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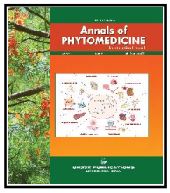

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Antimicrobial, antibiofilm and antioxidant activities of *Mukia maderaspatana* (L.) M. Roem. methanolic extract against catheter-associated urinary tract infectious agents

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Abstract

The biofilm related catheter-associated urinary tract infections (CAUTI) are gathering great attention due to severe complication resulting in increased morbidity rate. Therefore, alternative antimicrobials are urgently required for the treatment of CAUTI. Consequently, our study explored the *Mukia maderaspatana* (L.) M. Roem. antimicrobial and antibiofilm potentials against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans* and *Enterococcus faecalis*. The antipathogenic activities were documented against test pathogens and the activity was noted at 0.5 mg/ml against *E. coli* and *S. aureus* and also, 1 mg/ml against *C. albicans* and *E. faecalis* using microdilution method. The *M. maderaspatana* antibiofilm activity was examined using crystal violet staining method and effectively inhibited biofilm forming test pathogens. Similarly, 3X MIC of *M. maderaspatana* extract effectively eliminated the maximum of 86%, 86%, 86%, and 87% of *S. aureus*, *E. coli, E. faecalis*, biofilms *C. albicans* mature biofilms. The *M. maderaspatana* extract coated catheter tube showed antimicrobial activity against test pathogens. Further, antioxidant property of *M. maderaspatana* was investigated and cytotoxicity study revealed no toxic effect on normal L_{929} cells. Based on above results, *M. maderaspatana* could be a better alternative antimicrobial agent for CAUTI.

1. Introduction

The existing information and current technology in medical science guide clinicians in treating unmanaged diseases. Currently, several reported hospital-acquired infections are primarily related to medical devices used for life-saving purposes in many hospitalized patients who are admitted for various reasons. Moreover, the use of medical devices has significantly increased the chance of iatrogenic infections in immunocompromised patients admitted to hospitals when these devices are not handled or cared for properly (Medina and Castillo-Pino, 2019; Skelton-Dudley *et al*., 2019). One of the most commonly used medical devices is the indwelling catheter, which helps with urine drainage in patients undergoing surgery or other complicated treatments (Stickler, 2014). Continued catheterization creates an optimum environment for microbial entry into the sterile urinary tract *via* catheter implantation, making these devices prone to infection and resulting in catheter-associated urinary tract infections (CAUTIs). This continues to pose a risk during hospitalization (Cooper *et al*., 2016; Babich *et al*., 2018).

The infection rate is high among patients using catheters due to various risk factors, such as patient age and urinary incontinence, which increase the concern for CAUTI (Ramstedt *et al*., 2019; Yoo and Spencer, 2018). CAUTI develops when uropathogens enter the urethra or bladder, attaching to the catheter surface and forming biofilms. This biofilm formation makes CAUTI one of the most important nosocomial infections (Stærk *et al*., 2016; Papanikolopoulou *et al*., 2022; Saint *et al*., 2016; Wooller *et al*., 2018). Prolonged catheter use can lead to a range of complications, including bacteriuria and sepsis, resulting in extended hospital stays, which confirm the high morbidity and mortality rates associated with these infections (Flores-Mireles *et al*., 2019; Magill *et al*., 2018).

CAUTI encourages polymicrobial infections, including bacteria and fungi such as *E. faecalis*, *S. aureus*, *E. coli*, and *C. albicans*, all of which are capable of forming biofilms (Kim *et al*., 2017; Sharma *et al*., 2016; Chatterjee *et al*., 2014). The ability to form biofilms as three-dimensional structures produces extracellular polymeric substances that protect the microbes from antibiotic treatment as well as external stress, leading to the development of resistant strains, which makes treatment very challenging (Olivares *et al*., 2020; Hrvatin, 2017; Karigoudar *et al*., 2019; Kamali *et al*., 2020). Therefore, alternative antimicrobials are urgently needed to combat biofilm-forming organisms.

This alarming situation has triggered the development of antimicrobial agents from plant sources. Plant-based biomolecules have significant pharmaceutical value and applications in various fields, including antimicrobial, anticancer, and anti-inflammatory treatments, among others. Consequently, identified plant-based biomolecules have been used as traditional medicines to treat life-threatening diseases. In line with this evidence, *M. maderaspatana* (Cucurbitaceae) is used for treating asthma and respiratory infections and is widely distributed throughout India. It is known for its hepatoprotective, antirheumatic,

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antimicrobial, and anti-inflammatory properties (Harshiny*et al*., 2015). Therefore, our study documents the antimicrobial and antibiofilm activities of the methanolic extract of *M. maderaspatana* against CAUTI-causing organisms.

