Effectiveness of crude ethanolic extracts of stinging nettle (Urtica dioica) and roman chamomile (Chamaemelum nobile) in combating the multi-drug resistant pathogens isolated from wounds at surgical sites

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Article

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Abstract

Objectives

This study investigates the antimicrobial activities of ethanolic extracts of *Urtica dioica* and *Chamaemelum nobile* against highly resistant pathogens.

Methods

104 pus samples were collected from patients after 72 hours of their surgery; 138 pathogens were isolated, and 16 multidrug-resistant (MDR) strains were identified, 10 of which were *P. aeruginosa* and 06 of which were *S. aureus*. 100 µL of crude ethanolic extracts from plant materials were used in the well diffusion method against isolated resistant pathogens. In a 96-well plate, 70% ethanolic plant extracts were serially diluted (50 to 0.097 µg/ml) to determine the minimum inhibitory concentration (MIC) using broth microdilution method. Each well contained 50 µL Mueller Hinton broth (MHB) and 50 µL bacterial inoculum (OD_{600} = 0.09–0.1). After incubating for 24 hours at 37°C, 10 µL of resazurin dye solution were added. The lowest concentration at which there was no color change to determine the MIC.

Results

All the test strains were sensitive to the *U. dioica* (zone diameter 14.5 ± 0.5 to 29.5 ± 0.5 mm) and *C. nobile* (zone diameter 14.77 ± 0.25 to 29.93 ± 0.4 mm) extracts. The ciprofloxacin was utilized as a positive control in this investigation (both *P. aeruginosa* and *S. aureus* exhibit ciprofloxacin resistance). Minimum inhibitory concentrations of *U. dioica* and *C. nobile* extracts against MDR strains of *S. aureus* (MIC = 6.25 mg/mL), and *P. aeruginosa* (MIC = 3.125 mg/mL) were calculated by applying the micro-broth dilution technique with 50 µL (mg) of crude extract. Both plant extracts had the same minimum bactericidal concentration (MBC) values against isolated pathogens: 6.25 mg/mL for *P. aeruginosa*, and 12.5 mg/mL for *S. aureus*.

Conclusion

According to the context of the research, the ethanolic extracts of *C. nobile* (flower) and *U. dioica* (leaves) demonstrated antimicrobial efficacy against the MDR strains isolated from surgical site infections, suggesting that they could be used as natural antiseptics and antimicrobials in therapeutics to control post-operative surgical site wound infections.

Introduction

A surgical site infection (SSI) occurs due to contamination of surgical wards by microorganisms. Its occurrence is within 30 days post-surgery. SSIs are among the most prevalent types of hospital-acquired

infections [1]. The infections might be superficial or deep, as well as one that affects multiple organs or bodily parts. Postoperative SSI is one of the most prevalent surgical site issues in individuals following surgical operations [2, 3]. The most common surgeries performed in postoperative SSIs are exploratory laparotomy, followed by laparoscopic cholecystectomy; colectomy, hernia, ulcer, and cancer were collected as wound swab samples. Pathogenic bacteria can reach the wound region via a variety of routes, including direct contact, self-contamination, and air dispersion. However, direct contact, inadequate hand washing by healthcare professionals, and inadequate sterilization during the pre and postoperative stages are among the major sources of infection. At the same time, there is inadequate information to determine the most prevalent route for hazardous microorganisms to enter the wound [4]. Commonly isolated organisms from surgical site infections include *S. aureus* and *P. aeruginosa*, CONS, *Streptococcus pyogenes*, *Enterobacter cloacae*, *E. coli, K. pneumoniae*, and *Acinetobacter baumannii* are also frequently isolated from post-operative SSIs [5].

Multidrug-resistant bacteria which include *P. aeruginosa* and *S. aureus* have emerged and spread globally, posing serious public health implications. India is known for having one of the nation's highest rates of antimicrobial resistance, caused by antibiotic abuse and misuse, poor infection control methods, and insufficient public health measures [6]. These organisms can cause a wide range of illnesses, from skin infections to potentially lethal disorders, particularly in immunocompromised patients [7]. MDR strains pose a crucial obstacle to healthcare systems due to their resistance to prescribed antibiotics, necessitating the exploration of alternative treatment options [8, 9, 10]. Natural plant products have traditionally played an essential role in the production of antibacterial agents. Medicinal plants such as Stinging nettle (*Urtica dioica*) and Roman chamomile (*Chamaemelum nobile*) are receiving attention for their bioactive components with possible antibacterial capabilities [11]. Stinging nettle is renowned for its extensive content of flavonoids, phenolic compounds, and tannins, which exhibit anti-microbial and anti-inflammatory activities [12]. Similarly, Roman chamomile contains essential oils, terpenoids, and flavonoids, which have demonstrated inhibitory effects against various pathogenic microorganisms [13].

The herbaceous perennial flowering plant *U. dioica*, which is found in North Africa, Asia, America, and Europe, is a member of the family *Urticaceae*. Because of its multi-utilitarian nature, it has enormous economic potential [14]. Urticating hairs on its stem and leaves and the plant can reach a height of one meter. The elongated or round leaves have scattered margins. The tiny green blooms are arranged in spikes that protrude from the leaf axil [15]. The antimicrobial activity of *U. dioica* aqueous and alcoholic extracts of against *S. aureus*, *B. subtilis*, *C. albicans*, *P. aeruginosa*, and *E. coli* [16]. Extracts from *U. dioica* leaves were assessed for the flavonoid and polyphenols content, as well as their antibacterial and antioxidant properties. The hydroethanolic extract has the strongest antibacterial and antioxidant properties *in vitro* [17].

A perennial herbaceous plant, *C. nobile* is a member of the *Asteraceae* family. Chamomile, a plant native to temperate regions of Europe and Asia, is grown all over the world due to its great culinary and therapeutic properties [18, 19]. Chamomile is one of the world’s most well-known and ancient

therapeutic herbs. Numerous pharmacological characteristics, including anti-ulcer, antibacterial, and anti-inflammatory, effects have been demonstrated for *C. nobile* [20].

The natural antibacterial substances present in medicinal plants are significant sources that could help treat several problematic bacterial diseases [21]. World Health Organization claims that plant materials with medicinal properties are the most effective approach to obtaining a variety of medications [22]. Numerous studies have demonstrated how effective ethanolic plant extracts are at preventing microbial infections. Ethanol is often employed as an extraction solvent because of its ability to dissolve a broad spectrum of phytochemicals, enhancing the antimicrobial potency of the extract [23, 24, 25, 26]. Investigating the antibacterial efficacy of *U*. *dioica* and *C*. *nobile* against MDR pathogens could shed light on their potential as alternative therapeutic agents. This study evaluates the anti-bacterial efficacy of ethanolic plant extractions of Stinging nettle and Roman chamomile against MDR *P*. *aeruginosa* and *S*. *aureus*.

Materials and Methods

Collection of plants materials and processing of samples

During September-October 2023, fresh *U*. *dioica* leaves were collected from Manali region in Kullu District, Himachal Pradesh, India [Latitude: 32.2396 °N, Longitude: 77.1887 °E, and Altitude: approximately 2,050 meters (6,726 feet) above sea level] and *C*. *nobile* flowers were collected from Bageshwar District Kumaon region of Uttarakhand, India [Latitude: 29.8403606 °N, Longitude: 79.769426 °E, Altitude: approximately 934 meters (3,064 feet) above sea level]. Both plants were identified by the botanist (Dr. Garima Bartariya, botanist at IIMT University, Meerut) and the herbarium submitted to the department (*U*. *dioica* Collection No: IIMTU/BOT/PIN-04 and *C*. *nobile* Collection No: IIMTU/BOT/PIN-02). *U. dioica* and *C*. *nobile* has been deposited in Botany Department herbarium at CCS University, Meerut, India. (*U*. *dioica* Voucher No: Ref: Bot/PB/455 and *C*. *nobile* Voucher No: Bot/PB/540). The components of collected plants (leaves of *U*. *dioica* and flowers of *C*. *nobile*) were properly rinsed with sterilized water, dried in a shelter at room temperature for 72 hours, and then finely ground with an electric grinder. In a sterile reagent bottle, a total of 50 gm of both plant powder was macerated in 500 ml of 70% ethanol (1:10 Weight/Volume ratio). The solution was sealed and let to rest for 72 hours at room temperature, with stirring occasionally to enhance extraction. The containers were kept in a dark and cool place to prevent degradation of the bioactive compounds. After maceration, the extract was filtered using Whatman No. 1 filter paper to get rid of any last bits of solid particles. Any solid particles from in the extract were eliminated by centrifuging the filtrates for 15 minutes at 3500 RPM after filtration. The solvent was evaporated after three repetitions by exposing the yielded materials to room temperature for 7 consecutive days. The final yield was stored at 2–4 ℃ for further use [27]. The final yield was quantified using the following formula:

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

U. dioica

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

C. nobile

$$\% \text{ Yield (w/w)} = \frac{(5.4 \text{ g}) \times 100}{50} = 10.8 \% \text{ (w/w)}$$

Isolation of pathogens from post operative surgical site wound infections

MDR pathogens were isolated from the pus samples collected from the patients (72 hours after their surgery) admitted in surgical wards no. 9 and 10 in LLRM Medical College and Hospital, Meerut, (UP) India. Pus samples were collected from the patients undergone the following surgery: Exploratory Laparotomy, Laparoscopic Cholecystectomy, Colectomy, Hernia, Ulcer, and Cancer. Swab samples were taken from patients who had postoperative surgical site infections. Samples from the wounds were collected using sterilized cotton swab sticks. The area surrounding the surgical wound was cleansed with 70% ethanol, and exudates obtained from the inner surface of the infected area were gently swabbed before being sent to the microbiological laboratory. These samples were transported to IIMT University, Meerut for further identification of the pathogens. These isolates were identified using various culture media (mannitol salt agar, blood agar, and cetrimide agar), gram staining, and different biochemical tests (catalase, indole, coagulase, citrate, lactose, mannitol, oxidase, urease test).

