and MIC of 6.25 mg/mL, respectively, the reference strain *S. aureus* ATCC 25923 displayed a similar susceptibility profile (Table 1). The observed MBC/MIC ratio (2:1) suggests that MDR *S. aureus* isolates are susceptible to the bactericidal consequences of the *U. dioica* extract.

Table 1 MIC and MBC for MDR *Staphylococcus aureus*

		***S. aureus* MIC and MBC (mg/mL)**		
			U. dioica	
S.N.	**Strain I.D.**	**OD (λ = 600 nm)**	**MIC (in 50 mg/mL)**	**MBC (in 50 mg/mL)**
1	ATCC25923	0.0963±0.0013	6.25	12.5
2	IIMT/SA-01	0.0970±0.0017	6.25	12.5
3	IIMT/SA-04	0.0942±0.0031	6.25	12.5
4	IIMT/SA-06	0.0957±0.0017	6.25	12.5
5	IIMT/SA-07	0.0982±0.0004	6.25	12.5
6	IIMT/SA-11	0.0942±0.0018	6.25	12.5
7	IIMT/SA-13	0.0961±0.0012	6.25	12.5

*OD: Optical density [mean value of triplicate measurement ± Standard deviation (SD)]

that U. dioica is consistently effective against the strains of P. aeruginosa that were assessed.

The U. dioica leaf extract exhibited both bactericidal and bacteriostatic capabilities based on the concentration of MIC and MBC values. Extract was more effective against Gram's -ve MDR P. aeruginosa than Gram's +ve MDR S. aureus, as evidenced by the lower MIC and MBC values.

Discussion

The current study shows that Urtica dioica's crude ethanolic leaf extract exhibits substantial antimicrobial effectiveness against MDR S. aureus and P. aeruginosa have been identified from SSIs following surgery. Significant antimicrobial potency is indicated by the observed inhibition zones and low MIC/MBC values,

which in multiple cases approach or surpass the activity of ciprofloxacin, to which many isolates showed resistance. Such results are an aspect of the emerging problem of AMR in clinical practice and call for the immediate development of alternative therapies. The antibacterial abilities of U. dioica can be attributed to its full spectrum of phytochemicals composed of phenolics, flavonoids, tannins and terpenoids which can cause disruption of bacterial cell wall, modification of membrane permeability and inhibition of the bacterial essential metabolic activities. MDR P. aeruginosa was more sensitive than S. aureus indicating lower MIC and MBC values.

Table 2 Ethanolic extracts MIC and MBC for MDR *Pseudomonas aeruginosa*

		***P. aeruginosa* MIC and MBC (mg/ml)**		
			U. dioica	
S.N.	**Strain I.D.**	**OD=600 nm (Mean ±SEM)**	**MIC (in 50 mg/mL)**	**MBC (in 50 mg/mL)**
1	ATCC27853	0.0969±0.0020	3.125	6.25
2	IIMT/PA-01	0.0965±0.0013	3.125	6.25
3	IIMT/PA-02	0.0965±0.0009	3.125	6.25
4	IIMT/PA-07	0.0972±0.0010	3.125	6.25
5	IIMT/PA-11	0.0967±0.0018	3.125	6.25
6	IIMT/PA-12	0.0949±0.0040	3.125	6.25
7	IIMT/PA-13	0.0948±0.0034	3.125	6.25
8	IIMT/PA-16	0.0949±0.0028	3.125	6.25
9	IIMT/PA-19	0.0962±0.0030	6.25	12.5
10	IIMT/PA-22	0.0971±0.0016	3.125	6.25
11	IIMT/PA-24	0.0964±0.0025	3.125	6.25

*OD: Optical density [mean value of the triplicate measurement ± Standard deviation (SD)]

The inhibition zones of Urtica dioica for MDR P. aeruginosa (17.5–24.7 mm) and MDR S. aureus (14.5–29.5 mm) express its wide-spectrum activity. These results align with earlier research that shows the antibacterial properties of plant flavonoids and phenolic chemicals by denaturing proteins, rupturing bacterial cell membranes, and blocking critical enzyme processes. The multifaceted actions of flavonoids and phenolic compounds of medicinal plants could increase the effectiveness of antibiotics and have the capacity to decrease the resistance development. In vitro cultures of MDR S. aureus (6 isolates) and P. aeruginosa (10 isolates) were used to test the antibacterial properties of U. dioica's ethanol crude extract. Every MDR isolate was found to be vulnerable to U. dioica. As far as we know, there is no research paper on U. dioica's antibacterial action against human surgical site wound infections, although some studies reporting its antibacterial efficacy against the pathogens [17, 18].

Our findings align with a study by El Kahkahi et al., that demonstrated the inhibitory effects of U. dioica extracts on both Gram's +ve and Gram's -ve bacteria, including S. aureus and P. aeruginosa [19]. The occurrence of phenolic and flavonoid compounds, which could damage the integrity of bacterial cell walls and membrane function, was primarily responsible for this activity, according to the investigators [12]. Mirtaghi et al., confirmed the antibacterial potential of U. dioica, using agar well diffusion assays, that observed unique inhibition of S. aureus growth using this species [20]. Crucially, the originality of the study is due to the MDR isolates utilized in the study's clinical significance, which were directly isolated from postoperative surgical site infections, presenting the typical therapeutic dilemma of the current scenario in which conventionally used antibiotic are often not effective.