2. Materials and Methods

2.1 Collection and authentication of the plant material

The plant material used in the present study was collected from a local nursery and was identified as *Mukia maderaspatana* (L.) M. Roem. by Dr. Mamoon AlFakhi, Chief Scientist, DM Institute of Biological Research, Omdurman, Sudan with Authentication Number DMIBR/PA/07/2024.

2.2 Preparation of the inoculum

Overnight cultures adjusted to 0.5 McFarland units of *E. coli*, *S. aureus*, *E. faecalis*, and *C. albicans* were used in all experiments. *S. aureus* and *E. faecalis* were grown in Brain Heart Infusion (BHI) broth, while *C. albicans* and *E. coli* were grown in Sabouraud Dextrose Broth (SDB) and Mueller-Hinton Broth (MHB), respectively. Positive controls, such as ampicillin, rifampicin, and nystatin, as well as methanol (used as the vehicle control), were used in the study.

2.3 Preparation of crude methanolic *M. maderaspatana* extract

To prepare the crude extract of the plant, *M. maderaspatana* (purchased from the local market) was weighed (20 g) and placed in a cellulose thimble. The reaction was initiated by placing the thimble into a Soxhlet apparatus and adding methanol, as described earlier (Harley *et al*., 2022). A clear solution was obtained after several hours of reaction time, and the solvent-evaporated crude extract was used for further studies.

2.4 *M. maderaspatana* methanolic crude extract antimicrobial activity

The *M. maderaspatana* methanolic crude extract antimicrobial activity was studied against *E. coli, C. albicans, E. faecalis* and *S. aureus,* by well diffusion method as illustrated previously (Meiyazhagan *et al*., 2016). The sterile petri plates swabbed with overnight cultures were drilled and loaded with *M. maderaspatana* two different concentrations (1mg and 2 mg/well) and allowed for 24 h to form zone inhibition around the well which indicating antimicrobial activity against test pathogens.

2.5 *M. maderaspatana* methanolic crude extract MIC determination

The antimicrobial activity of the *M. maderaspatana* methanolic crude extract was studied against *E. coli*, *C. albicans*, *E. faecalis*, and *S. aureus* using the well diffusion method, as described previously (Meiyazhagan *et al*., 2016). The varying concentrations of *M. maderaspatana* crude extract from 4 mg/ml was serially diluted 0.031mg/ml in respective broth followed by cultures addition and allowed for 24 h for growth. Later, optical density (OD) of each well was measured at 600 nm.

2.6 Effect of *M. maderaspatana* methanolic crude extract on biofilm formation

The effect of *M. maderaspatana* on biofilm formation by *C. albicans*, *E. coli*, *S. aureus*, and *E. faecalis* was studied using the crystal violet method, as described previously (Meiyazhagan *et al*., 2015). Biofilm formation was allowed for overnight test cultures up to 5 days in the presence of varying concentrations (4 mg to 0.031 mg/ml) of *M. maderaspatana* methanolic crude extract and methanol fixation for attached cells, followed by crystal violet staining. The final purple color product was measured at 570 nm after adding an ethanol-acetone mixture.

2.7 *M. maderaspatana* methanolic crude extract effect on mature biofilm eradication

The effect of *M. maderaspatana* methanolic crude extract on mature biofilms of *C. albicans, E. coli, S. aureus*, and *E. faecalis* was studied using the crystal violet method, as described previously (Meiyazhagan *et al*., 2015). Briefly, biofilms of the test pathogens grown for 5 days were treated with *M. maderaspatana* methanolic crude extract at 1X, 2X, and 3X MIC concentrations for 24 h, and the attached biofilms were stained with crystal violet after methanol fixation. The final product, after adding an ethanol-acetone mixture, was measured at 570 nm.

2.8 Antimicrobial activity of *M. maderaspatana* methanolic crude extract coated catheter tube

The antimicrobial activity of *M. maderaspatana* methanolic crude extract-coated catheter tubes was examined against *E. coli*, *S. aureus*, *C. albicans*, and *E. faecalis* using an *in vitro* bladder model, as described previously (Goda *et al*., 2022). The sterile small silicone catheter tubes were coated with *M. maderaspatana* methanolic crude extract, placed on overnight culture-swabbed petri plates, and incubated to assess growth inhibition.