Screening of MDR Pseudomonas aeruginosa and MDR Staphylococcus aureus

Although we have isolated eight different types of bacterial pathogens from pus samples our study was focused on *P. aeruginosa* and *S. aureus* because both pathogens are a serious concern for human health because of their nature of multi-drug resistance. For the screening of MDR *P. aeruginosa* and MDR *S. aureus* Kirby-Bauer disk-diffusion technique was applied. In this study following antibiotics were selected (those were prescribed by the doctor to the patients after their surgery): ampicillin/sulbactam (10/10 mcg), linezolid (30 mcg), tetracycline (30 mcg), gentamicin (100 mcg), levofloxacin (5 mcg), piperacillin (100 mcg), ofloxacin (5 mcg), ciprofloxacin (5 mcg), and amikacin (30 mcg). *Staphylococcus aureus* ATCC25923 and *Pseudomonas aeruginosa* ATCC27853 strains were used as standard control.

Identification of MDR Pseudomonas aeruginosa and MDR Staphylococcus aureus using 16S rRNA sequencing

MDR strains were sent to a commercial facility, Biokart India Pvt. Ltd. (Bangaluru, Karnataka, India), for sequencing. For molecular identification, the 16S rRNA genes were amplified using PCR. A 1.5 kbp fragment of the 16S rDNA were amplified using high-fidelity PCR polymerase to ensure accuracy. The

PCR products were sequenced in bi-directionally (forward and reverse) using the ABI3130xl platform. The resulting sequence data were aligned and analyzed to determine bacterial identity and nearest phylogenetic relatives. Strict precautions were taken to prevent cross-contamination during amplification, and appropriate controls were included at each step to ensure data integrity.

The 16S rRNA gene (nucleotide) sequences data generated in this study have been deposited in the GenBank repository, a member of the International Nucleotide Sequence Database Collaboration (INSDC), and are publicly accessible via the National Center for Biotechnology Information (NCBI) database.

The 16S rRNA gene sequences of MDR *Staphylococcus aureus* isolates are available under accession number [PX023482 and PX024849], while sequences of *Pseudomonas aeruginosa* isolates are available under accession number [PX458929 and PX480726].

Antibacterial Activity Assay of U. dioica and C. nobile

The study included 16 MDR strains (10 MDR *P. aeruginosa* and 06 MDR *S. aureus*). By applying a well diffusion technique, the antibacterial efficacy of extraction of both plants was evaluated. Test microorganisms were inoculated into Mueller-Hinton agar, which were used to spread 0.5 McFarland standard suspensions (10^8 CFU/mL) that had been spread out using sterile swabs. Sterile borers were used to make 6 mm diameter wells in the agar plate. After that, 100 µL (mg) of crude extractions of the plant were added to them. The positive control was ciprofloxacin (5 mcg), while the negative control was 70% ethanol. After keeping the plates at room temperature (15 minutes), the plates were incubated at 37°C for 24 hours to enable the plant extract to penetrate the agar [28]. We measured the inhibitory zones in millimeters (mm). Every experiment was conducted in triplicate.

Determination of minimum inhibitory concentration and minimum bactericidal concentration

Applying the micro-broth dilution technique, MIC and MBC were determined. Extracts of both plants were serially diluted in a twofold dilution in a 96-well microtiter plate. The test was performed in 1–10 wells of a sterile 96-well micro-titer plate (round-bottom) with 50 µL of Mueller Hinton broth. 100 µL of 70% ethanolic plant extracts were added to well 11, and then transfer the 50 µL of highest to the lowest (50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.19, 0.097 µL) concentration into 11 − 2 microplate wells respectively, and finally from well 2, 50 µL was discarded. 50 µL of bacterial inoculums were then added to the well 1–11. The optical density (OD) of each inoculum was 0.09 to 0.1 (at λ = 600 nm). In each series, well No. 1, which contained bacterial inoculums and culture media, was considered a positive control, whereas well No. 12, which contained sterile MHB, was considered a negative control. As an indicator, 10 µL of resazurin dye solution are added to each well after being incubated at 37°C for 24 hours, and the wells were then incubated for 30 minutes to 2 hours. The plates were examined for color shifts, which were considered positive, from purple to pink/colorless. The lowest concentrations of plant

extract at which a color shift takes place are known as MIC values. Every experiment was conducted in triplicate. Using average values, the MIC of the test material were calculated.

On clinical bacterial isolates, MBC of the plant extract were conducted with the National Committee for Clinical Laboratory Standards (NCCLS) guidelines [29]. Each non-turbid tube provided a loopful of inoculums, which had been examined for 24 hours at 37°C after being streaked on a Nutrient Agar plate. When no observable growth was observed at the lowest concentration after an overnight incubation, the MBC value was calculated. To determine the extract concentration needed to kill 99.9% of the isolates of bacteria, the plates were incubated and checked for bacterial growth.

Statistical analysis

The mean ± the standard error of the mean (SEM) is used to display values. A two-way ANOVA variability was used to evaluate the data, and the variance was determined to be significant in statistical terms if $P \leq 0.05$.

Results

Diversity of pathogens in surgical site wound infections

A total of 104 swab samples were collected from post-operative surgical site wound infections. Out of these 104 samples, 96 (92.307%) were culture-positive and there was no growth on 8 (7.693%) samples. The total isolated pathogens were 138, among these pathogens, 13.10% were *S. aureus* and *P. aeruginosa* 17.40%, Coagulase-negative staphylococci were 10.15%, *S. pyogenes* was 5.80%, *E. coli* was 11.60%, *K. pneumoniae* was 18.70% *E. cloacae* was 13.10%, and *A. baumannii* was 10.15% (Fig. 1). For further study we focused on *P. aeruginosa* and *S. aureus* only because according to the literature survey these two pathogens are highly resistant to various antibiotics. 24 strains of *P. aeruginosa* and 18 strains of *S. aureus* were isolated during the study.

Morphological and Biochemical Analysis of Pathogens

All the isolates were cultivated on suitable culture for their morphological examination. All the isolates of *P. aeruginosa* were catalase positive, oxidase positive, citrate positive, were non-lactose fermenter on MacConkey's Agar, and fluorescence and pyocyanin pigment production were also observed on Cetrimide agar (Fig. 2B). All the isolates of *S. aureus* were catalase positive, co-agulase positive, showed mannitol fermentation on Mannitol salt agar (Fig. 2A), golden yellow colonies on NAM and beta haemolytic on blood agar. Pure cultures of all pathogens were further identified with the help of 16S rRNA sequencing.

Screening of MDR pathogens

Kirby-Bauer methods were using the screening of multi-drug resistant pathogens. Out of 24 isolated strains of *P. aeruginosa*, 10 were MDR strains [highly resistant to ciprofloxacin (87.50%), ampicillin/sulbactam (62.50%), gentamicin (50%), and amikacin (41.67%) (Fig. 3B and Fig. 4A)], and out of 18 *S. aureu*s isolates, 06 were MDR strains [highly resistant to ciprofloxacin (88.89%), gentamicin (55.56%), ampicillin/sulbactam (50%), and levofloxacin (38.89%), (Fig. 3A and Fig. 4B)]. Two ATCC standard control strains (*P. aeruginosa* ATCC27853, and *S. aureus* ATCC25923) were used for this study. All the MDR strains of *P. aeruginosa* were named as IIMT-PA01 to IIMT-PA10) and MDR strains of *S. aureus* were named as IIMT-SA01 to IIMT-SA06.

[**A**: *Staphylococcus aureus* (ampicillin/sulbactam (10/10 mcg), tetracycline (30 mcg), linezolid (30 mcg), levofloxacin (5 mcg), ciprofloxacin (5 mcg), and gentamicin (10 mcg); **B**: *Pseudomonas aeruginosa* (ampicillin/sulbactam (10/10 mcg), piperacillin (100 mcg), ciprofloxacin (5 mcg), ofloxacin (5 mcg), gentamicin (10 mcg), and amikacin (30 mcg)]

A: *Pseudomonas aeruginosa* (total isolates-24). **B**: *Staphylococcus aureus* (total isolates- 18).