Based on the current study MIC and MBC tests demonstrated that U. dioica extract revealed more potent effect on MDR Pseudomonas aeruginosa compared to MDR Staphylococcus aureus. The extract at 3.125 mg/mL were effective in inhibition of growth of nine P. aeruginosa isolates and IIMT/PA-19 isolate (was the only one) needed a slightly higher concentration equal or higher than 6.25 mg/mL for effectiveness of inhibition. Most isolates had an MBC value of 6.25 mg/mL, while IIMT/PA-19 MBC was 12.5 mg/mL. However, all the isolates of S. aureus showed higher susceptibility breakpoints and the values of MIC was 6.25 mg/mL while MBC was 12.5 mg/mL and these results indicated that U. dioica was a more bactericidal agent against MDR P. aeruginosa than MDR S. aureus.

The wide-spectrum antibacterial potential of U. dioica in actual clinical settings where antibiotic resistance makes patient management extremely difficult is demonstrated by the consistency of MIC and MBC values against a number of MDR clinical isolates [21]. A stable antimicrobial activity against various MDR strains is suggested by the comparatively constant inhibitory and bactericidal concentrations. However, compared to the other isolates tested by Zhu et al., one P. aeruginosa isolate required significantly higher MIC and MBC values, serving as a reminder that there is inherent variation in susceptibility between laboratory commensal strains and clinical infectious isolates. This difference serves to emphasize the variable resistance patterns frequently encountered in

a hospital setting and further supports the need to test plant-derived antimicrobial agents against clinically derived microorganisms.

The development of globally disseminated multidrug-resistant microorganisms represents a significant problem to clinical management, particularly for P. aeruginosa and S. aureus [22]. The Global Antimicrobial Resistance and US Surveillance Systems report from 2018 emphasized the significance of creating innovative tactics to stop the spread of antibiotic resistance. This report noted that antibiotics are becoming increasingly ineffective. This has led to the search for adjuvant therapies, including evidence-based herbal therapies, which are increasingly seen as having the potential to contribute to the management of infection and antimicrobial stewardship [23, 24].

Studies have suggested that U. dioica has antimicrobial effects, although the potency of the extracts varies. Kukrić et al. indicated a MIC of 9.05 mg/mL for U. dioica against S. aureus, while Salehzadeh et al. indicated a MIC of 15 mg/mL and minimum bactericidal value of 20 mg/mL against S. aureus ATCC 6538 [21]. For comparison, the present study showed that the MIC value was lower at 6.25 mg/mL against multidrug-resistant S. aureus, suggesting relatively better antibacterial potency of the ethanolic extract under study. Variations in antibacterial efficacy of plant extracts are often associated with the plant chemotype, geographic origin, conditions of harvest, extraction solvent and technique, and the bacterial strains used in the study. Standardization of extraction and testing procedures is a key determinant in accurate phytopharmacological research results since these variables are collectively responsible for changes to the phytochemical composition and bioactivity.

The current study's MIC and MBC results for U. dioica are similar to those found in other species, such as the multidrug-resistant P. aeruginosa and S. aureus. The findings are consistent with prior research demonstrating the antimicrobial qualities of an ethanolic extract of U. dioica in the prevention of wound infection [25]. The current research focused on multidrug-resistant (MDR) clinical isolates, which tend to display a greater level of resistance, unlike previous research, which frequently tested standard laboratory strains. With respect to the current research, the MIC values for MDR S. aureus (6.25 mg/mL) tend to fall within the upper range of previous research, possibly as a reflection of reduced susceptibility, a characteristic of clinical wound isolates. Here, we show that U. dioica is similar to a clinically significant gram's +ve and gram's -ve pathogen, demonstrating the clinical significance of our findings and justifying more research as an adjuvant or replacement antimicrobial agent for infections that have developed drug resistance.

The potent antibacterial activity of ethanolic extracts from U. dioica is explained by this advantageous phytochemical profile. Flavonoids, phenolic compounds, and terpenoids natural chemicals with special rights to be utilized as antimicrobials are among the many bioactive components found in U. dioica. By permanently denaturing proteins, rupturing the integrity of bacterial cell membranes, and blocking essential metabolic processes necessary for bacterial viability, these phytochemicals have bactericidal effects [11, 19].

Although phytochemical profiling was not included in this work, future research should focus on identifying and characterizing these components to ascertain which bioactive composition is in charge of the observed antibacterial action. Examples of cutting-edge analytical techniques that may reveal specific mechanisms of action and clarify structural activity connections are chromatographic and spectrometric profiling. Additionally, these studies would enable the logical enhancement of antimicrobials produced from plants and their possible medicinal use.

Conclusion

Similar to clinical isolates of drug-resistant pathogens, antimicrobial efficacy against MDR S. aureus and P. aeruginosa that were isolated from patients experiencing surgical site infections with diarrheal symptoms suggests that Urtica dioica may be helpful in the development of plant-derived antimicrobial agents. Its effectiveness, especially against P. aeruginosa, indicates that it may have potential as an adjunct to conventional antibiotics. This study also emphasizes the significance of medicinal herbs as a source of antibacterial substances.

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