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Antibacterial activity of *Erythrina indica* Lam. methanolic extract against catheter-associated urinary tract infections by *Staphylococcus aureus*

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Abstract

Indwelling catheters play a major role in device-associated bacterial infections which cause complications in the treatment, resulting in severe suffering to the patients by prolonged morbidity, higher hospital expenses and comparatively higher mortality rates. Catheter-associated urinary tract infections are among them and their management poses challenges as most of the causative bacteria form biofilms resulting in the development of antibiotic resistant strains. As the scientific world is searching for potential compounds with novel modes of action, the present study investigates the antibacterial potentials of the extract (methanolic) of the traditionally used medicinal plant *Erythrina indica* Lam. against one of the most challenging, biofilm forming CAUTIs causing bacterial pathogens - *Staphylococcus aureus*. The methanolic extract of *E. indica* was screened to find its antibacterial activity, minimum inhibitory concentration, antibiofilm activity, *etc*. The extract showed promising bioactivity by suppressing the growth of *S. aureus* and it is MIC was found to be 4.8 mg/ml. Also, the extract could reduce the *S. aureus* biofilms on the catheters up to 71%. The above-mentioned findings indicates that the methanol-based extract of *E. indica* contains bioactive compounds capable of eradicating the catheter biofilms formed by *S. aureus*. Therefore, the authors recommend for further studies to purify the compounds responsible and to develop antibacterial coatings from the extract for its clinical use.

1. Introduction

The use of indwelling medical devices has been in practice for several centuries and the application of indwelling urinary catheters gained huge attention owing to their use in hospitalized patients to overcome diseases (Milo *et al*., 2019; Wooller *et al*., 2018; Saint *et al*., 2016; Guggenbichler *et al*., 2011). But most nosocomial infections are associated with medical devices, and the lifesaving indwelling urinary catheters also significantly increase the risk for iatrogenic infections in hospitalized patients, particularly in immune-compromised patients (Medina and Castillo-Pino, 2019). Such CAUTIs (catheter-associated urinary tract infections) are more prevalent even though they may vary from uncomplicated to severe infections, they affect several millions of people worldwide (Papanikolopoulou *et al*., 2022; Flores-Mireles *et al*., 2019). The indwelling catheters which are mainly used to drain the urine from urinary system create an opportunity for microbial entry resulting in the development of CAUTIs (Yisiak *et al.,* 2021; Skelton-Dudley *et al*., 2019). Once the microbial entry is happened from the urine to the bladder through the lumen, it can form a surface colonization which results in mild to severely complicated urinary tract infections (UTIs) leading to a lengthy hospital stay, higher economic burden and comparatively higher rate of mortality (Magill *et al*., 2018).

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Many etiological microorganisms like Gram-negative and Gram-positive bacteria as well as fungal species are responsible for CAUTIs and the formation of biofilms by these agents on the catheter surfaces makes CAUTI management critically challenging (Kurmoo *et al*., 2020; Di Martino, 2018). The biofilm forming ability of prevalent microorganisms is the primary reason for making the treatment process harder, as these biofilms produce an extracellular polymeric substance that protects the pathogens from external attack including antibiotic treatment (Peng *et al*., 2018; Tenke *et al*., 2017). The antibiotic treatment tolerance creates a huge problem in destroying the biofilm structures resulting in the development of antibiotic resistant strains causing treatment failures in many cases of CAUTIs (Walker *et al*., 2020; Maharjan *et al*., 2018). Therefore, the development of potential antibacterial agents with novel modes of action is needed to fight against such biofilms including against those caused by *S. aureus* in CAUTIs.

Since ancient times, plants of various kinds as they possess potential activities, have been used in curing and managing human diseases including microbial infections, and even in the age of modern medicine, most of the world's population is dependent on medicinal plants for their major health care (Sahil *et al.,* 2023; Bhawana and Afroz, 2022; WHO, 2012). The plant genus *Erythrina* which grows in tropical and subtropical areas is rich with compounds having pharmacological properties like sedative, laxative, and diuretic activities. *E. indica* is one of the major members of the genus with biological activities and the phytochemicals like alkaloids, flavonoids and phenolic compounds are found to be responsible for its antimicrobial properties (Zhang *et al.,* 2016). Hence, the present study investigates the antibacterial and antibiofilm activities of the methanolic extract of the medicinal plant *E. indica* against one of the major CAUTI biofilm forming bacterial pathogens - *S. aureus*.

2. Materials and Methods

2.1 Preparation of methanolic crude extract of *E. indica*

The collected *E. indica* plant was cleaned, dried and powdered for the extraction which was performed as per standard protocols (Harley *et al.,* 2022). In brief, 20 gm of the powder was added into the thimble cellulose tube and was placed inside the Soxhlet apparatus. An adequate volume of the solvent methanol was added into the flask and the temperature was set at 60^0C to run the cycles for several hours. The extraction procedure was continued till the colorless solvent was obtained. The obtained crude extract was subjected to solvent evaporation. The collected product was weighed and used for further analyses.