*Blue: sensitive; Red: resistant [A/S: ampicillin/sulbactam (10/10 mcg), PC: piperacillin (100 mcg), RC: ciprofloxacin (5mcg), ZN: ofloxacin (5mcg), GM: gentamicin (10 mcg), AK: amikacin (30 mcg), TE: tetracycline (30 mcg), QB: levofloxacin (5 mcg), LZ: linezolid (30 mcg)]

Antibacterial activity of U. dioica and C. nobile

Using well diffusion method, the antimicrobial activity of 70% ethanolic extracts of *U. dioica* and *C. nobile*, were determined against 16 MDR isolates. 10 MDR isolates of *P. aeruginosa* and 06 MDR isolates of *S. aureus* were found to be susceptible to the effects of these extracts. All the 10 MDR isolates of *P. aeruginosa* were susceptible to the ethanolic extraction *U. dioica* (zone of inhibition diameter 17.5 ± 0.5 to 24.7 ± 0.36 mm) and *C. nobile* (zone of inhibition diameter 15.7 ± 0.36 to 29.93 ± 0.4 mm) (Fig. 5).9 out of 10 isolated MDR *P. aeruginosa* strains were resistant to the antibiotic ciprofloxacin (5 mcg), were used as a positive control for this assay (Table 1). Similarly, all the 06 MDR *S. aureus* were also sensitive to both the plant extracts. The ethanolic extract of *U. dioica* demonstrated efficacy against *S. aureus* (zone of inhibition diameter 14.5 ± 0.5 to 29.5 ± 0.5 mm), while the ethanolic extract of *C. nobile* demonstrated activity (zone of inhibition diameter 16.56 ± 0.6 to 21.93 ± 0.4 mm) (Fig. 6). Ciprofloxacin (5 mcg) was used as positive control and this antibiotic was resistant to all 6 isolated MDR *S. aureus* (Table 2). The positive control, ciprofloxacin, showed the inhibition zone range (00 ± 00 to 26.6 ± 0.36 mm) against *P. aeruginosa*. The positive control, ciprofloxacin, showed the inhibition zone range (00 ± 00 to 16.6 ± 0.4 mm) against *S. aureus*. All these extracts showed a weak to moderate activities.

*Well 1 *U. dioica* (18–25 mm), well 2 *C. nobile* (16–30 mm), 3 (ciprofloxacin) used as positive control, and well 4 (70% ethanol) used as negative control

*Well 1 *U. dioica* (15–30 mm), well 2 *C. nobile* (17–22 mm), 3 (ciprofloxacin) used as positive control, and well 4 (70% ethanol) used as negative control

Minimum inhibitory concentration and Minimum bactericidal concentration of Urtica dioica and Chamaemelum nobile

MIC and MBC values shows the 70% ethanolic crude extracts of *U*. *dioica* and *C*. *nobile* have antibacterial activity against *P*. *aeruginosa* and *S*. *aureus* (Tables 3 and Table 4). For 06 MDR *S*. *aureus* isolates, *U*. *dioica* leaves and *C*. *nobile* flowers extracts had MICs and MBCs of 6.25 and 12.5 μL, respectively (Fig. 7). MIC and MBC of *U*. *dioica* leaf extracts were 3.125 and 6.25 μL and *C*. *nobile* flower extracts were 3.125 and 6.25 μL concentrations for 10 MDR *P*. *aeruginosa* isolates respectively (Fig. 8). Two control strains were used, one for Gram-positive *S*. *aureus* (ATCC25923) MIC and MBC of the extract *U*. *dioica* (6.25 and 12.5 μL) and *C*. *nobile* (6.25 and 12.5 μL) and therefore Gram-negative *P*. *aeruginosa* (ATCC27853) there MIC and MBC of the extracts of *U*. *dioica* (3.125 and 6.25 μL) and *C*. *nobile* (3.125 and 6.25 μL) respectively.

***** *In* each series, well no. 1 (positive control) shows pink color or colorless, well no. 2 (negative control) shows purple color, well 2–11 used as test for MIC and MBC which color shifts from purple to pink or colorless (use of Resazurin dye for the responsible of color shift)

***** *In* each series, well no. 1 (positive control) shows pink color or colorless, well no. 2 (negative control) shows purple color, well 2–11 used as test for MIC and MBC which color shifts from purple to pink or colorless (use of Resazurin dye for the responsible of color shift)

As a result, the *U*. *dioica* leaves and *C*. *nobile* flower extracts indicated an anti-bacterial effect on the MDR *P*. *aeruginosa* and MDR *S. aureu*s isolates of post-operative surgical site wound infections.

Table 1

Inhibitory effects of various ethanolic leaves extract of *U. dioica* and flower extract of *C. nobile* and positive control Ciprofloxacin against *Pseudomonas aeruginosa* strains

Mean diameter of inhibitory zone (in mm ± SD)					
Gram Negative Bacteria (*P. aeruginosa*)					
S.N.	**Strain I.D.**	**Source**	***U. dioica* (leaves extract) (100 mg/mL)**	***C. nobile* (flower extract) (100 mg/mL)**	**Ciprofloxacin (5 mcg) (Positive Control)**
1	IIMT -PA01	Exploratory laparotomy	22.5 ± 0.5	22 ± 0.2	26.6 ± 0.36
2	IIMT -PA02	Hernia	19.77 ± 0.68	22.5 ± 0.5	00 ± 00
3	IIMT -PA03	Exploratory laparotomy	21.7 ± 0.75	25.8 ± 0.26	22.7 ± 0.3
4	IIMT-PA04	Laparoscopic cholecystectomy	24.7 ± 0.36	29.93 ± 0.4	11.77 ± 0.25
5	IIMT-PA05	Exploratory laparotomy	22.9 ± 0.85	19.6 ± 0.52	22.97 ± 0.05
6	IIMT-PA06	Exploratory laparotomy	23.37 ± 0.6	20.47 ± 0.83	00 ± 00
7	IIMT-PA07	Exploratory laparotomy	17.5 ± 0.5	15.7 ± 0.36	00 ± 00
8	IIMT-PA08	Ulcer	20.4 ± 0.52	17.67 ± 0.3	12.53 ± 0.5
9	IIMT-PA09	Laparoscopic cholecystectomy	19.97 ± 0.45	24.6 ± 0.4	7.3 ± 0.6
10	IIMT-PA10	Laparoscopic cholecystectomy	21.6 ± 0.36	18.4 ± 0.6	7.63 ± 0.4
11	ATCC27853		19.67 ± 0.41	14.77 ± 0.25	25.77 ± 0.32
*****Mean value of triplicate measurement ± Standard deviation (SD)					
*P–value (P = 0.0032) statistically significant					

Table 2

Inhibitory effects of various ethanolic leaves extract of *U. dioica* and flower extract of *C. nobile* and positive control Ciprofloxacin against *Staphylococcus aureus* strains

Mean diameter of inhibitory zone (in mm ± SD)					
Gram's-Positive Bacteria (*S. aureus*)					
S.N.	**Strain I.D.**	**Source**	***U. dioica* (leaves extract) (100 mg/mL)**	***C. nobile* (flower extract) (100 mg/mL)**	**Ciprofloxacin (5 mcg) (Positive Control)**
1	IIMT -SA01	Exploratory laparotomy	29.5 ± 0.5	19.77 ± 0.68	15.93 ± 0.4
2	IIMT -SA02	Ulcer	21.67 ± 0.57	17.77 ± 0.25	00 ± 00
3	IIMT -SA03	Hernia	15.57 ± 0.51	17.5 ± 0.5	15.5 ± 0.5
4	IIMT-SA04	Laparoscopic cholecystectomy	14.5 ± 0.5	16.57 ± 0.6	7.67 ± 0.57
5	IIMT-SA05	Exploratory laparotomy	15.27 ± 0.87	18.73 ± 0.3	14.8 ± 0.26
6	IIMT-SA06	Laparoscopic cholecystectomy	14.67 ± 0.57	21.93 ± 0.4	16.6 ± 0.4
7	ATCC25923		29.4 ± 0.65	21.5 ± 0.5	27.43 ± 0.6
*****Mean value of triplicate measurement ± Standard deviation (SD)					
* P–value (P = 0.0057) statistically significant					

Table 3
MIC and MBC of ethanolic crude plant extracts against MDR *Pseudomonas aeruginosa*

	***P. aeruginosa* MIC and MBC (mg/mL)**					
			U. dioica		***C. nobile***	
S.N.	**Strain I.D.**	**OD (λ = 600 nm)**	**MIC (in 50 mg/mL)**	**MBC (in 50 mg/mL)**	**MIC (in 50 mg/mL)**	**MBC (in 50 mg/mL)**
1	ATCC27853	0.0969 ± 0.0020	3.125	6.25	3.125	6.25
2	IIMT-PA01	0.0965 ± 0.0013	3.125	6.25	3.125	6.25
3	IIMT-PA02	0.0965 ± 0.0009	3.125	6.25	3.125	6.25
4	IIMT-PA03	0.0972 ± 0.0010	3.125	6.25	3.125	6.25
5	IIMT-PA04	0.0967 ± 0.0018	3.125	6.25	3.125	6.25
6	IIMT-PA05	0.0949 ± 0.0040	3.125	6.25	3.125	6.25
7	IIMT-PA06	0.0948 ± 0.0034	3.125	6.25	3.125	6.25
8	IIMT-PA07	0.0949 ± 0.0028	3.125	6.25	3.125	6.25
9	IIMT-PA08	0.0962 ± 0.0030	6.25	12.5	3.125	6.25
10	IIMT-PA09	0.0971 ± 0.0016	3.125	6.25	3.125	6.25
11	IIMT-PA10	0.0964 ± 0.0025	3.125	6.25	3.125	6.25

