

Developing Biofilm Resistant Catheter Coatings: Effect of *Mimosa pudica* L. Extract on Catheter-associated Urinary Tract Infections causing Agents

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Abstract

Background: Among several nosocomial infections, catheter-associated urinary tract infection (CAUTI) is a very common one, and it often results in high morbidity as well as mortality, especially when the causative organisms are multidrug-resistant biofilm-forming pathogens. To prevent such biofilms and to make the treatment and management of the infection more effective, the scientific world is searching for novel antimicrobial catheter coating agents, and due to many factors, including multitarget actions, phytochemicals pose one of the promising options. Hence, our aim is to investigate the antibiofilm, antibacterial, and antiadhesive properties of a plant – *Mimosa pudica* L methanolic crude extract against major CAUTI-causing biofilm-forming microorganisms like *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans*. **Methods and Results:** The crude extract showed potent antimicrobial activity against tested pathogens and also had excellent minimal inhibitory concentrations (MIC) as low as 0.312 mg/ml against all the tested microbes. The activity of antibiofilm was proved through crystal violet staining method, which revealed effective activity against all the test pathogens through biofilm inhibition and mature biofilm reduction. The antimicrobial activity of the catheter coated with *M. pudica* methanolic extract showed activity by growth inhibition around the catheter. In addition, *M. pudica* extract exhibited anti-oxidant property and also revealed no toxic effect to L₉₂₉ cells. **Conclusion:** Based on these findings, the authors suggest detailed *in vitro* and *in vivo* investigations to develop *M. pudica* extract as a potential agent in treating and managing CAUTI.

Key words: Antiadhesive, biofilms, catheter-associated urinary tract infections, catheter-coating, *Mimosa pudica* L

INTRODUCTION

Nosocomial infections are common and important among hospitalized patients whose underwent prolonged usage of catheter for various reasons. Among them, 40% of the nosocomial infections are related to catheter-associated urinary tract infection (CAUTI), which gained considerable courtesy due to high morbidity and mortality.^[1,2]

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The catheter usages are short or long term, it provides an appropriate micro environment for infection, resulting mild to severe complication which affecting more than million people globally.^[3-5] The microbial entry promotes the colony formation on in and outer surface of the catheter through lumen makes the initiation of infection by attachment and later on biofilm development in the catheter, which adjust the host defense as well as normal microbiota of the urinary system resulting renal failure when its untreated.^[6] One of the important reasons for CAUTI treatment failure is biofilm-forming ability of prevalent uropathogens.^[7] Among the numerous frequently isolated uropathogens, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans* are mainly involved in CAUTI development.^[8,9] These pathogens form biofilm, which produces the slimy extracellular polymeric substance that protects the bacteria's being attacked from various environmental factors such as stress, antibiotic treatment, pH, and so on.^[10] The complex three-dimensional structure of biofilm can able to protect the microbes from antibiotic treatment by expelling the antibiotics or slowdown the antibiotic uptake or alteration in target site may hinder the treatment process resulting long stay and high therapeutic cost.^[11-13] Many complications are encountered due to recurrent or inappropriate use of antibiotics for CAUTI treatment resulting antibiotic resistant strain development makes treatment challenges.^[14-16] Usually, the catheter replacement or proper antibiotic treatments are an alternative option for CAUTI prevention, but when it is undiagnosed or left untreated increasing the infection severity. This dreadful condition activated the development process for an alternative antimicrobial agent with potential antibiofilm activity against organisms accountable for CAUTI.

In this scenario, nature has been acting as a very potential source of medicine for several 100 years. Therefore, the plant source for phytochemicals and their potency against many diseases attracted the researcher to explore the many medicinal plants for novel antimicrobial drug development. WHO says, 80% world's population are still using the old-style medication for their prime health condition.^[17] Keeping this in mind, researchers have documented the requirement for medicinal plant screening to discover novel drugs with excellent treatment efficacy to overcome the current situation. Therefore, an abandoned weed *Mimosa pudica* - touch me not plant from Fabaceae family has been investigated against CAUTI pathogens. It is an annual or perennial creeping plant and also one of the desirable plants used in Ayurveda for their various pharmacological properties, including antioxidant, antidiabetic, anti-hepatotoxin, antitoxin, as well as wound healing property.^[18,19] Based on the evidence for the potential pharmacological property of the plant, our study inspected the crude methanolic extract of *M. pudica* antimicrobial, antibiofilm activities against test microbes.