2.2 Antibacterial activity of crude methanolic extract of *E. indica*

The crude methanolic extract of *E. indica* was analysed for its antibacterial activity against *S. aureus* as per standard procedures (Meiyazhagan *et al*., 2016). Briefly, 0.5 MacFarland units of the overnight culture of the bacterial pathogen which was grown in Mueller Hinton Broth (MHB) was swabbed on sterile Mueller Hinton Agar plates and 6 mm wells were drilled on the agar surface. Two different concentrations of methanolic crude extract were put into each of the wells. At 37^0C, the plates were incubated for a period of 36 h. Methanol was used as vehicle control and the antibiotic ampicillin was used as positive control for *S. aureus*. The antibacterial activity was determined based on the formation of zones of growth inhibition around the well and was measured in millimeters (mm).

2.3 *E. indica* MIC determination

The minimal inhibitory concentration (MIC) of the crude methanol-based extract of the plant against *S. aureus* was determined by the microdilution method as explained by Meiyazhagan *et al*. (2015). In brief, 4.8 mg/ml of the crude extract was added into the wells, and using MHB, they were serially diluted up to 0.03 mg/ml. The culture was put into all the wells and subjected for incubation in standard conditions. After incubation, the ODs of the plates were measured at 600 nm. All the experiments were done thrice.

2.4 Effect of *E. indica* crude extract on *S. aureus* colony formation

The experiment to determine the effect of methanolic extract of the plant on *S. aureus* colonization was conducted as explained by Meiyazhagan *et al*. (2015). The methanolic crude extract of the plant (4.8 mg/ml) was serially diluted using MHB until it reached a concentration of 0.03 mg/ml and the bacterial culture was put to all the wells, and at 37^0C for a period of 96 h, the plate was incubated. After incubation, the wells were washed with the Phosphate buffer saline (PBS) to remove the unattached cells, followed by cell fixation with methanol. The fixed cells were stained (with 0.1% crystal violet) and de-stained with an acetone-ethanol mixture. The obtained coloured product was read at 570 nm. The experiment was done in triplicates. The well contained untreated cell served as negative control.

2.5 Antibiofilm effect of *E. indica* crude extract against *S. aureus*

The effect of methanolic crude extract of *E. indica* on *S. aureus* biofilm formation was studied using the biofilm formation assay as described by Gowri *et al.* (2020). In short, *S. aureus* biofilm formation was attained on polystyrene surfaces after 96 h of incubation of the bacterial culture on it. The formed biofilm was treated for 24 h with 1 X and 2 X MICs of the crude extract of *E. indica*. After the treatment, the unattached cells were removed from wells by PBS wash and was followed by the cell fixation with methanol. The staining of the fixed cells was performed using 0.1% crystal violet solution and then de-stained with acetone and ethanol mixture. The obtained purple coloured product was read at 570 nm. The experiment was done in triplicates. The well containing untreated cells served as the negative control.

2.6 Antibacterial activity of *E. indica* crude extract coating on the catheter

The antibacterial activity of the methanolic crude extract of *E. indica* coated catheter against *S. aureus* was investigated in *in vitro* bladder model as per standardized protocols (Goda *et al.,* 2022). For the assay, the sterile silicone catheter tube pieces were made and dipped into the crude extract for 2 h, followed by air drying. The air-dried catheter pieces were placed over the surface of sterile MHA plates swabbed with *S. aureus* and incubated in standard conditions. Later, the incubated plates were analysed for the formation of zones of growth inhibition around them. The experiments were done in duplicate.

3. Results

3.1 Determination of antibacterial activity

The antibacterial potentials of crude methanolic extract of *E. indica* were investigated against one of the major CAUTI biofilm forming bacterium, *S. aureus,* and the result is presented in Figure 1. The antibacterial activity was achieved at a concentration of 4.8 mg/ml of the plant extract. The diameters of the zones of inhibition were found to be increasing with the increase in the concentration of the plant extract. Here, the vehicle control was not able to form any zone of growth inhibition.

