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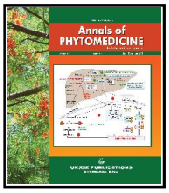

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Antibacterial activity of methanolic crude extracts from *Aerva lanata* (L.) A.L. Juss. ex Schultes against *Staphylococcus aureus* and *Escherichia coli* using *in vitro* bladder model for catheter-associated urinary tract infection

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Abstract

Catheter-associated urinary tract infections (CAUTI) represents the most important bacterial infection associated with catheter usage, which originates in uropathogens and results in biofilm formation. The study investigated the antibacterial activity of methanolic crude extract of *Aerva lanata* (L.) A.L. Juss. ex Schultes against *Staphylococcus aureus* and *Escherichia coli*. Since the crude extract possessed antibacterial activity against the tested microbes, the microdilution method was adopted to determine the minimum inhibitory concentration (MIC) of *A. lanata*. Further, the *A. lanata* was investigated against colony forming ability of *S. aureus* and *E. coli* on polystyrene surfaces after treatment with different concentrations. The *A. lanata* coated catheter was also evaluated against *S. aureus* and *E. coli* in *in vitro* bladder model and the activity was observed as clear zone formation around the well. It was revealed that *A. lanata* effectively inhibited the colony-forming ability of *S. aureus* and *E. coli* up to the MIC level. The *A. lanata* showed a marked biofilm reduction after treatment. It was also evident that a catheter tube coated with an antibacterial agent is an excellent method to prevent biofilm formation. This suggested that methanolic crude extract of *A. lanata* can be an alternative antibacterial agent for *S. aureus* and *E. coli*.

1. Introduction

Patients admitted to healthcare facilities for a long time with health complications need to be surrounded by lifesaving and non-contagious medical devices. If, a medical device is not handled or maintained properly, it will be associated with hospital-acquired infections, proving harmful for the patient. Among several medical devices, the indwelling catheter is the most commonly deployed medical device used for urine drainage during surgery or other urinary tract complications (Stickler, 2014). The urinary tract is although a sterile environment but during a urinary catheter implantation, it becomes vulnerable paving the way for the bacterial entry through the urinary tract to the bladder, thus making the implant devices carriers of infection (Cooper *et al.,* 2016).

Such an infection is commonly known as catheter-associated urinary tract infection (CAUTI), which is also one type of hospitalization risk (Babich *et al*., 2018). The number of infection due to catheter's usage has increased worldwide to more than 150 million individuals annually (Glenn, 2022; Ramstedt *et al*., 2019; Yoo and Spencer, 2018). In addition, the catheterized patients also suffer from other risk factors like ageing and urinary incontinence which makes CAUTI a matter of great concern. The development of CAUTI starts when bacteria enter into the bladder during catheterization and stick to the surface to form colonies with a three-dimensional biofilm structure (Stærk *et al.,* 2016). It has also been observed that long-term catheter usage causes a thicker biofilm formation which produces a kind of polymeric substance that helps the bacteria for further colonization and the development of a biofilm community (Singha *et al*., 2018). Several predominant organisms are responsible for CAUTI including Gram-negative bacteria (*Escherichia. coli, Proteus mirabilis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) (Lo *et al.,* 2014).

The objective of this study was to draw attention to the biofilm-forming ability of the uropathogens attached to the surface of the catheter and show how those pathogens use the catheter as a medium for colonization. The problem grows bigger when the infection gets much deeper and harder, leading to the production of extra polymeric substances and treatment challenges. These substances further inhibit the antibiotic entry and guard the uropathogen from antibiotics and other treatment agents (Stærk *et al*., 2021). This alarming situation paves a dire need for effective antibiotic drug development, with the potential to eradicate the biofilm-forming organisms involved in CAUTI. Although, CAUTI is preventable by catheter replacement or antibiotic therapy but if it is not treated properly or the infection severity is allowed to increase, it aggravates the disease, requiring to look for alternative solutions.