MATERIALS AND METHODS

Plant authentication

The plant used in the present investigation was identified as *M. pudica* L. by Dr. Mamoon AlFakhi, Chief Scientist, Prince Sattam bin Abdulaziz University Institutional Review Board (SCBR-093-2024).

Preparation of inoculum

The standard 0.5 MacFarland unit of test cultures was used throughout the study. The media, such as brain heart infusion broth for *S. aureus* and *E. faecalis* growth, MHB (Mueller Hinton) broth for *E. coli*, and Sabouraud dextrose broth for *C. albicans*, were used. The experiments were done in triplets.

Methanolic crude extract preparation of *M. pudica*

In a Soxhlet apparatus, the cellulose thimble filled with *M. pudica* (20 g) powder was placed to prepare crude extract as dictated before.^[20] The bottom flask was occupied with enough volume of methanol to start the reaction, and it was continued for several hours to get colorless solution in the apparatus. The product was undergone solvent evaporation to get crude extract.

Antimicrobial activity of *M. pudica*

For the determination of *M. pudica* crude extract antimicrobial activity against test microbes, agar diffusion method was adopted as mentioned earlier.^[21] The wells made on the sterile petri plates lawned with respective overnight test pathogens were received various *M. pudica* concentrations, followed by incubation. The *M. pudica* antimicrobial activity against *S. aureus*, *E. faecalis*, *E. coli*, and *C. albicans* was determined through clear growth inhibition around the well.

Minimal inhibitory concentration (MIC) determination for *M. pudica*

The *M. pudica* MIC was determined towards test microbes using micro microdilution method as indicated before.^[22] For the MIC determination, two-fold serial dilution of 1.25 mg/mL of *M. pudica* was done up to 0.009 mg/mL in respective broth, followed by culture addition and incubation. The turbidity optical densities were read at 600 nm.

Effect of *M. pudica* on biofilm formation

The *M. pudica* effects on biofilm formations of test microbes were inspected by crystal violet method, as illustrated earlier.^[22] For the study, test pathogens added in the well containing various *M. pudica* concentrations (1.25 mg/mL to 0.009 mg/mL) for 5 days to form biofilm followed by

methanol fixation. The fixed cells were stained with crystal violet and destained using ethanol-acetone mixture, and the resultant was read at 570 nm.

Effect of *M. pudica* on biofilm eradication

The *M. pudica* effects on mature biofilms of test microbes were studied by crystal violet method as mentioned before.^[22] In short, three different *M. pudica* concentrations (1X, 2X, and 3X MIC) were used for treating 5-day-old biofilms and incubated. The unattached biofilms were removed and methanol fixation. The fixed cells stained by crystal violet and destain with ethanol and acetone combination and resulting purple color was measured at 570 nm.

Antimicrobial activities of *M. pudica*-coated catheters

The antimicrobial activity of *M. pudica*-coated catheter against *S. aureus*, *E. faecalis*, *C. albicans*, and *E. coli* studied as dictated before.^[23] *M. pudica* coated a small catheter tube was placed over respective test pathogens' lawn surface and incubated overnight. The microbial inhibition around the catheter as zone indicates the activity of *M. pudica*-coated catheter against the tested organisms.