2.9 Antioxidant properties of *M. maderaspatana* methanolic crude extract

The antioxidant properties of *M. maderaspatana* methanolic crude extract were determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, as described previously (Gayathri and Sathish Kumar, 2016). Briefly, various concentrations of *M. maderaspatana* methanolic crude extract (5 mg/ml, 4 mg/ml, 3 mg/ml, 2 mg/ml, 1 mg/ml, and 0.5 mg/ml) were allowed to react with DPPH solution for 30 min. The percentage of radical scavenging activity was calculated after measuring the final product at 517 nm.

2.10 Cytotoxicity of *M. maderaspatana* methanolic crude extract

The cytotoxicity of *M. maderaspatana* methanolic crude extract was investigated towards L_{929} (mouse fibroblast) cells using the MTT assay, as described earlier (Meiyazhagan *et al*., 2015). Briefly, various concentrations of *M. maderaspatana* methanolic crude extract (4 mg/ml, 3 mg/ml, 2 mg/ml, 1 mg/ml, and 0.5 mg/ml) were added to DMEM (Dulbecco's Modified Eagle's Medium) containing cells for 24 h, followed by the addition of MTT to measure formazan product formation. The percentage of cell viability was calculated after measuring the final product at 570 nm.

2.11 Statistical analysis

The mean and standard deviations were used to calculate error bars for all experiments.

3. Results

3.1 Antimicrobial Activity of *M. maderaspatana* methanolic crude extract

The antimicrobial activity of *M. maderaspatana* methanolic crude extract against the test pathogens is shown in Figure 1. As observed, the two tested concentrations clearly exhibited growth inhibition around the well, with the zone size increasing as the concentration increased, indicating that the activity is dose-dependent.

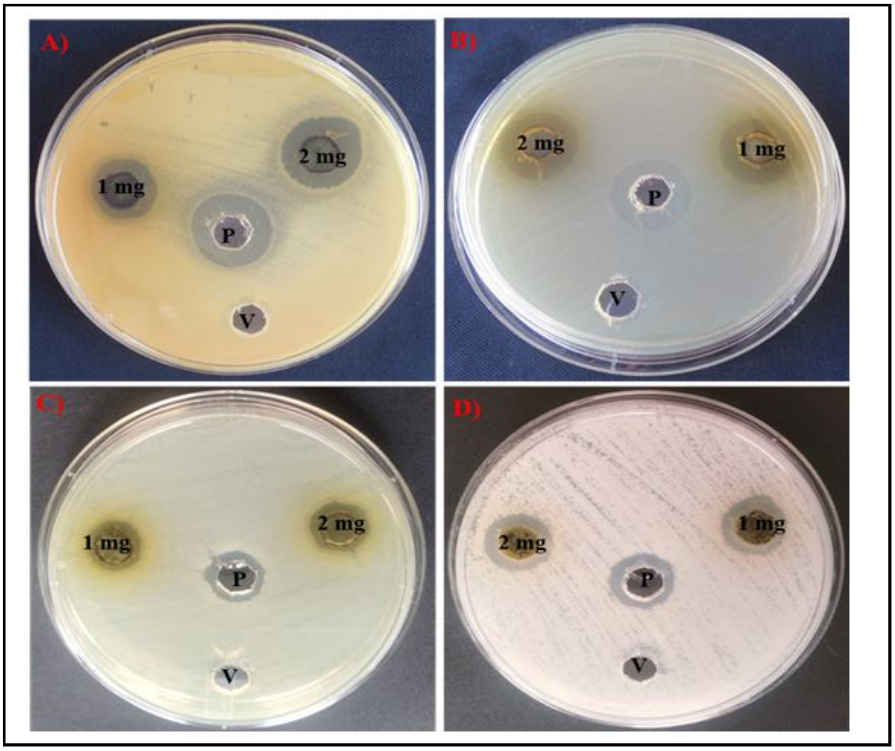

Figure 1: *M. maderaspatana* methanolic crude extract antimicrobial activity. (A) *S. aureus* (B) *E. faecalis* (C) *E. coli* and (D) *C. albicans* Note: P-positive controls and V-vehicle control.

3.2 *M. maderaspatana* methanolic crude extract MIC determination

The MIC of *M. maderaspatana* methanolic crude extract against the pathogens is shown in Figure 2. As depicted in the figure, the minimal growth inhibitory concentrations of *M. maderaspatana* methanolic crude extract were calculated for the test pathogens. Additionally, the growth of *S. aureus* and *E. coli* was inhibited at 0.5 mg/ml, while 1 mg/ml of *M. maderaspatana* methanolic crude extract was required to stop the growth of *C. albicans* and *E. faecalis*.