Nature provides most miraculous solutions for health hazards, one of which are the medicinal plants, comprising components such as

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antimicrobial compounds to treat chronic diseases (Arasu *et al*., 2019). A wide variety of natural compounds are isolated from these natural resources for the treatment of infectious diseases (Mittal and Dixit, 2013). Consequently, researchers worldwide have paid immense attention on the screening of medicinal plants for such phytochemicals that have antimicrobial properties as prospective novel therapeutic applications. Contextually, since the 1990s, crude plant extracts have gained much attention owing to the antibiotic abuse or overdosing of traditional antibiotics. This led to the emergence of antibiotic-resistant microbes. It was proven that phytochemicals present in plant crude extract have potential antimicrobial agents that are more effective than individual compounds (Al-Dhabi *et al*., 2019).

For the current study, the antibiofilm and antiadhesive properties of *A. lanata* (L.) A. L. Juss. ex Schultes (henceforth *A. lanata*) have been tested against prevalent organisms such as *S. aureus and E. coli* in CAUTI for investigation. The study specifically investigated the antibacterial activity caused by the methanolic extract of *A. lanata* against *S. aureus* and *E. coli*. *A. lanata* is a vertically grown shrub of long tap-root family found in the wild. Traditionally considered a medicine plant, it is used in many ailments like urolithiasis and considered to be very effective treatment due to its phytochemical, antimicrobial, hepaprotective and antiurolithiasis properties (Zhu *et al*., 2019). This study explores whether this plant can be used as antibiofilm and antiadhesive material against organisms such as *S. aureus* and *E. coli* in catheter-associated urinary tract infection.

2. Materials and Methods

2.1 Preparation of methanolic crude extract of *A. lanata*

A. lanata whole plant powder, approximately weighing 20 g was purchased from local market and added into a thimble made up of cellulose. Cellulose tubing was chosen for its permeability and its passive transport quality and passive diffusion, used effectively for organic compound extraction and dust sampling and placed inside the Soxhlet apparatus for crude extract preparation as described earlier (Harley *et al.,* 2022). The flask was filled with methanol and the temperature was set at 60^0C to run the cycles for several hours. The extraction procedure was completed after obtaining the colourless solvent. Soon after the solvent evaporation was done, the obtained product was weighed and used for further analysis. The methanol was used in this experiment to extract the *A. lanata* due to its low boiling point, high volatility and higher extraction efficiency when compared to other solvent extraction media.

2.2 *A. lanata* and its antibacterial activity

The methanolic crude extract of *A. lanata* was utilized in this experiment to ascertain the antibacterial activity against *S. aureus* (ATCC 25923) *and E. coli* (ATCC 25922) (Meiyazhagan *et al*., 2016). In short, the overnight cultures (0.5 MacFarland units) of *S. aureus and E. coli* were swabbed on previously prepared sterile MHA plates followed by the well drill. Each well received two different concentrations of *A. lanata* crude extract followed by plate incubation. The antibacterial activity was determined based on the zone formation around the well. In this procedure, methanol functioned as "vehicle control" and ampicillin and rifampicin were used as "positive controls" for *S. aureus and E. coli.*

2.3 MIC determination of *A. lanata*

The methanolic crude extracts of *A. lanata* MIC against *S. aureus and E. coli* were determined using the microdilution method (Meiyazhagan *et al*., 2015). For the microdilution assay, the methanolic crude extract (5 mg/ml) was sequentially diluted in MHB and it continued until the final concentration of extract was reduced to 0.03 mg/ml. The microtiter plate was incubated whose "optical density" of the plate reached 590 nm. The experiment was performed in triplicate.