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

Table 4
MIC and MBC values of ethanolic crude plant extracts against MDR *Staphylococcus aureus*

	***S. aureus* MIC and MBC (mg/mL)**					
			U. dioica		***C. nobile***	
S.N.	**Strain I.D.**	**OD (λ = 600 nm)**	**MIC (in 50 mg/mL)**	**MBC (in 50 mg/mL)**	**MIC (in 50 mg/mL)**	**MBC (in 50 mg/mL)**
1	ATCC25923	0.0963 ± 0.0013	6.25	12.5	6.25	12.5
2	IIMT-SA01	0.0970 ± 0.0017	6.25	12.5	6.25	12.5
3	IIMT-SA02	0.0942 ± 0.0031	6.25	12.5	6.25	12.5
4	IIMT-SA03	0.0957 ± 0.0017	6.25	12.5	6.25	12.5
5	IIMT-SA04	0.0982 ± 0.0004	6.25	12.5	6.25	12.5
6	IIMT-SA05	0.0942 ± 0.0018	6.25	12.5	6.25	12.5
7	IIMT-SA06	0.0961 ± 0.0012	6.25	12.5	6.25	12.5

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

Discussion

Crude ethanolic extracts of *U. dioica* and *C. nobile* are tested for their antimicrobial activity against MDR pathogens isolated from surgical site wounds. Drug-resistant bacteria and increase in antibiotic resistance in recent years have emerged to find novel antimicrobial agents. The effects of *U. dioica* and *C. nobile* Crude ethanolic extracts on MDR *P. aeruginosa* and *S. aureus* were investigated *in vitro*. The results demonstrated that these extracts have antimicrobial properties. All test organisms of *P. aeruginosa* and *S. aureus* were susceptible to extracts of *U. dioica* a with inhibition zone (17.5 ± 0.5 to 24.7 ± 0.36 mm and 14.5 ± 0.5 to 29.5 ± 0.5 mm), and all the test organisms of *P. aeruginosa* and *S. aureus* were susceptible to extract of *C. nobile* with inhibition zone (15.7 ± 0.36 to 29.93 ± 0.4 mm and 16.57 ± 0.6 to 21.93 ± 0.4 mm).

Surgical site wound infections occur in the area where the surgery has been performed and these infections generally are treated using antibiotics. According to recent data, SSIs considerably contribute to postoperative morbidity and mortality, as evidenced by recent data showing that they cause over 2 million of nosocomial infections in the United States [20]. To combat these infections antibiotics are given to the patients and in recent years, the emergence of drug-resistant microorganisms is among the

most problematic causes to worldwide growth and human health. In 2019, bacterial resistance is expected to have kill 1.27 million human beings and contribute to 4.95 million deaths globally [30]. A new study is centered on extracts of the leaves of *U*. *dioica* and the flower of *C*. *nobile* as a source of antimicrobial agent (topical use) to treat post-operative surgical site wound infections and both the plants were tested for their antibacterial activity against isolated pathogens.

U. *dioica* has a significant role in traditional treatment of blood pressure, arthritis, diabetes, and allergic rhinitis and its extract has been utilized in conventional medicine to treat eczema, joint pain, digestion, and anemia [31, 32]. In our study, the antibacterial impact of ethanolic crude extract of *U*. *dioica* was examined on the in vitro growth of MDR *P*. *aeruginosa* (10 isolates) and MDR *S*. *aureus* (6 isolates). All the MDR isolates (isolated from surgical site wound infections) were sensitive to *U*. *dioica*. Although some scientists reported its antibacterial activity against the pathogens as per our knowledge, we have not found any research paper related to the anti-bacterial activity of *U*. *dioica* against surgical site wound infections of human beings [33, 34].

It is thought that *C*. *nobile* has calming, anti-inflammatory, carminative, and antispasmodic capabilities. Although *C*. *nobile* has been demonstrated to have *in vivo* anti-bacterial and wound-healing activity [35], its antibacterial properties of *C*. *nobile* against the pathogens isolated from surgical site infections have not been reported yet. In our investigation, we discovered that *C*. *nobile* had extremely good antibacterial activity against MDR *P*. *aeruginosa* and MDR *S*. *aureus* isolated from surgical site wound infections.

U. *dioica* and *C*. *nobile* have a stronger antibacterial activity due to high number of flavonoids, phenolic compounds, and tannins, which are known to disrupt bacterial cell membranes and interfere with important microbial enzymes [12]. These bioactive compounds likely act synergistically, enhancing the overall antimicrobial effect. *C*. *nobile* exhibited notable antimicrobial activity against MDR pathogens that is higher than *U*. *dioica*.

Nowadays, plant extracts are widely available and considered an effective alternative for wound healing. After an injury, the wound healing process is a very intricate restoration process. Angiogenesis may be delayed by *P. aeruginosa* and *S. aureus* infections, which can also disrupt the normal clotting mechanisms and delayed the inflammatory phase. The current investigation discovered that the ethanolic crude extract of *U. dioica* and *C. nobile* were discovered to show exceptional antimicrobial properties *in vitro* [36].

MIC value of *U. dioica and C. nobile was* 3.125 mg/mL for all 10 isolated MDR strains of *P*. *aeruginosa* but it was 6.25 mg/mL for strain no. IIMT-PA08 while the MBC value of both the plant extract for all 10 isolates of *P*. *aeruginosa* was 6.25 mg/mL (except strain no. IIMT-PA08; MBC was 12.5 mg/mL). If we discuss the MDR *S*. *aureus*; the MIC value of *U*. *dioica* and *C*. *nobile* was 6.25 mg/mL and the MBC value was 12.5 mg/mL against all 06 isolates strains of *S*. *aureus* (IIMT-SA01 to IIMT-SA06). This value suggests that both the plant extracts were more effective against MDR *P. aeruginosa* as compared to MDR *S. aureus*.

In their investigation of *U*. *dioica* antimicrobial activity against several clinical isolates, Salehzadeh et al. reported that the MIC 15 mg/ml and MBC 20 mg/ml against the reference strain of *S*. *aureus* ATCC 6538 [37]. In another study, MIC against the *S*. *aureus* was reported to as 9.05mg/mL Kukric et al., However, this study revealed the MIC value of 6.25mg/mL against MDR *S*. *aureus* [24].

This study shows that *Urtica dioica's* ethanolic extraction has antibacterial properties against both of the isolated MDR pathogens, but Harrison et al.'s investigation of *U*. *dioica's* antibacterial activity with vinegar, oil, and butter revealed no antimicrobial activity against *S*. *aureus* and *P*. *aeruginosa* [38].

The antibacterial properties of German chamomile against *P*. *aeruginosa* and MRSA were examined [39]. They have not found any inhibitory activity against *P*. *aeruginosa*, while the MIC and MBC values for MRSA were 6.25 and 12.5 mg/mL respectively. All *P*. *aeruginosa* isolates that produced metallo-beta-lactamases were MIC and MBC of 12.5 and 25 mg/mL, for chamomile leaf extract (not specifically *C*. *nobile*) [40]. The disc diffusion method and ethanolic chamomile extract were utilized by Solidonio et al. in Brazil to test for antimicrobial activity; however, no inhibitory activity against *P. aeruginosa* was observed [41]. However, they have not mentioned the species of chamomile. As per our knowledge, we have not found any research paper claiming the antimicrobial activity of *C*. *nobile* against these pathogens.

For MDR *S. aureus* and *P. aeruginosa*, the MIC and MBC values of *U. dioica* and *C. nobile* in our investigation are mostly consistent with other recent studies. Our results are in line with the substantial antibacterial activity of *U*. *dioica* ethanolic extracts against wound-healing microorganisms, which had MIC values ranging from 3.12 mg/mL to 6.25 mg/mL [42]. Similarly, recent work by Prado et al., on *C. nobile* demonstrated MIC values in the 2.5-5 mg/mL range against Gram-negative bacteria, showing comparable potency [43]. Minor discrepancies in MIC and MBC values observed across studies could be attributed to numerous factors, including differences in plant origin, extraction method, solvent polarity, and pathogen resistance profiles.

Conclusion

This study found that crude ethanolic extracts of *U*. *dioica* and *C*. *nobile* have a significant antimicrobial impact on surgical site isolates of MDR *P*. *aeruginosa* and *S*. *aureus*. The zone of inhibition is large and the MIC and MBC values are low, suggesting a high bacteriostatic and bactericidal capability. These findings suggest that these plants could be effective, natural alternatives to traditional antibiotics, particularly in the development of topical medications like antimicrobial gels, wound dressings, or rinses in post-surgical infection control. Future studies should concentrate on discovering active phytochemicals, analyzing toxicity and pharmacokinetics *in vivo*, and investigating synergistic combinations with existing antibiotics to improve therapeutic efficacy.