Antioxidant properties of *M. pudica*

M. pudica antioxidant property was studied using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay as illustrated before.^[24] The DPPH solution was mixed with different concentrations of *M. pudica* and allowed for 30 min. The resulting product read at 517 nm.

$$\text{Scavenging effect} = 100 \times \frac{(\text{OD of blank} - \text{OD of sample})}{\text{OD of blank}}$$

Cytotoxicity of *M. pudica*

The cytotoxic effect of *M. pudica* was investigated on L₉₂₉, normal mouse fibroblast cells using MTT assay as designated earlier.^[22] The cell attachment in the Dulbecco's Modified Eagle Medium, which was treated with *M. pudica* various concentrations for 24 h, followed by the formation of formazan product by adding MTT solution. The final purple product obtained after adding DMSO was read at 570 nm, and the percentage of cell viability after treatments with *M. pudica* was calculated.

RESULTS

Antimicrobial activities of *M. pudica* methanolic crude extract

The antimicrobial activity investigated for various *M. pudica* concentrations against test microbes using agar

diffusion method is displayed in Figure 1. As seen in figure, antimicrobial activity was indicated as zone of inhibition around the well against test pathogens, and the zone diameter was increased in response to concentration increase represent that the activity is concentration dependent.

MIC determination for *M. pudica*

The *M. pudica* MIC determined against test microbes through micro-dilution method is represented in Figure 2. As observed in figure, the plotted graph indicated the least growth inhibitory concentration of *M. pudica* against test pathogens and to stop the *S. aureus*, *E. faecalis*, and *E. coli* growth, 0.312 mg/mL of *M. pudica* concentration was needed, whereas 0.156 mg/mL of *M. pudica* extract was essential to inhibit *C. albicans* growth.

Effect of *M. pudica* on biofilm formation

The biofilm-forming ability of *S. aureus*, *E. faecalis*, *E. coli*, and *C. albicans* in the presence of varying ranges of *M. pudica* concentrations investigated by crystal violet, is mentioned in Figure 3. The figure represented the calculated biofilm formation percentage after treatment with *M. pudica* against test pathogens, and all the test pathogens biofilm formation was not reported till their MIC level indicating antibiofilm activity against test pathogens. Further, biofilm formation gradual increase observed below the MIC of *M. pudica* demonstrating the trace of crude extract may hinder the biofilm-forming ability of test pathogens.

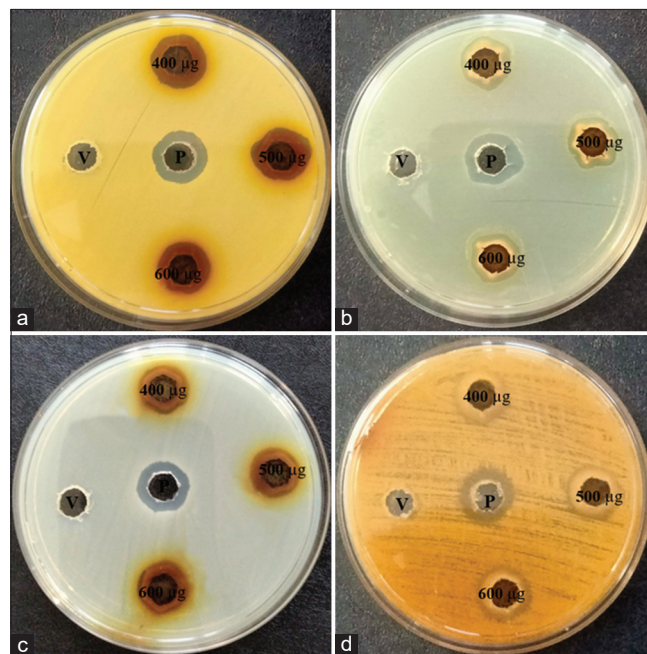


Figure 1: (a-d) Methanolic crude extract *Mimosa pudica* antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans*. P- Positive controls (rifampicin, nystatin, and ampicillin) and V- methanol (vehicle control)