2.4 Effect of *A. lanata* crude extracts on microbial colonization

The impact of methanolic crude extracts of *A. lanata* on *S. aureus and E. coli* colonization was studied using polystyrene plates (Meiyazhagan *et al*., 2015). As mentioned earlier, when the crude extract of 5 mg/ml was sequentially diluted in MHB to reach 0.03 mg/ml concentration, the colonization of both microbes was studied. The microtitre plates were incubated for 96 h for colonization. The methanol was used to fix the attached cells after removing the unattached cells with PBS wash. Fixed cells were stained using crystal violet solution (0.1%) and destained with mixture of ethanol acetone. In the end, the purple output was measured at 560 nm until the untreated cells were seen as negative control. The test was performed in triplicate.

2.5 *A. lanata* crude extracts effect on biofilm formation

The biofilm formation assay was also performed to study the effect of methanolic crude extract of *A. lanata* against *S. aureus and E. coli* in a micrtitre plate as found in a previous study (Gowri *et al.,* 2020). In brief, biofilm formation of both microbes was achieved when the cultures were grown in a microtitre plate for 96 h. The matured biofilm received the 1X and 2XMIC of crude extract treatment for 24 h. Eventually, the methanol fixation was done for the attached cells after removing the cells. Fixed cells were stained with crystal violet and destaining was done using the mixture of ethanol and acetone. The plates were then measured at 560 nm until the untreated cells were seen as negative control. The test was performed in triplicate.

2.6 Antibacterial activity on catheter coated with *A. lanata*

The catheter coating with methanolic crude extract of *A. lanata* antibacterial activity was investigated against *S. aureus and E. coli* using the "*in vitro* bladder model" as mentioned in a previous study (Goda *et al.* 2022). The sterile silicone tube pieces were made and immersed in the solution of methanolic crude extract of *A. lanata* for 2 h, followed by air drying. In order to evaluate the antibacterial activity of the coated catheter, the air-dried catheter was kept over a sterile MHA plate swabbed with respective microbes and incubated overnight. The antibacterial activity of the methanolic extract of *A. lanata* was determined by the observation of zone formation around the piece. The test was performed two times.

3. Results

3.1 Determination of antibacterial activity

Figure 1 exhibits zone formation taken place by different concentrations of crude extract of *A. lanata* against prevalent organisms determined. It was evident from the result that the methanolic crude extract performed an antibacterial activity in 5 mg against tested microbes. When the concentration increased, say to 10 mg, the size of the zone formation would also increase against *S.aureus and E. coli* involved in CAUTI.

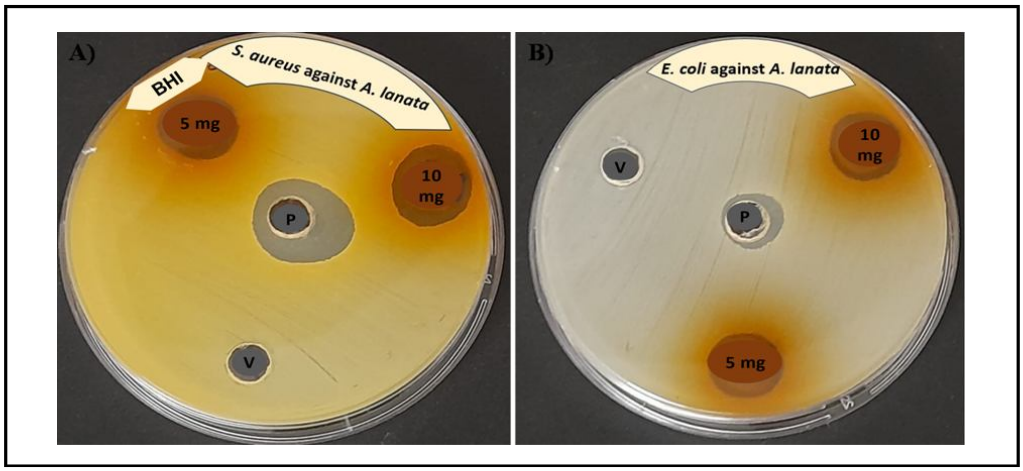

Figure 1: Antibacterial behavior of methanolic crude extract of *A. lanata* against (A) *S. aureus and* (B) *E. coli.*

Note: V: Vehicle Control; P: Positive Control; BHI: Brain Heart Infusion.