Abbreviations

AMR

Antimicrobial resistance
CLSI
Clinical and laboratory standard institute
CONS
Coagulase negative *Staphylococci*
MBC Minimum bactericidal concentration
MDR
Multidrug-resistant
MHB
Mueller Hinton agar
MIC
Minimum inhibitory concentration
NAM
Nutrient agar medium
NCBI
National center for biotechnology information
OD
Optical density
rRNA
Ribosomal ribonucleic acids
SSIs
Surgical site infections.

Declarations

Ethical permission

This research was conducted under ethical clearance certificate, provided by the Research Ethical Committee at IIMT University [Ref. No: IIMTU/REC/2025/003], and LLRM Medical College Hospital, Meerut [Rec. No: 15171, Date: 30/11/2023] Patients, or parents in the case of children, were informed of the study's objectives, and those who gave their consent were part of this study.

The collection of plant materials, such as Stinging nettle (*Urtica dioica*) and Roman chamomile (*Chamaemelum nobile*), was done in line with applicable institutional guidelines. Before collecting samples, the appropriate authorities gave permission and collection licenses. The study did not include endangered or protected species, and all methods followed rules for protecting biodiversity.

Ethics approval and consent to participate: This study was approved by the IIMT Research ethical Committee, IIMT University, Meerut Uttar Pradesh, India. All procedures involving human participants were conducted in accordance with the ethical standards of the committee and the Declaration of Helsinki. The informed consent was obtained from all of the participants.

Consent for publication: Applicable
Availability of data and materials: All data generated or analysed during this study are included in this published article.

The datasets generated and analysed during the current study are available in the GenBank repository, under accession number (*Staphylococcus aureus*) **PX023482** and **PX024849**, and (*Pseudomonas aeruginosa*) **PX458929** and **PX480726.**
Competing Interests: No competing Interests
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Authors' contributions: Atul Singh: Writing- original draft, investigation, Data curation, Conceptualization. **Mukta Sharma:** Conceptualization,Formal analysis, Supervision.

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References

1. Upula, S. A. et al. Characterization of Virulent Bacterial Isolates Associated with Multi-Drug Resistance among Patients with Surgical Site Infections in Selected Specialist Hospitals in Calabar, Nigeria. *Annual Res. Rev. Biology*. **37** (12), 75–85. https://doi.org/10.9734/arrb/2022/v37i1230559 (2022).
2. Sattar, F., Sattar, Z., Zaman, M. & Akbar, S. Frequency of Post-operative Surgical Site Infections in a Tertiary Care Hospital in Abbottabad, Pakistan 2019. *Cureus*, **24**(3). https://doi.org/10.7759/cureus.4243
3. Sway, A. et al. Prospective cohort study on timing of antimicrobial prophylaxis for post-cesarean surgical site infections. *Surg. Infect.* **21** (6), 552–555 (2020).
4. ElHawary, H., Hintermayer, M. A., Alam, P., Brunetti, V. C. & Janis, J. E. Decreasing Surgical Site Infections in Plastic Surgery: A Systematic Review and Meta-analysis of Level 1 Evidence. Aesthet Surg J. 2021; Jun 14; 41(7):NP948-NP958. 10.1093/asj/sjab119. PMID: 33693462.
5. Kaye, K. S. et al. The deadly toll of invasive Methicillin resistant *Staphylococcus aureus* infection in community hospitals. *Clin. Infect. Dis.* **46**, 1568–1577 (2008). (PubMed: 18419491).
6. Laxminarayan, R. et al. Antibiotic resistance—the need for global solutions. *Lancet. Infect. Dis*. **13** (12), 1057–1098 (2013).
7. Gordon, R. J. & Lowy, F. D. Pathogenesis of Methicillin-Resistant *Staphylococcus aureus* Infection. *Clin. Infect. Dis.* **46** (S5), S350–S359 (2008).

8. Kasra-Kermanshahi, R. & Mobarak-Qamsari, E. Inhibition effect of lactic acid bacteria against foodborne pathogen, *Listeria monocytogenes*. *Appl. Food Biotechnol.* **2** (4), 11–19 (2015).
9. Ventola, C. L. The antibiotic resistance crisis: part 1: causes and threats. *Pharm. Ther.* **40** (4), 277–283 (2015).
10. Meshkibaf, M. H. et al. Antibacterial effects of hydroalcoholic extracts of *Ziziphoratenuior*, *Teucrium polium*, *Barberiscorcorde*, and *Stachys inflate*. *J. Semnan Univ. Med. Sci.* **11** (4), 24–245 (2010).
11. Nostro, A., Germano, M. P., D'Angelo, V., Marino, A. & Cannatelli, M. A. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett. Appl. Microbiol.* **30** (5), 379–384 (2000).
12. Gülçin, İ., Küfrevioğlu, Ö. İ., Oktay, M. & &Büyükokuroğlu, M. E. Antioxidant, antimicrobial, anti-inflammatory, and anticancer activities of *Urtica dioica* L. *J. Ethnopharmacol.* **90** (2–3), 205–215 (2004).
13. McKay, D. L. & Blumberg, J. B. A review of the bioactivity and potential health benefits of chamomile tea (*Matricaria recutita L*). *Phytother. Res.* **20** (7), 519–530 (2006).
14. Kasouni, A. I. et al. The unexplored wound healing activity of *Urtica dioica* l. Extract: An in vitro and in vivo study. *Molecules* **26** (20), 1–20. https://doi.org/10.3390/molecules26206248 (2021).
15. Velasco, R. et al. Antibacterial activity of three Urtica species. *Int. J. Pharm. Sci. Res.* **12** (2), 1293–1299. https://doi.org/10.13040/IJPSR.0975-8232.12 (2021).
16. Of, E. et al. *Evaluation Antibact. Activities Urtica Dioica Against* ; **4**(7), 216–220. (2015).
17. Zouari Bouassida, K. et al. &Trigui M. Exploring the Urtica dioica Leaves Hemostatic and Wound-Healing Potential. *Biomed. Res. Int.* https://doi.org/10.1155/2017/1047523 (2017).
18. Dai, Y. L. et al. Chamomile: A Review of Its Traditional Uses, Chemical Constituents, Pharmacological Activities and Quality Control Studies. *Molecules* **28** (1). https://doi.org/10.3390/molecules28010133 (2023).
19. Filipović, V. et al. &Ugrenović V. The First Study on Cultivating Roman Chamomile (*Chamaemelum nobile*(L.) All.) for its Flower and Essential Oil in Southeast Serbia. *Horticulturae* **10** (4). https://doi.org/10.3390/horticulturae10040396 (2024).
20. Kazemian, H. et al. In vivo Antibacterial and Wound Healing Activities of Roman Chamomile (*Chamaemelum nobile*). *Infect. Disord Drug Targets*. **18** (1), 41–45 (2018). Doi: 10.2174/1871526516666161230123133. PMID: 28034365.
21. Iwu, M. W., Duncan, A. R. & Okunji, C. O. New Antimicrobials of Plant Origin. In: (ed Janick, J.) Perspectives on New Crops and New Uses, ASHS, Alexandria, ; 457–462. (1999).
22. W.H.O. Traditional Medicine Strategy (2002–2005). WHO/EDM/TRM ; 1. (2002).
23. Cowan, M. M. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* **12** (4), 564–582 (1999).
24. Kukrić, Z. Z. et al. Characterization of antioxidant and antimicrobial activities of nettle leaves (*Urtica dioica* L). *Acta Periodica Technologica*. **43**, 257–272. https://doi.org/10.2298/APT1243257K (2012).