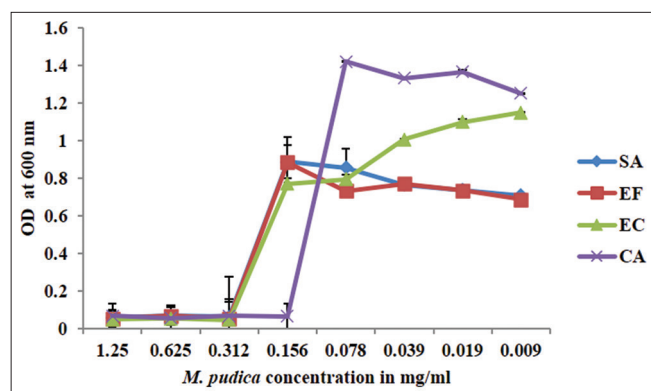


Figure 2: Graph indicating *Mimosa pudica* minimum inhibitory concentration against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans*

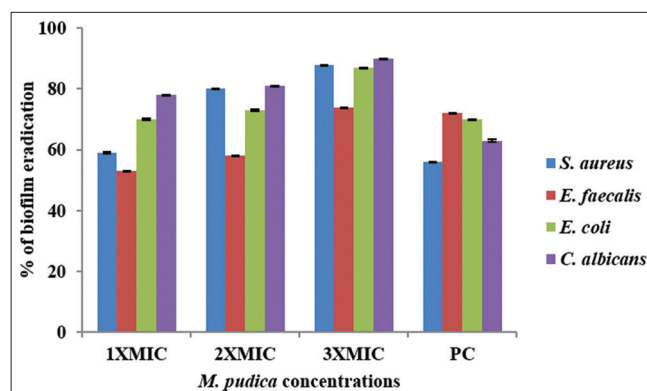


Figure 4: *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans* biofilm eradication percentage after *Mimosa pudica* treatment

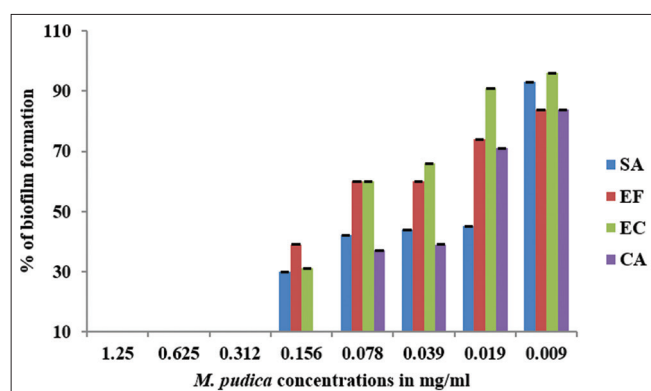


Figure 3: Percentage of *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans* biofilm formation after treatment with *Mimosa pudica*

Effect of *M. pudica* on mature biofilm

The calculated test microbe's biofilm eradication percentage after treating with three different concentrations of *M. pudica* is documented in Figure 4. The figure indicates, *M. pudica* eradicates a maximum of 90%, 87%, 74% and 88% of *C. albicans*, *E. coli*, *E. faecalis*, and *S. aureus* biofilm after 3× MIC of *M. pudica* treatment suggesting potency to eliminate the biofilms on any non-living surfaces.

Antimicrobial activity of *M. pudica*-coated catheters

The catheter coated with *M. pudica* evaluated for antimicrobial activity against *S. aureus*, *E. faecalis*, *E. coli*, and *C. albicans* by *in vitro* bladder model is represented in Figure 5. The formation of zone in the catheter surroundings proved activity of *M. pudica* against test pathogens.