3.2 MIC determination

The methanolic crude extract of *A. lanata* MIC, determined against *S. aureus and E.coli,* used the microdilution method and the minimum concentration of *A. lanata* needed to inhibit the growth of *S. aureus and E. coli,* as presented in Figures 2 and 3. As seen in the figures, the MIC calculated for *A. lanata* methanolic crude extract against *S. aureus and E. coli* was found to be 5 mg/ml.

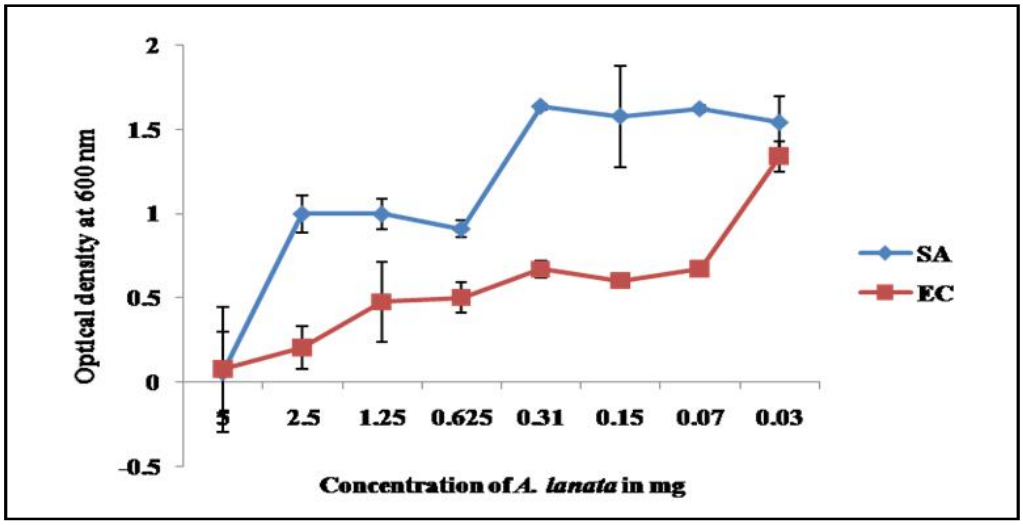

Figure 2: MIC of methanolic crude extract of *A. lanata* against *S. aureus and E. coli.*

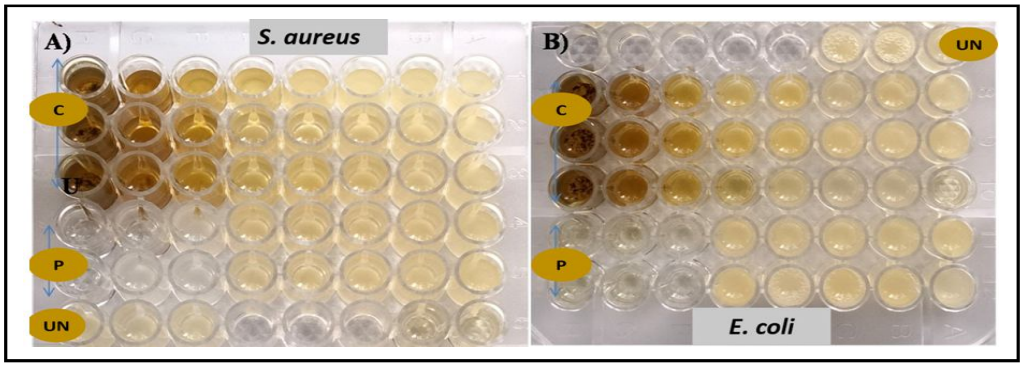

Figure 3: Visualization of MIC determination of methanolic crude extract of *A. lanata* against (A) *S. aureus and* (B) *E. coli.*

Note: C: Crude; P: Positive Control; Un: Untreated.