25. Sidaoui, F., BelghithIgueld, S., Barth, D., Trabelsi-Ayadi, M. & Cherif, J. K. Study of Tunisian nettle leaves (*Urtica dioica* L.): mineral composition and antioxidant capacity of their extracts obtained by maceration and supercritical fluid extraction. *Ijppr* **7**, 707–713 (2015).
26. Riehemann, K., Behnke, B. & Schulze-Osthoff, K. Plant extracts from stinging nettle (Urtica dioica), an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-κB. *FEBS Lett.* **442** (1), 89–94. 10.1016/S0014-5793(98)01622-6] (1999). [DOI:.
27. Kiani, M. et al. Antimicrobial Activity of Ethanolic and Methanolic Extract of *Urtics dioica*, *Mentha longifolia*, and *Bacteriocin* produced by *actobacillus casei* against Antibiotic-resistant Bacteria. *Res. Mol. Med.* **8** (4), 163–170. https://doi.org/10.32598/rmm.8.1062.2 (2020).
28. Riose, J. L., Recio, M. C. & Villar, A. Screening methods for natural products with antimicrobial activity: a review of the literature. *J. Ethnopharmacol.* **23**, 127–149 (1988).
29. National Committee for Clinical Laboratory Standards (NCCLS). ; Vol.32, Issue: 2.Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard. National Committee for Clinical Laboratory Standards, Wayne, Pennsylvania, USA (2012).
30. Zabaglo, M., Leslie, S. W. & Sharman, T. Postoperative Wound Infections. In: StatPearls [Internet]. Treasure Island (FL). ; (2024). Available from: https://www.ncbi.nlm.nih.gov/books/NBK560533/
31. WHO. *Antimicrobial resistance: accelerating national and global responses* (World Health Organization, December 2023). https://apps.who.int/gb/ebwha/pdf_files/EB154/B154_13-en
32. Fattahi, S. et al. Total Phenolic and Flavonoid Contents of Aqueous Extract of Stinging Nettle and In Vitro Antiproliferative Effect on Hela and BT-474 Cell Lines. *Int. J. Mol. Cell. Med.* **3** (2), 102–107 (2014).
33. Chrubasik, J. E., Roufogalis, B. D., Wagner, H. & Chrubasik, S. A. A comprehensive review on nettle effect and efficacy profiles, part I: herba urticae. *Phytomedicine* **14** (6), 423–435 (2007). [DOI] [PubMed] [Google Scholar].
34. Aydin, H. B., Korkmaz, S. & Korkmaz, B. I. O. Antibacterial and Antiviral Activities of Stinging Nettle (*Urtica Dioica* L.) Leaf Extract on Norovirus and *Campylobacter jejuni* as Foodborne Pathogens. *J. Microbiol. Biotechnol. Food Sci.* **13** (1). https://doi.org/10.55251/jmbfs.9768 (2023).
35. Sándor, Z. et al. Evidence supports tradition: The in vitro effects of Roman Chamomile on smooth muscles. *Front. Pharmacol.* **9** (APR), 1–11. https://doi.org/10.3389/fphar.2018.00323 (2018).
36. Süntar, I., Akkol, E. K., Nahar, L. & Sarker, S. D. Wound healing and antioxidant properties: do they coexist in plants? *Free Radicals Antioxid.* **2** (2), 1–7. https://doi.org/10.5530/ax.2012.2.2.1 (2012).
37. Salehzadeh, A., Asadpour, L., Naeemi, A. S. & Houshmand, E. ; 11(1), 38–40. (2014).
38. Harrison, F., Furner-Pardoe, J. & Connelly, E. An assessment of the evidence for antibacterial activity of stinging nettle (*Urtica dioica*) extracts. *Access. Microbiol.* **4** (3). https://doi.org/10.1099/acmi.0.000336 (2022).
39. Azari, A. A. & Danesh, A. Antibacterial Effect of Matricaria chamomilla Alcoholic extract against Drug-resistant Isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Infect. Epidemology Microbiol.* **7 910**, 29–35. 10.52547/iem.7.1.29 (2021).

40. Eslami, G. et al. Antibacterial effects of *Zataria multiflora*, *Ziziphus*, Chamomile and *Myrtus communis* methanolic extracts on IMP-type metallo-beta-lactamase-producing Pseudomonas aeruginosa. Archives of Clinical Infectious Diseases, ; 11(1), 1–5. (2016). https://doi.org/10.5812/archcid.32413
41. Solidônio, E. G. et al. &Colaço W. Evaluation of antimicrobial activity of extracts of chamomile (*Matricaria recutita* L.) obtained from irradiated and non-irradiated samples. The Battle against Microbial Pathogens: Basic Science, Technological Advancements and Educational Programmes, ; 151–156. (2015).
42. Ahmad, M., Khan, R. A. & Ali, S. Therapeutic efficacy of *Urtica dioica* extract in diabetic wound infection models. *J. Ethnopharmacol.* **320**, 116876. https://doi.org/10.1016/j.jep.2023.116876 (2023).
43. Prado, P. R., Alves, L. F. A., Pereira, L. P. & Silva, B. M. Antibacterial and healing properties of Roman chamomile: A review. *Phytother. Res.* **36** (5), 2235–2248. https://doi.org/10.1002/ptr.7430 (2022).

Figures

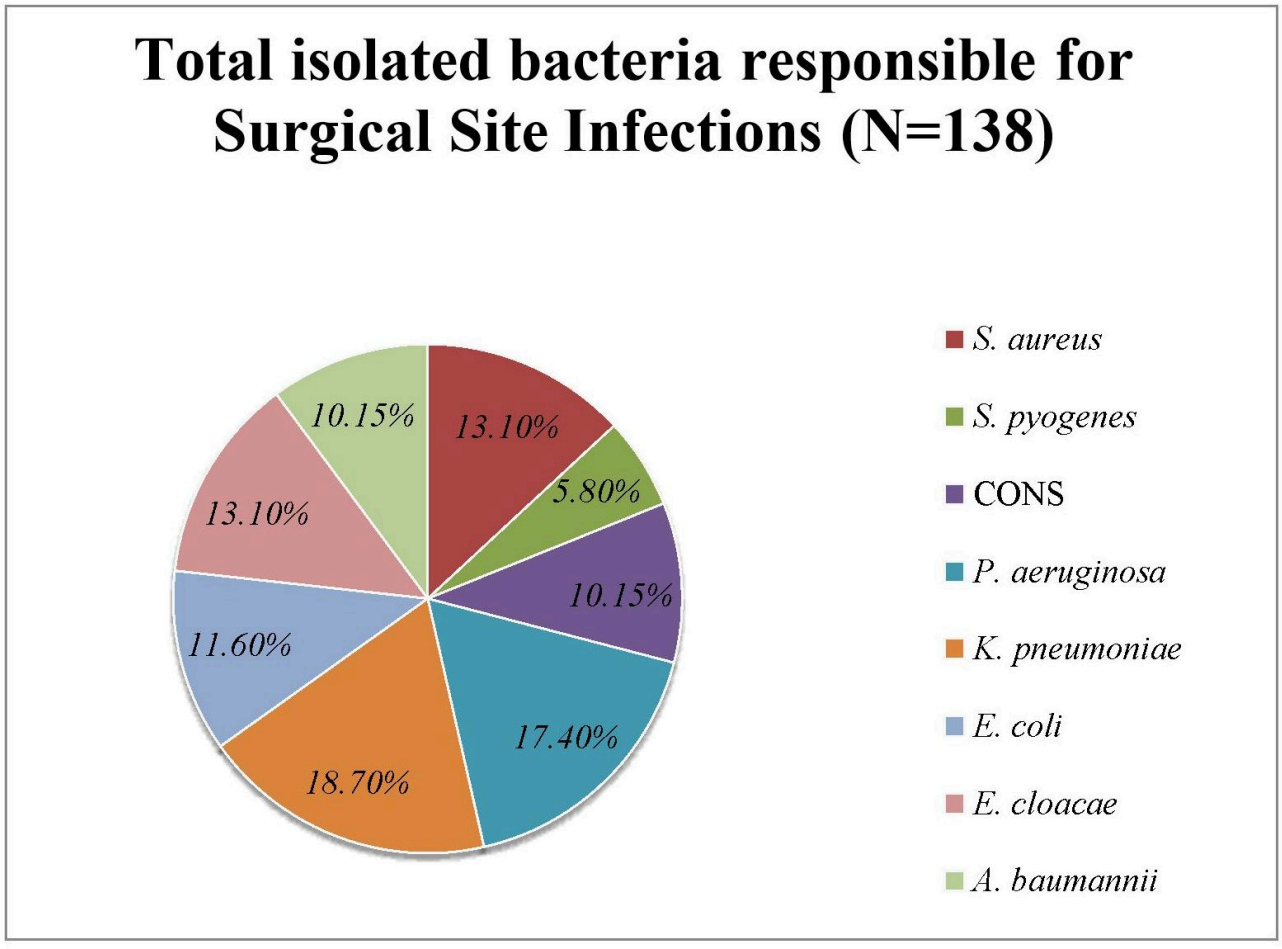

Fig. 1 Diversity of pathogens in post operative surgical site wound infections

Figure 1

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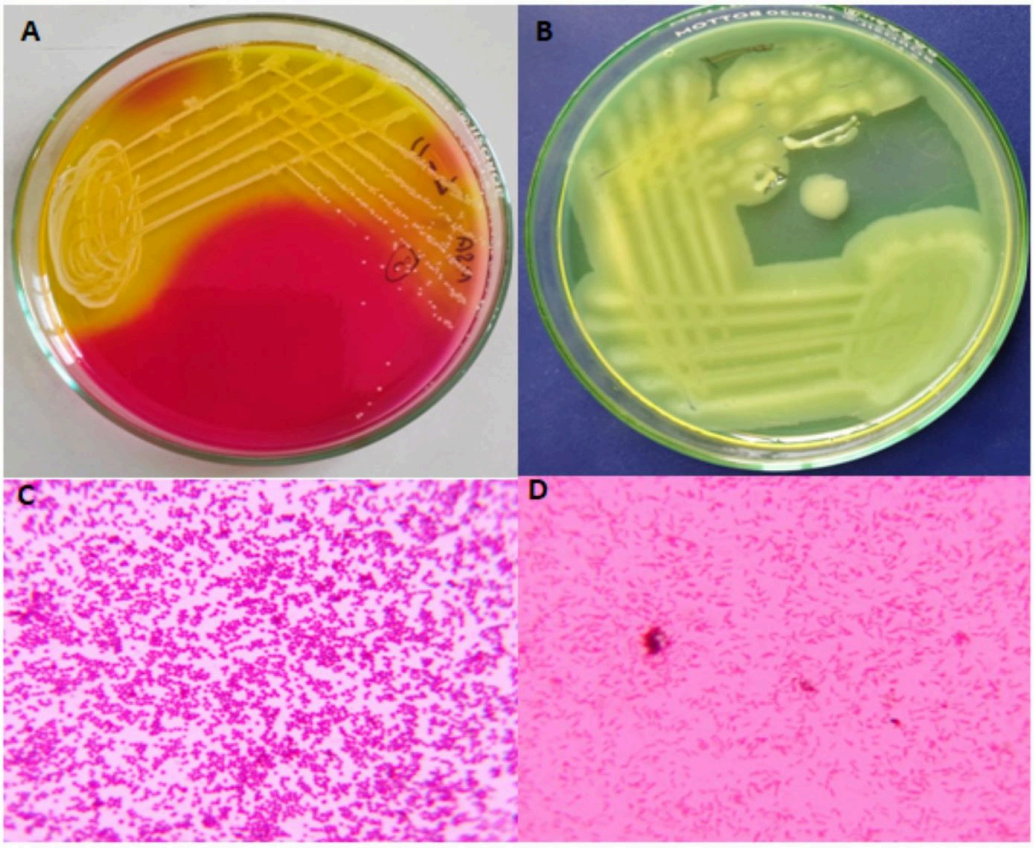