Antioxidant properties of *M. pudica*

M. pudica methanolic crude extract antioxidant property studied using DPPH is presented in Figure 6. As exposed in

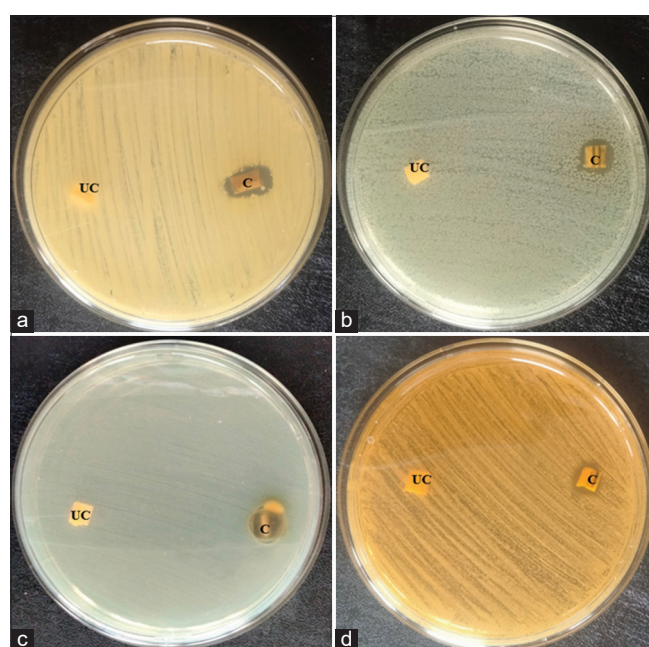


Figure 5: (a-d) *In vitro* bladder model to show antimicrobial activity of *Mimosa pudica* against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans*. UC- uncoated, C-coated with *M. pudica*

figure, the percentage of *M. pudica* various concentrations free radical scavenging was calculated, and the highest activity as 85% was observed at 2.5 mg/mL of *M. pudica*.

Cytotoxicities of *M. pudica*

The L_{929} cell viability after treatment with varying concentrations of *M. pudica* investigated for human purpose is documented in Figure 7. The graph representing the cell viability percentage after *M. pudica* crude extract and the 0.032 mg/mL of *M. pudica* crude extract showed 89% L_{929} cell viability after treatment indicating the *M. pudica* was not cytotoxic towards normal cells when compared to untreated cells.

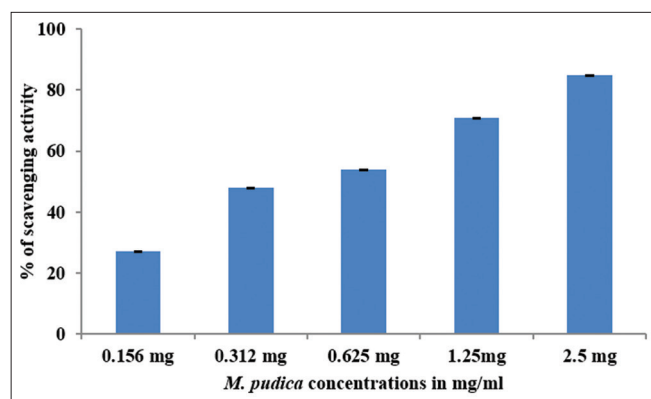


Figure 6: *Mimosa pudica* antioxidant property

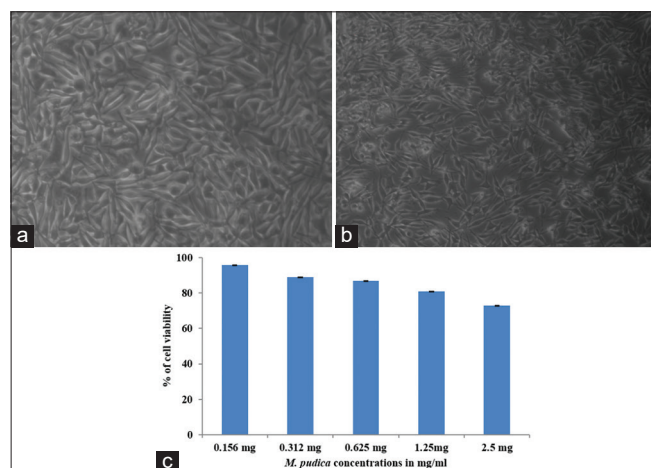


Figure 7: (a-c) *Mimosa pudica* induced cytotoxic effect on mammalian cells.