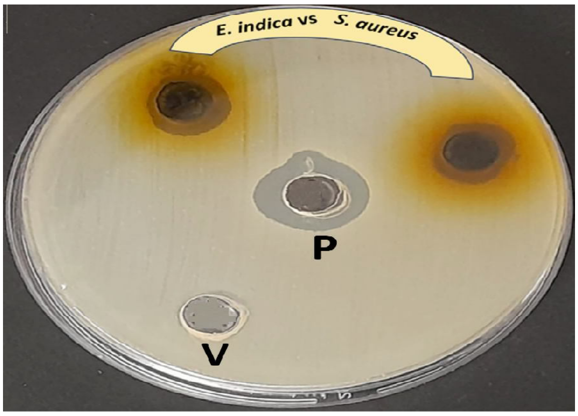

Figure 1: Antibacterial activity of *E. indica* against *S. aureus.*

3.2 Determination of MIC of methanolic extract of *E. indica* against *S. aureus*

MIC of the methanolic extracts of *E. indica* was determined against *S. aureus* using the microdilution method is presented in Figure 2 and Figure 3. As it is indicated, the graph represents the least concentration needed to inhibit the growth of *S. aureus* was calculated that the MIC, and was 4.8 mg/ml of the extract.

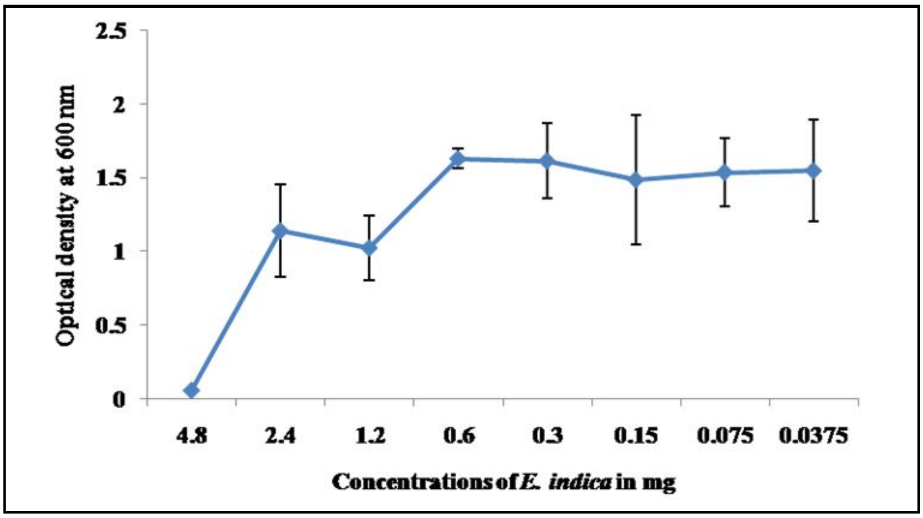

Figure 2: MIC determination of *E. indica* against *S. aureus*.

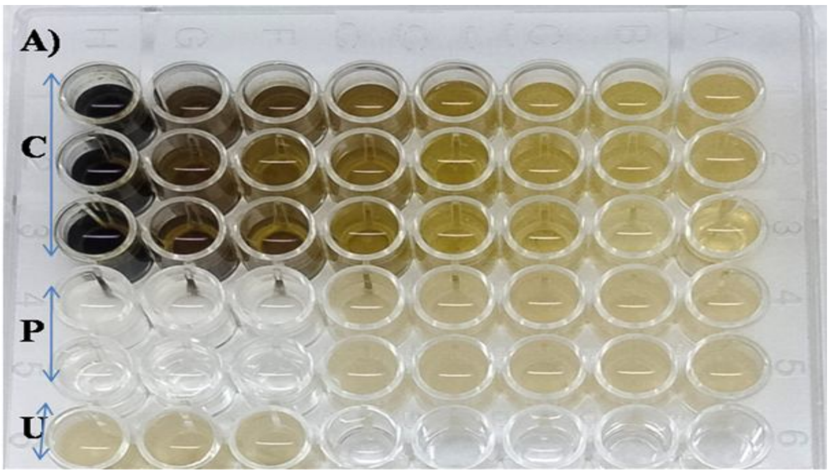

Figure 3: Visual effect of MIC determination of *E. indica* against *S. aureus*.

3.3 Effect of *E. indica* extract on colony formation by *S. aureus*

The colony forming ability of *S. aureus* on the polystyrene surfaces was studied after processing with different concentrations of the methanolic extract of *E. indica* and the results are shown in Figure 4 and Figure 5. As clear from the figures, the plant extract was able to hinder colony formation up to its MIC level. As the *E. indica* concentration decreased, a gradual increase in the *S. aureus* colony formation was also visible.