Fig. 2 Culture characteristics and microscopic identification **(A)** *S. aureus* on Mannitol salt agar, **(B)** *P. aeruginosa* on Cetrimide agar, **(C)** Microscopic view of *S. aureus* and **(D)** Microscopic view of *P. aeruginosa*

Figure 2

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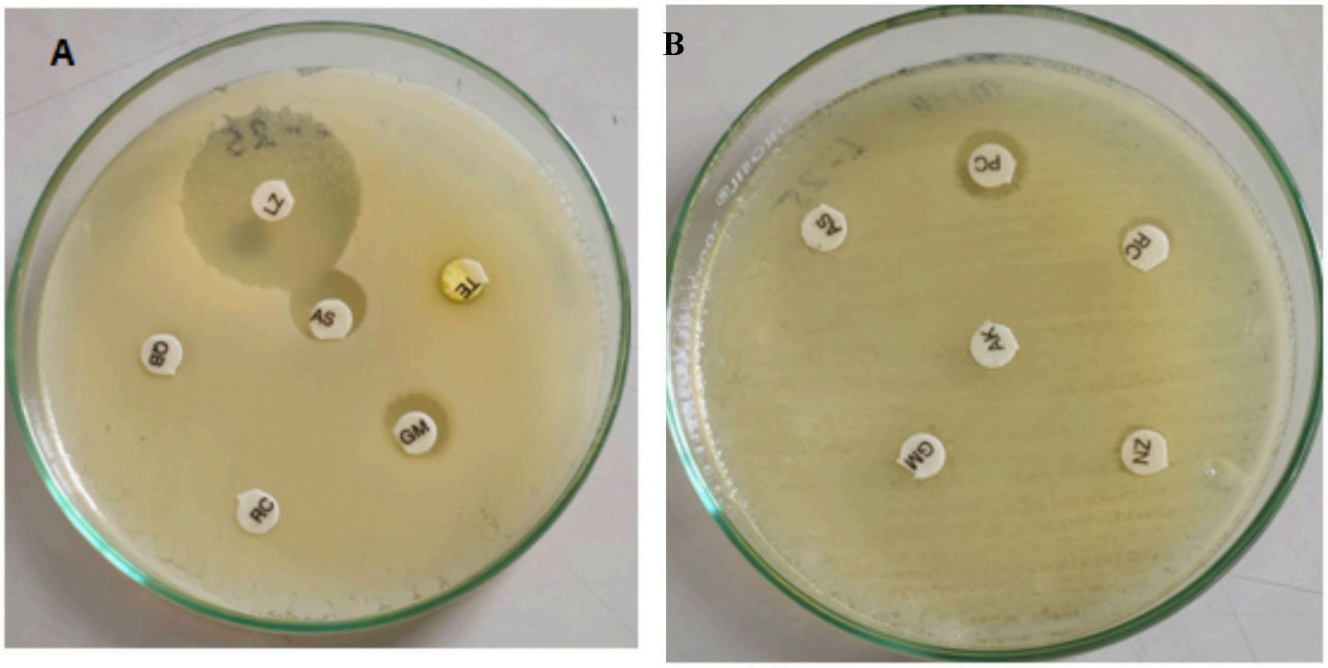

Fig. 3 Antibiotics sensitivity test on Mueller Hinton agar plate showing multi-drug resistance in isolated pathogens.

[**A** = *Staphylococcus aureus* (ampicillin/sulbactam (10/10 mcg), tetracycline (30 mcg), linezolid (30 mcg), levofloxacin (5 mcg), ciprofloxacin (5 mcg), and gentamicin (10 mcg); **B** = *Pseudomonas aeruginosa* (ampicillin/sulbactam (10/10 mcg), piperacillin (100 mcg), ciprofloxacin (5 mcg), ofloxacin (5 mcg), gentamicin (10 mcg), and amikacin (30 mcg)]

Figure 3

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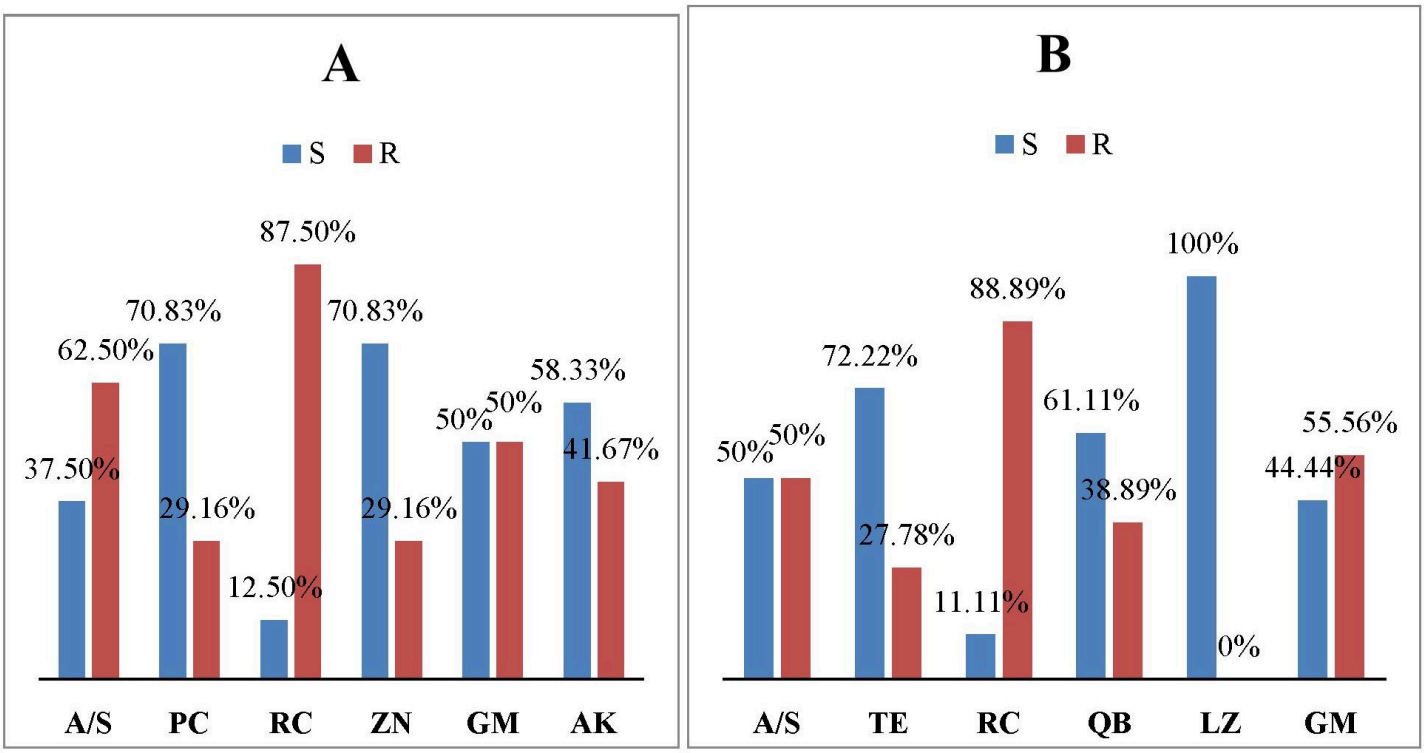

Fig. 4 Result of antibiotic sensitivity test of various antibiotics against isolated pathogens

A: *Pseudomonas aeruginosa* (total isolates-24) **B**: *Staphylococcus aureus* (total isolates- 18)

*Blue: sensitive; Red: resistant [A/S: ampicillin/sulbactam (10/10 mcg), PC: piperacillin (100 mcg), RC: ciprofloxacin (5mcg), ZN: ofloxacin (5mcg), GM: gentamicin (10 mcg), AK: amikacin (30 mcg), TE: tetracycline (30 mcg), QB: levofloxacin (5 mcg), LZ: linezolid (30 mcg)]