3.3 *A. lanata* crude extracts effect on colony formation

A. lanata methanolic crude extract's effect on *S. aureus and E. coli* colony formation was calculated using polystyrene surface, which is represented in Figures 4 and 5. As exhibited in the figures, the crude extract can resist the colony formation of *S. aureus and E. coli* at the MIC level (5 mg/ml) on a polystyrene surface which supports the growth of bacteria. Interestingly, Figure 4 exhibits that the colony formation of *S. aureus* does not increase when the *A. lanata* crude extract concentration is decreased, which indicates that even the minimal concentration present in the well can decrease the colonization when compared to controls. In contrast, when the concentration decreases the colonizing ability of *E. coli* increases.

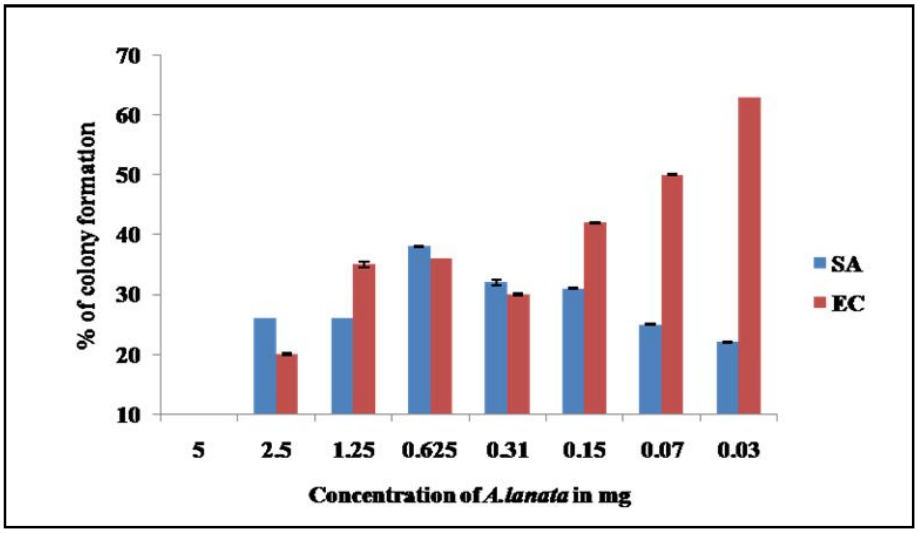

Figure 4: Graph representing percentage colony formation after treatment with *A. lanata* methanolic extract against *S. aureus and E. coli.*

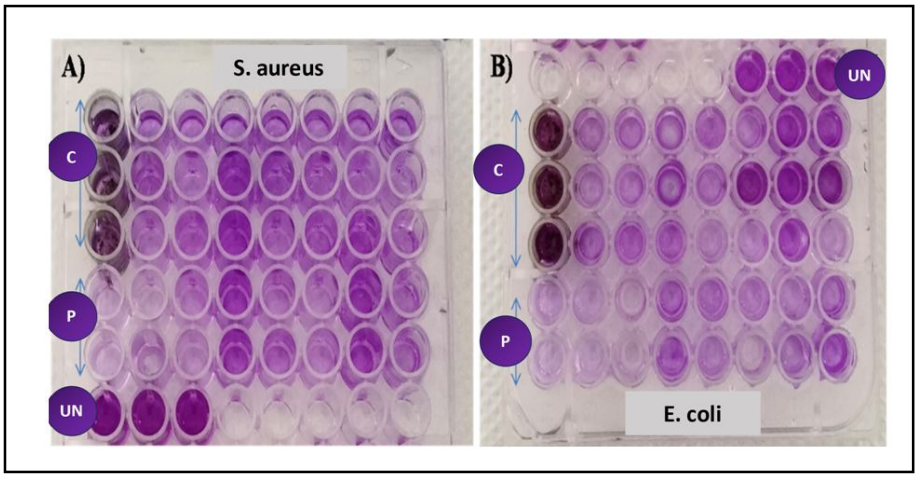

Figure 5: Photographic representation of colony formation after treatment with *A. lanata* against *S. aureus and E. coli.*

Note: C: Crude; P:Positive Control; Un: Untreated.