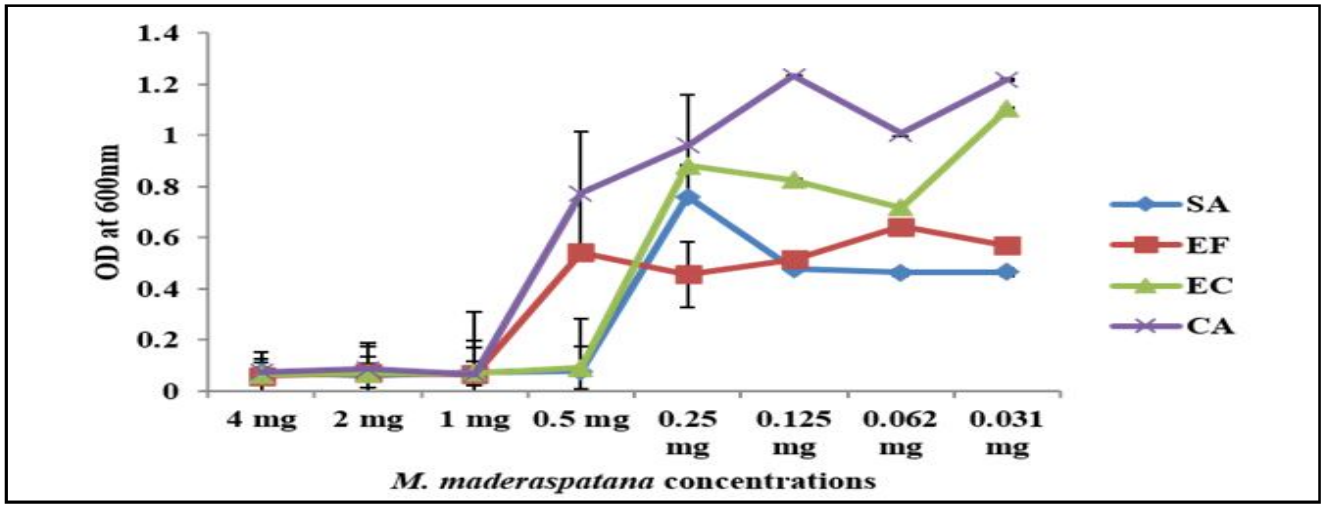

Figure 2: *M. maderaspatana* methanolic crude extract MIC determination against *E. coli, S. aureus, C. albicans* and *E.faecalis* using microdilution method. Note: SA-*S. aureus*, EF-*E. faecalis*, EC-*E. coli* and CA-*C. albicans*.

3.3 *M. maderaspatana* methanolic crude extract effect on biofilm formation

The effect of *M. maderaspatana* methanolic crude extract on the biofilm-forming ability of the organisms was evaluated, and biofilm formation was quantified after treatment with different concentrations (4 mg/ml to 0.031 mg/ml), as shown in Figure 3. Biofilm formation was observed only after reaching the MIC level, and a gradual increase in biofilm formation was noted beyond the MIC level. However, complete biofilm formation was not observed in any of the test pathogens, indicating that even small quantities of *M. maderaspatana* methanolic crude extract delayed biofilm formation on non-living surfaces.