Figure 4

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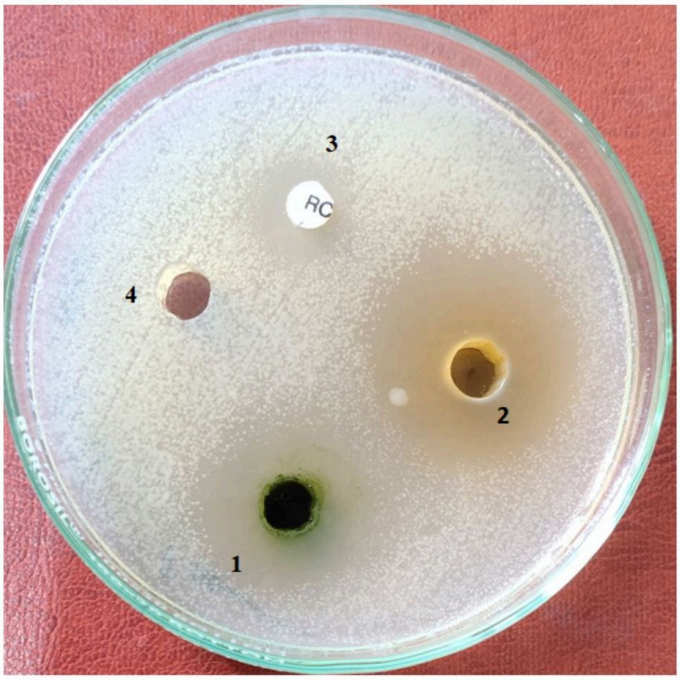

Fig. 5 Anti-bacterial effect of *U. dioica* and *C. nobile* against MDR *Pseudomonas aeruginosa*
*well 1 *U. dioica* (18-25 mm), well 2 *C. nobile* (16-30 mm), 3 (ciprofloxacin) used as positive control, and well 4 (70% ethanol) used as negative control

Figure 5

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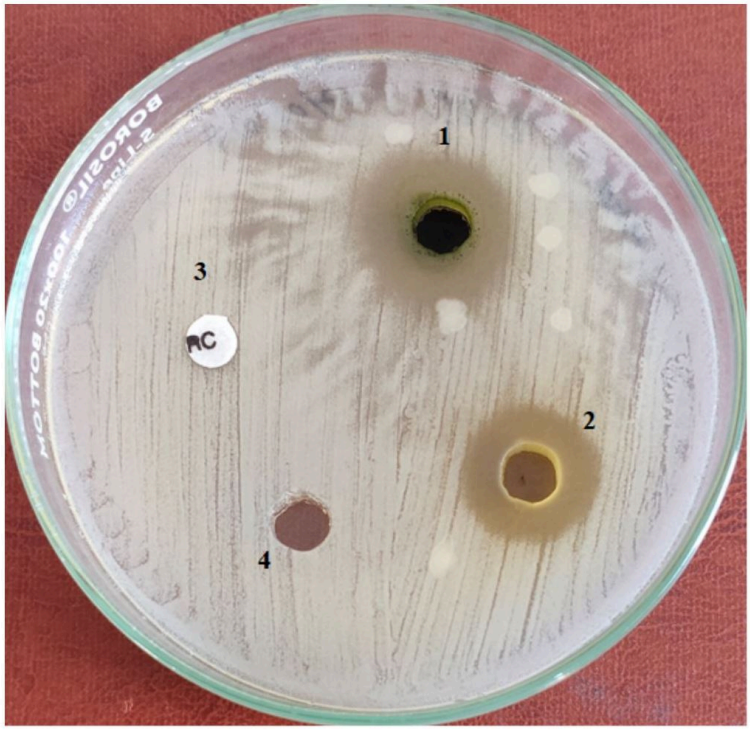

Fig. 6 Anti-bacterial effect of *U. dioica* and *C. nobile* against MDR *Staphylococcus aureus*

*well 1 *U. dioica* (15-30 mm), well 2 *C. nobile* (17-22 mm), 3 (ciprofloxacin) used as positive control, and well 4 (70% ethanol) used as negative control

Figure 6

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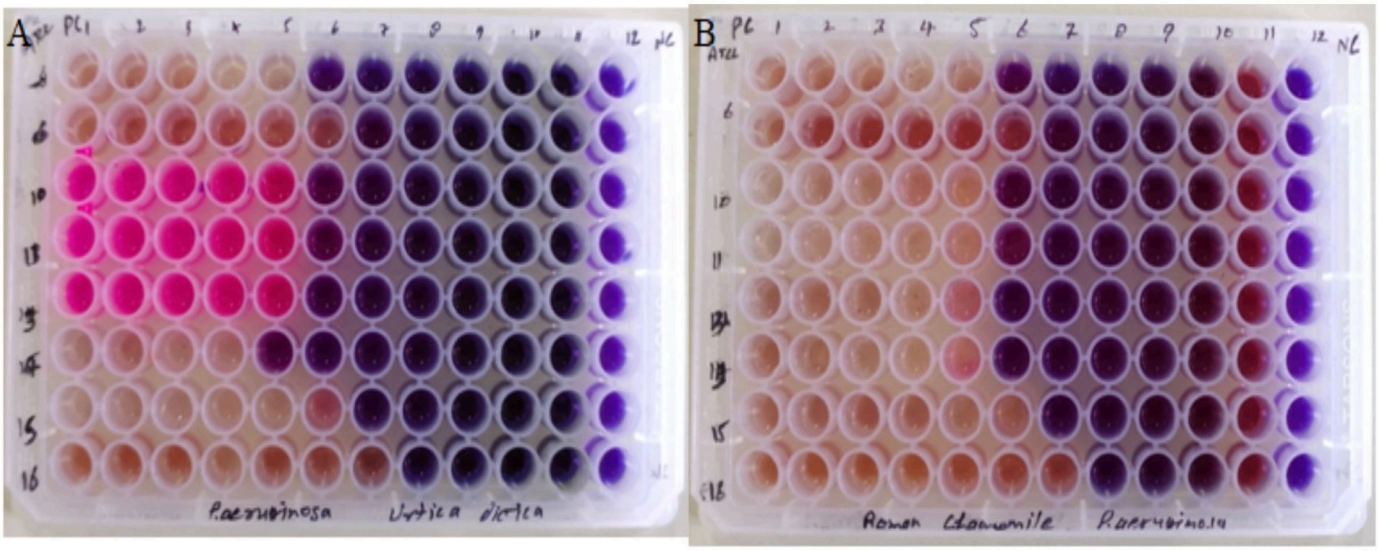

Fig. 7 MIC of *U. dioica* **(A)** and *C. nobile* **(B)** against *Pseudomonas aeruginosa*

**In* each series, well no. 1 (positive control) shows pink color or colorless, well no. 2 (negative control) show purple color, well 2-11 used as test for MIC and MBC which color shifts from purple to pink or colorless (use of Resazurin dye for the responsible of color shift)

Figure 7

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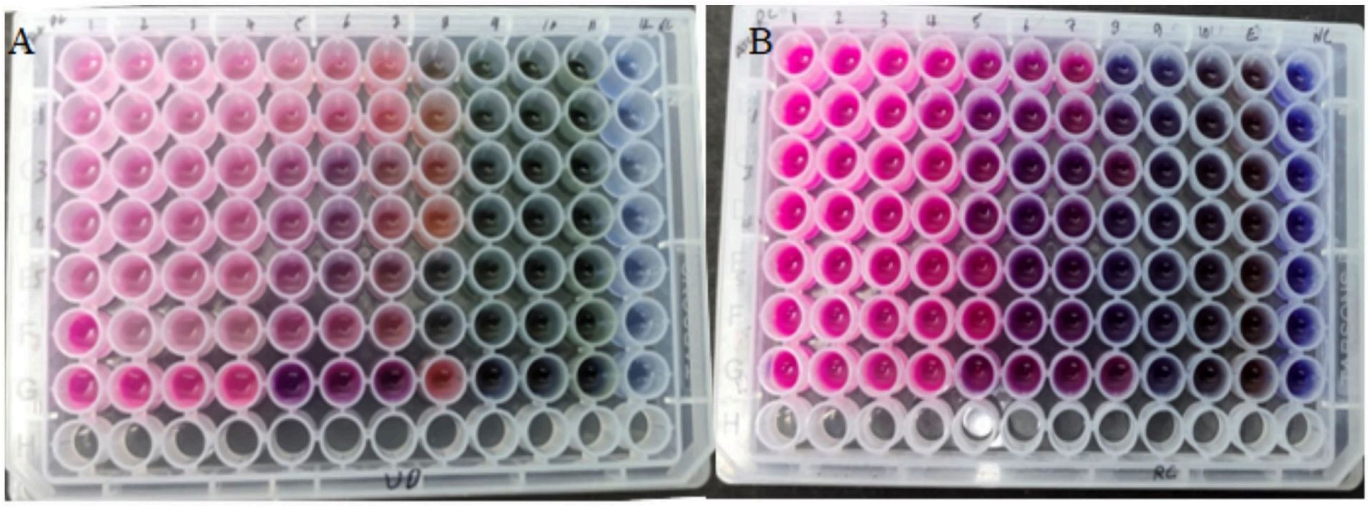

Fig. 8 MIC of *U. dioica* **(A)** and *C. nobile* **(B)** against *Staphylococcus aureus*

**In* each series, well no. 1 (positive control) shows pink color or colorless, well no. 2 (negative control) show purple color, well 2-11 used as test for MIC and MBC which color shifts from purple to pink or colorless (use of Resazurin dye for the responsible of color shift)

Figure 8

See image above for figure legend