3.4 Effect of *A. lanata* extracts on biofilm formation

Various concentrations of methanolic extracts of *A. lanata* were studied on biofilm formation caused by *S. aureus and E. coli,* and the percentages were calculated for biofilm inhibition after treatment as presented in Figures 6 and 7. As mentioned in the figures, the percentage of biofilm inhibition of *S. aureus* after treatment with crude extract exhibited 67% and 79% reductions for 1XMIC and 2X MIC, respectively; while *E. coli* was reduced to 85% and 87% after being treated with 1X and 2X MIC of methanolic crude extract of *A. lanata.* These results revealed the antibiofilm ability of *A. lanata* against *S. aureus and E. coli.*

3.5 Antibacterial activity of *A. lanata* on coated catheter

The catheter coating of *A. lanata* methanolic extract antibacterial activity was studied against *S. aureus and E. coli* in the *in vitro* bladder model which mimics the suitable condition presented in Figure 8. The zone formation surrounding the catheter coated with extract suggests the anti-adhesive property of crude extract of *A. lanata.*

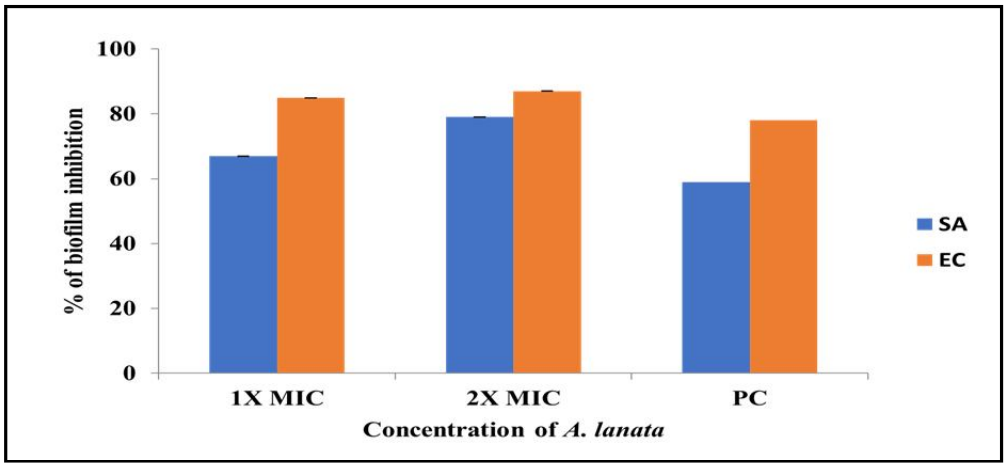

Figure 6: Exhibition of the percentage of biofilm inhibition after treatment with *A. lanata* against *S. aureus and E. coli.*