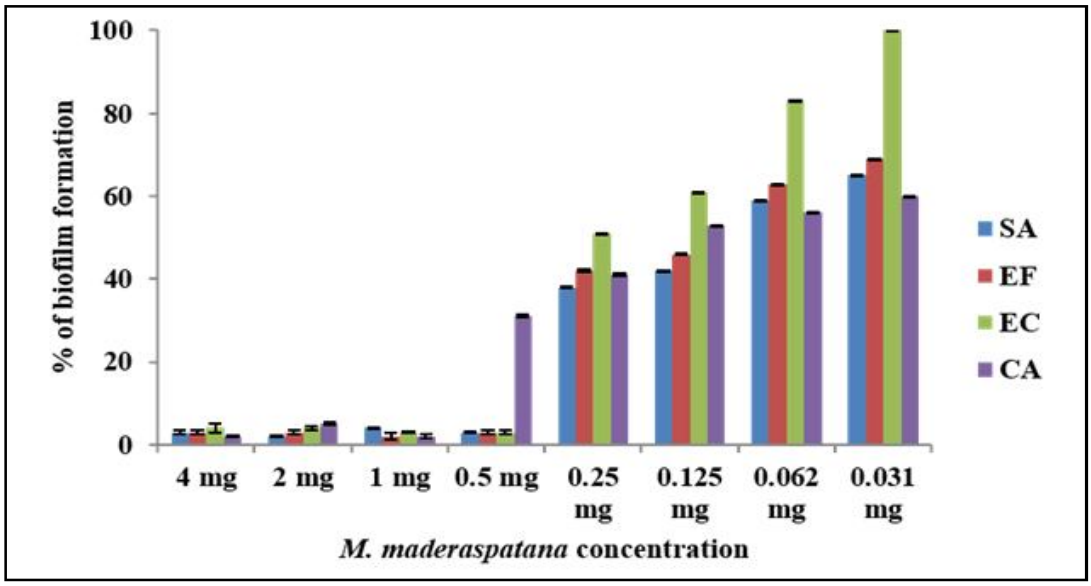

Figure 3: ***M. maderaspatana*** **methanolic crude extract effect on** ***E. coli, S. aureus, C. albicans and E. faecalis*** **biofilm formation. Note: SA-*S. aureus*, EF-*E. faecalis*, EC-*E. coli* and CA-*C. albicans*.**

3.4 *M. maderaspatana* crude methanolic extract effect on mature biofilm eradication

The effect of *M. maderaspatana* crude methanolic extract on mature biofilms of the pathogens was quantified, and the percentage of biofilm eradication by the extract is displayed in Figure 4. The figure shows that three different concentrations of *M. maderaspatana* methanolic crude extract (1X, 2X, and 3X MIC) effectively eradicated the biofilms of the test pathogens. The extract eliminated 76%, 79%, and 86% of *S. aureus* mature biofilms. Similarly, 68%, 81%, and 86% of *E. faecalis* biofilms were eradicated after treatment. Likewise, *M. maderaspatana* extract treatment eradicated 71%, 78%, and 86% of *E. coli* mature biofilms, and 77%, 84%, and 87% of *C. albicans* mature biofilms were effectively eliminated, indicating the antibiofilm activity of the extract against the test pathogens.

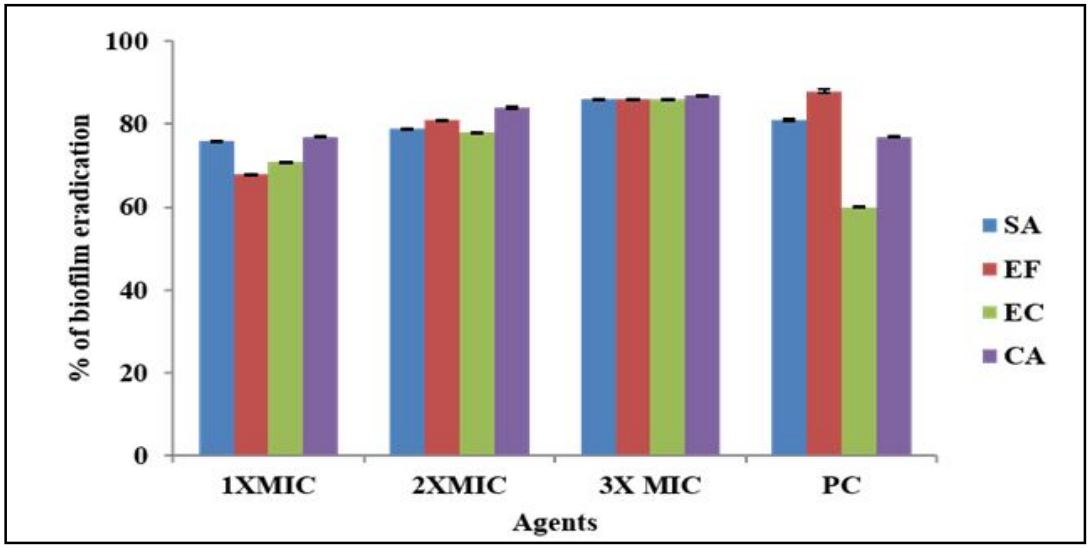

Figure 4: Quantitative biofilm eradication of ***M. maderaspatana*** **methanolic crude extract against** ***E. coli, S. aureus, C. albicans and E. faecalis.*** **Note: SA-*S. aureus*, EF-*E. faecalis*, EC-*E. coli* and CA-*C. albicans*. PC-positive controls.**

3.5 *M. maderaspatana* methanolic crude extract coated catheter antimicrobial activity

Using an *in vitro* bladder model, the antiadhesive property of *M. maderaspatana* methanolic extract-coated catheters was analyzed against the selected microorganisms, and the observed results are displayed in Figure 5. The *M. maderaspatana* methanolic crude extract-coated catheter showed clear zone formation around the catheter when compared to the untreated catheter tube, which exhibited no clear zone. Hence, the antiadhesive property of *M. maderaspatana* methanolic crude extract was confirmed.