DISCUSSION

CAUTI caused by multi drug-resistant biofilm-forming microbes is gaining much attraction owing to high morbidity as well as mortality rate. Therefore, the drug development is an urgently required to overcome the drug resistance. Henceforth, our study intended for *M. pudica* methanolic crude extract antibacterial as well as antibiofilm properties against test microbes and showed better antimicrobial activity against all the test pathogens with the least inhibitory concentration as 0.312 mg/mL against bacterial pathogens and 0.156 mg/mL for fungal pathogens. In support of this, a human humble plant, *M. pudica* is one of the important traditional plant investigated for antimicrobial activity against *Pseudomonas aeruginosa*, *E. coli*, *Bacillus cereus*, *Klebsiella pneumoniae*, and *C. albicans*, and the ethanolic crude extract showed the antimicrobial activity against all the test pathogens with MICs as 100 µg/well, indicating the antimicrobial activity is depending on the phytochemicals such as saponins, alkaloids, flavonoids, coumarins, glycosides, and terpenoids present in *M. pudica* ethanolic crude extract.^[25] The differences in the MIC value may vary depend on the clinical strains. Similarly, *M. pudica* from Terai region of Nepal assessed for phytochemicals and antimicrobial activity

against *S. aureus*, *B. cereus*, *E. coli*, and *K. pneumoniae* and found activity against toward the pathogens. The report says, the phytochemicals like phenolic and flavonoids are responsible for antibacterial activity, and the binding affinity of the extract with bacterial protein, along with binding energy, was calculated by *in silico* method, which suggest L-mimosine is the potent antibacterial compound.^[26] The various solvents such as methanol, chloroform, petroleum ether, ethyl acetate, and water extracts were studied against *Proteus vulgaris*, *S. aureus*, *E. coli*, *Staphylococcus albus*, *Shigella flexneri*, *Salmonella typhi*, *Salmonella paratyphi A* and *B* and *Aspergillus fumigates* and all the solvents showed good antimicrobial activity against all the screened pathogens.^[27,28]

Additionally, the *M. pudica* crude extract was analyzed for their antibiofilm activity against *S. aureus*, *E. faecalis*, *E. coli*, and *C. albicans*. During the catheterization, the bacterial entry was initiated through catheter lumen from outer environment and makes biofilm formation by several stages, which possess complex structure resulting treatment challenges for management.^[29,30] Hence, our study reported the antibiofilm effect by biofilm inhibition and eradication of mature biofilms. Further, bacterial entry was initiated through catheter lumen resulting biofilm formation, which created the treatment process inefficient. Therefore, the coating of catheter outer and inner surface with any antimicrobial agents is an excellent alternative method for biofilm prevention. Later, our study proved the antimicrobial activity of catheter coated with methanolic extract of *M. pudica* against test pathogen in suitable environment. In support of this, numerous antimicrobials such as polymer, zinc oxide, some antibiotic combinations, silver, and Fosfomycin-coated catheter were analyzed for antimicrobial activity against various test pathogens.^[31-34]

Further, *M. pudica* antioxidant property was investigated and found potent antioxidant property. Same way, recent findings reported that the various extracts of *M. pudica* showed *in vitro* as well as *in vivo* antioxidant property.^[26] Another study finds the *M. pudica* antioxidant property by enzymatic and non-enzymatic antioxidant, such as catalase, superoxide dismutase, glutathione peroxidase, Vitamin C, and glutathione in serum and liver of rat revealed significant antioxidant property by lowering lipid peroxidation level. In addition, *M. pudica* had no cytotoxic effect toward normal cells.

CONCLUSION

Our study exposed the *M. pudica* methanolic extract antibacterial, antibiofilm activity, along with antioxidant properties. Moreover, the test parameters and test pathogens chosen were infection-specific, and the antioxidant property possessed by *M. pudica* was the standard for infection-related to reactive oxygen species. In addition, *M. pudica* showed no

cytotoxic effect to L_{929} cells. Finally, the *M. pudica* can be an effective antibacterial as well as antibiofilm coating agent, and created an interest for new antimicrobial drug discovery to fight against CAUTI.

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ETHICAL APPROVAL

The experiments followed the protocol approved by the Institutional Animal Care and Use Committee at the Prince Sattam bin Abdulaziz University Institutional Review Board (SCBR-093-2024).

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