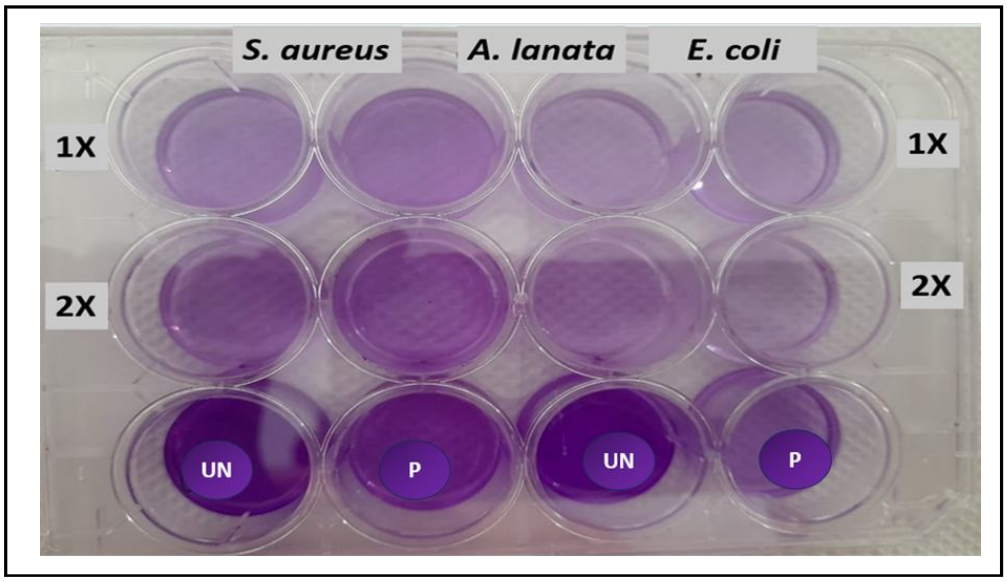

Figure 7: Visual images of the impact of biofilm inhibition after treatment with *A. lanata* against *S. aureus and E. coli.*
Note: UN: Uncoated; P:Positive control.

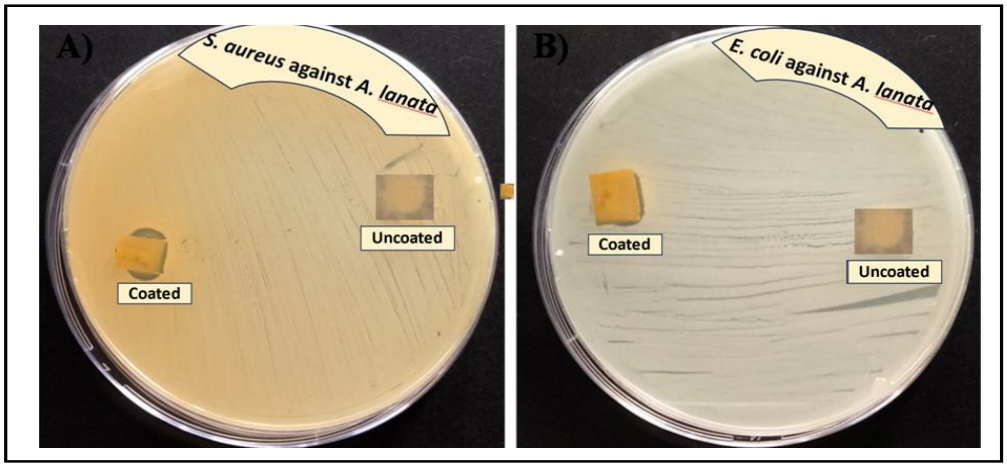

Figure 8: The antibacterial activity of methanolic extract of *A. lanata* coated catheter against *S. aureus and E. coli.*

4. Discussion

Catheter-associated urinary tract infections representing leading nosocomial infection causes mild to severe complication in immune-compromised patients and makes the high rate of morbidity and mortality due to its polymicrobial structure which makes the development of antibiotic-resistant strain and creates treatment challenges (Jordan *et al*., 2015; Sanchez *et al*., 2022). Consequently, to overcome the antibiotic resistance exhibited by the organism, attention is drawn towards discovering the novel antibacterial activity in natural sources (Khumalo *et al*., 2021; Gunda *et al.,* 2021). Among others, the plant resources have gained much attention due to their ample medical values in the healthcare system. Methanol was also used to extract the *A. lanata* due to its low boiling point required to ensure higher extraction efficiency. In the current study, the antibacterial activity was seen as the methanolic activity of *A. lanata* against *S. aureus and E. coli,* which are prevalent organisms involved in CAUTI. The main finding of experiment was exhibited in the form of the antibacterial activity of the methanolic crude extract of *A. lanata,* which showed antibacterial activity against the tested organism, and achieved the lowest concentration needed for *A. lanata* to stop the growth of *S. aureus and E. coli*.