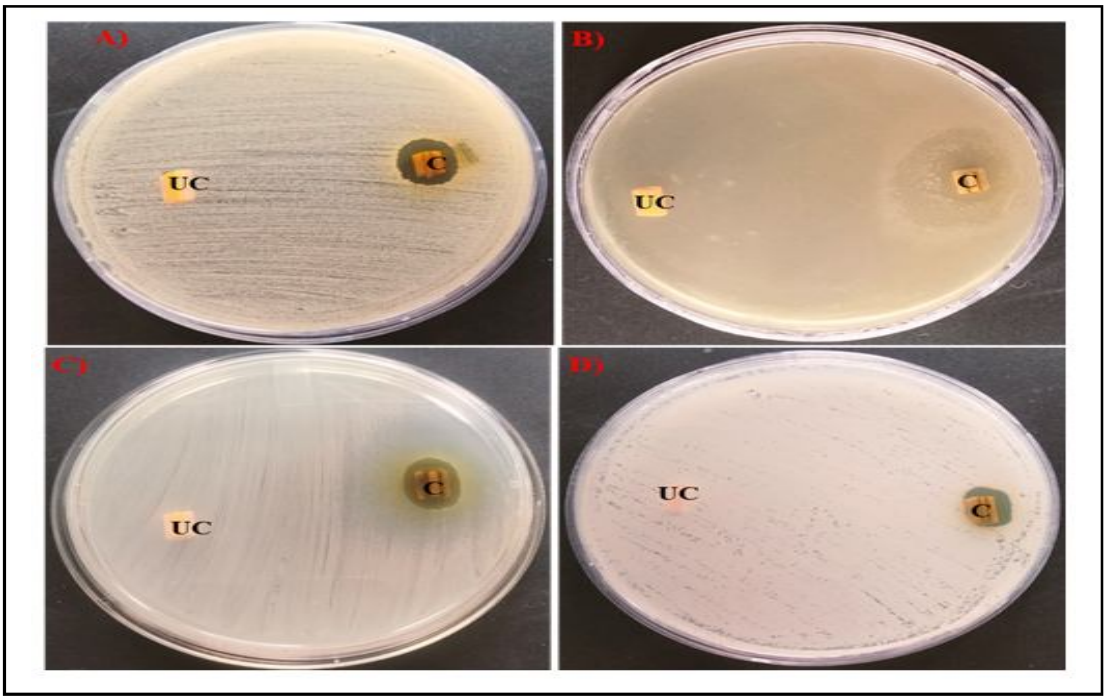

Figure 5: The anti-adhesive property of *M. maderaspatana* methanolic crude extract coated catheter was evaluated against *E. coli, S. aureus, C. albicans and E. f aecalis*. (A) *S. aureus,* (B) *E. faecalis,* (C) *E. coli,* and (D) *C. albicans*. Note: UC-uncoated, C-coated with *M. maderaspatana* methanolic crude extract.

3.6 *M. maderaspatana* methanolic crude extract antioxidant property

The antioxidant property of *M. maderaspatana* methanolic crude extract, as determined by the DPPH assay, is shown in Figure 6. The figure presents the free radical scavenging percentage of various concentrations (5 mg/ml, 4 mg/ml, 3 mg/ml, 2 mg/ml, 1 mg/ml, and 0.5 mg/ml) of *M. maderaspatana* extract: 61%, 58%, 45%, 31%, 27%, and 15%, respectively, after treatment.

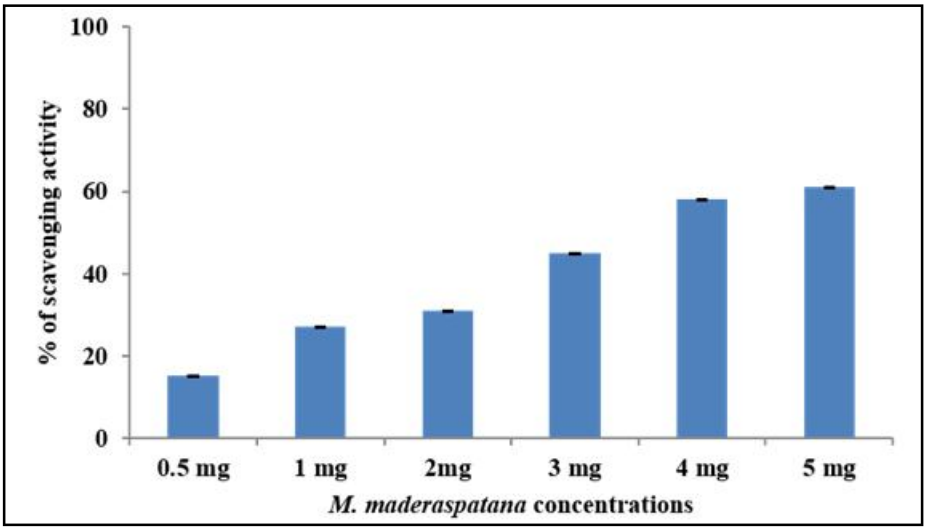

Figure 6: The antioxidant property of *M. maderaspatana* methanolic crude extract.

3.7 *M. maderaspatana*crude methanolic extract cytotoxicity

The cytotoxicity of *M. maderaspatana* crude methanolic extract was studied on L_{929} cells, and the percentage of cell viability after treatment with the extract is shown in Figure 7. Various concentrations of *M. maderaspatana* crude methanolic extract-treated cells exhibited maximum cell viability of 97% at 0.5 mg/ml, indicating that the extract was not cytotoxic to L_{929} cells at this concentration.