These findings are consistent with a few earlier studies wherein the antibacterial activity of *A. lanata* was determined using various organic solvents like methanol, acetone, water, and chloroform. In a study by Al-Ansari *et al*. (2019), the antibacterial activity was exhibited against *E. aerogenes* and *E. coli,* while the crude extract was analysed for various phytochemicals to confirm the presence of terpenoids, alkaloids, *etc*., and thereby proving the antibacterial activity of *A. lanata*. Likewise, in another study (Olufunmiso and Anthony, 2012), the antimicrobial activity of ethanolic extracts of *Erythrina caffra* was investigated against *Proteus vulgaris and Micrococcus luteus* using various experiments indicating them as the most potent folk medicine for the treatment of gastrointestinal infection. In contrast, the antimicrobial and antioxidative property of *A. lanata* was investigated against various microbes including *E. coli*, *Acinetobacter*, *S. aureus,* and *M. luteus,* and antimicrobial activity at 50 µg/ml (Behera and Ghosh, 2018).

In addition to being a source for the antibacterial activity, *A. lanata* was also investigated for its antibiofilm activity because CAUTI is a biofilm-associated infection. Biofilm formation can play a major role in eliminating the bacterial infection from the host. The infection gets started when the adherence of pathogen on the catheter surface facilitating microbial colonization and resulting in a quick biofilm formation. The biofilm produces a thick polymeric substance which avoids antibiotic entry resulting in antibiotic-resistant strain development, making the treatment critical (Zhu *et al*., 2019; Arnoldo *et al.,* 2013; Pelling *et al.,* 2019). These studies conclude that there should be a focus on each stage of biofilm formation from attachment to maturation when studying biofilm prevention (Muhammad *et al*., 2020).

The current study has reported the effect of the methanolic crude extract of *A. lanata* on colonization of *S. aureus and E coli*. It was found that *A. lanata* has the potential to inhibit colonization up to its MIC level; while, colonization cannot take place in the presence of a trace of methanolic crude extract indicating the potency of *A. lanata* in eliminating colonization. This was further confirmed by biofilm formation assay wherein the *A. lanata* effectively reduced the biofilm formation.

Further, to prevent biofilm formation on the catheter surface, coating with an antibacterial agent on the inner and outer surfaces of the catheter is an excellent method that estimate the clinical condition for evaluation of innovation to prevent catheter associated urinary infection. For this purpose, the silicone catheter tube is coated with methanolic extract of *A. lanata,* and the antibacterial activity is evaluated against *S. aureus and E. coli* using the *in vitro* bladder model showing the potent activity. Similarly, polymer-based catheter coating provides excellent antimicrobial and antibiofilm activity against *E. coli, S. aureus,* and *P. aeruginosa* in the urinary catheter environment (Jia *et al*., 2021). The catheter surfaces coated with several antibiotic combinations are evaluated against *S. aureus, E. coli,* and *P. mirabilis* and are able to control colonization for more than a week thereby reducing infection (Fisher *et al*., 2015).

5. Conclusion

The antibacterial activity of methanolic crude extract of *A. lanata* was investigated for antibacterial and antibiofilm activity against *S. aureus and E. coli* which are commonly encountered organisms in CAUTI. The antibacterial activity and least inhibitory concentration were determined against tested organisms. The *A. lanata* effect on colonization was studied and the biofilm reduction was observed after treatment with crude extract of *A. lanata*. The biofilm formation prevention on the catheter surface and the coating of the catheter with extract showed antibacterial activity against *S. aureus and E. coli.* Altogether, the methanolic crude extract of *A. lanata* proved its antibiofilm activity and that it can be used for coating purposes to prevent biofilm formation.

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Conflict of interest

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

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