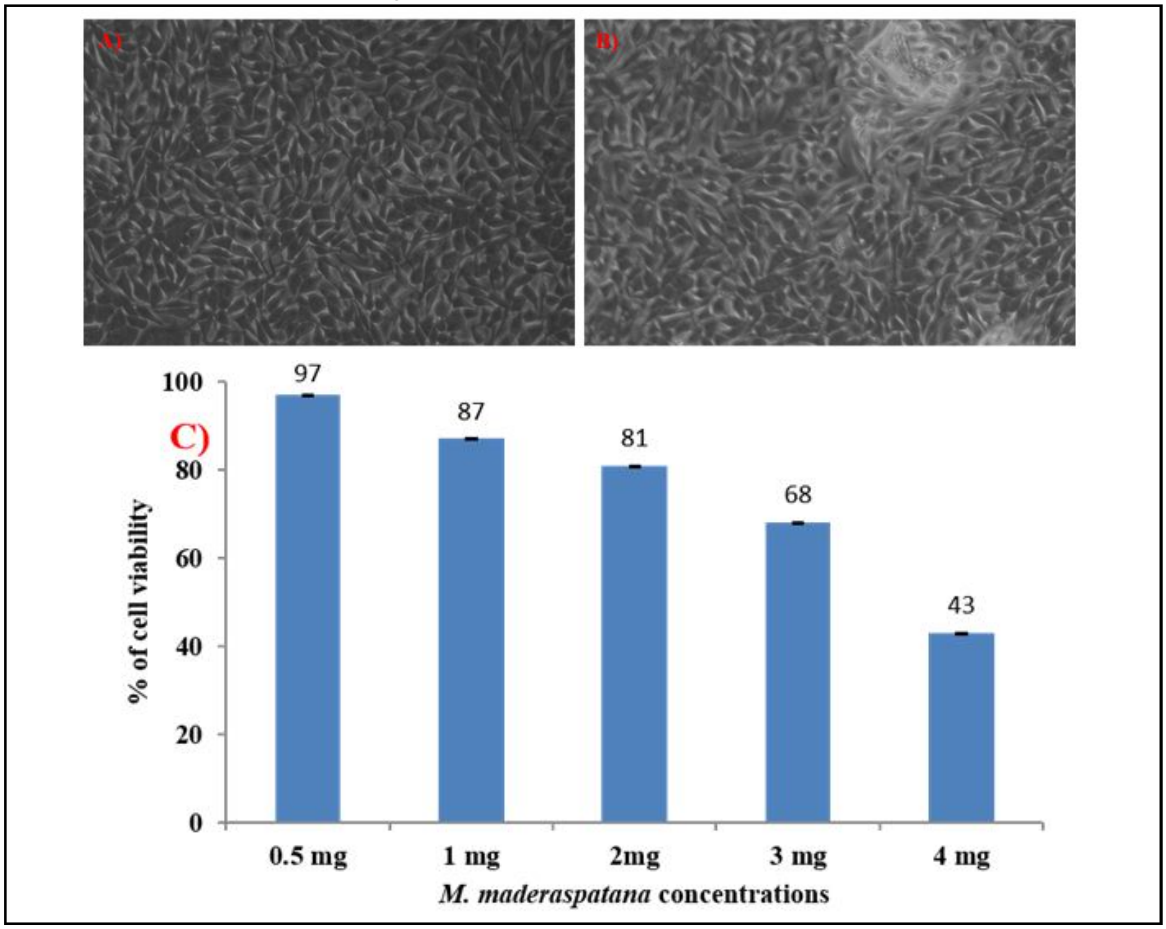

Figure 7: The cytotoxicity *M. maderaspatana* methanolic crude extract on L_{929} cells. (A) Untreated cells (B) Treated with *M. maderaspatana* methanolic crude extract, (C) Graph represents cell viability percentage after treatment with *M. maderaspatana* various concentrations.

4. Discussion

Medical device-associated infections are gaining increasing attention in hospitalized patients. Among these, CAUTI is a significant biofilm-related infection that causes drug resistance, leading to treatment failure. Therefore, there is an urgent need for the development of drugs with potential antimicrobial and antibiofilm activities to combat these pathogens. In this context, our study explored the antimicrobial potential of *M. maderaspatana* methanolic crude extract against *C. albicans*, *E. coli*, *S. aureus*, and *E. faecalis* and found it to exhibit activity at low concentrations.

In support of our findings, previous studies have shown that extracts of *M. maderaspatana* obtained using different solvents such as hexane, ethyl acetate, methanol, and water demonstrated antimicrobial potential against *S. aureus*, *Klebsiella pneumoniae*, and *E. coli*. The results revealed that the methanolic extract of *M. maderaspatana* exhibited the maximum inhibition against *K. pneumoniae*, attributed to various phytoconstituents, including flavonoids, alkaloids, phenolics, and saponins, which are responsible for its antimicrobial effects (Kumar *et al*., 2013; Moumita and Thankamani, 2013). The ethanolic extract of the leaf and root parts of *M. maderaspatana* was also investigated for antimicrobial activity against common human pathogens, including *Streptococcus pyogenes*, *E. coli*, *P. aeruginosa*, *Helicobacter pylori*, *C. albicans*, *Aspergillus niger*, *Fusarium* spp., and *Trichoderma viride*. Compared to the root extract, the leaf extract exhibited dose-dependent activity against the test pathogens (Dhanaraj *et al*., 2012).

In addition to antimicrobial activity, the antibiofilm activity of *M. maderaspatana* was evaluated against test pathogens. The catheterization process allows microbial entry *via* the catheter lumen, which initiates biofilm formation through several stages, from attachment and colony formation to maturation, resulting in a complex structure that challenges treatment for clinicians (Zhu *et al*., 2019; Pelling *et al*., 2019). Our focus was on targeting biofilms at each stage, and we found that *M. maderaspatana* exhibited antibiofilm activity by inhibiting biofilm formation and eradicating fivedayold mature biofilms. Given that catheters provide an opportunity for

bacterial colonization, coating them with an antimicrobial agent can prevent biofilm formation. Our study showed that coating catheter surfaces with the methanolic extract of *M. maderaspatana* is an excellent method to eradicate biofilm on catheter surfaces. This finding aligns with several studies that have reported the antimicrobial activity of various agents, such as polymer coatings, zinc oxide, antibiotic combinations, silver, and fosfomycin, in preventing catheter-associated infections caused by *E. faecalis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *C. albicans* (Aleksandra *et al*., 2021; Abbott *et al*., 2020; Fisher *et al*., 2015; Jia *et al*., 2021; Rahuman *et al*., 2021).

Overall, this study demonstrated that *M. maderaspatana* methanolic extract exhibits excellent *in vitro* antimicrobial and antibiofilm activity, suggesting its potential as a therapeutic agent for preventing and treating CAUTI infections. Further studies are necessary to explore the practical application of coating catheters with this extract to combat CAUTI.

5. Conclusion

The *M. maderaspatana* methanolic extract exhibited antimicrobial activity against the tested pathogens with minimal inhibitory concentrations. Biofilm inhibition and eradication of mature biofilms were achieved following treatment with *M. maderaspatana*, confirming its antibiofilm activity. The catheter coating with *M. maderaspatana* extract showed antimicrobial activity in an *invitro* bladder model. Overall, *M. maderaspatana* may serve as an alternative agent for preventing and treating CAUTI infections.

Acknowledgments

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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