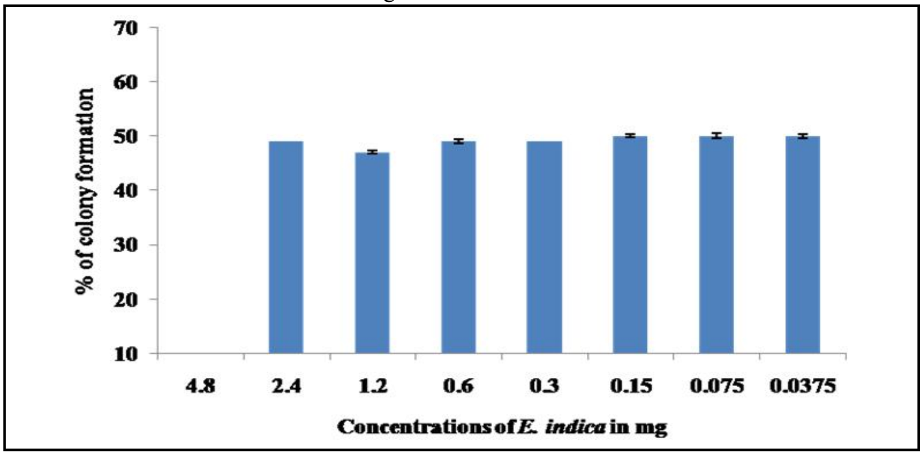

Figure 4: Percentage of *S. aureus* colony formation after treatment with *E. indica*.

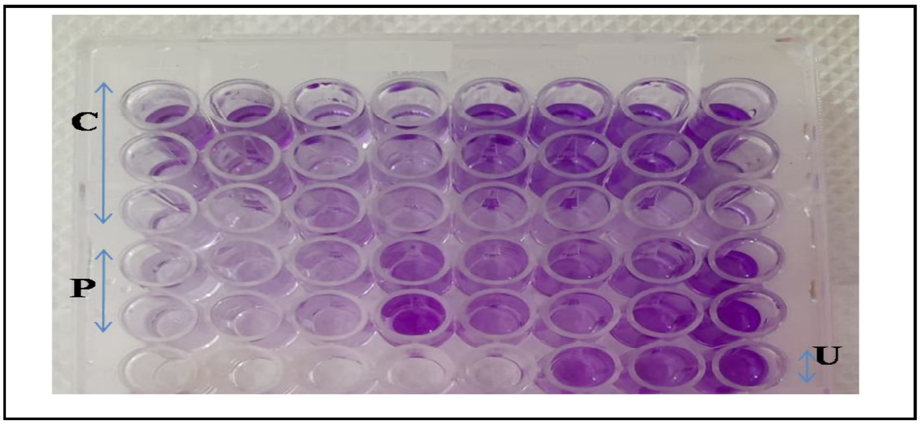

Figure 5: Pictorial representation of *E. indica* effect on *S. aureus* colony formation.

3.4 Effect of the of *E. indica* methanolic extract on *S. aureus* biofilm formation

The antibiofilm effect of the *E. indica* methanolic extract on *S. aureus* was analysed using biofilm formation assay and the percentage of biofilm inhibition after processing with different extract concentrations is presented in Figure 6 and in Figure 7. The graph shows that, when the methanolic extract concentration increases, the biofilm formation efficiency of the bacterium *S. aureus* decreases. The biofilms were reduced by 26% and by 71% when treated with 1 X MIC and 2 X MIC concentrations of the extract, respectively. The result proved the *E. indica* has the ability to destroy the biofilms by the CAUTI pathogen *S. aureus*.

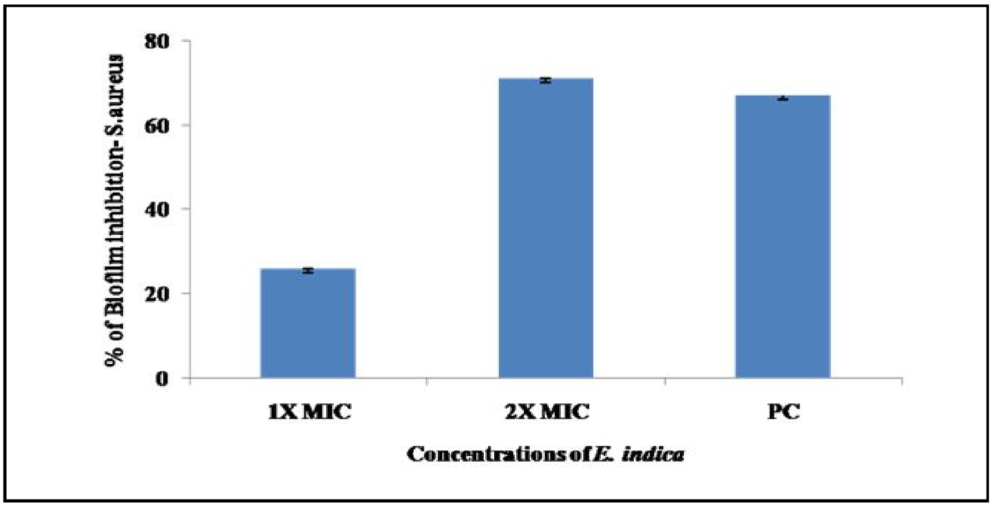

Figure 6: Graphical representation of *S. aureus* biofilm formation after treatment with *E. indica*.

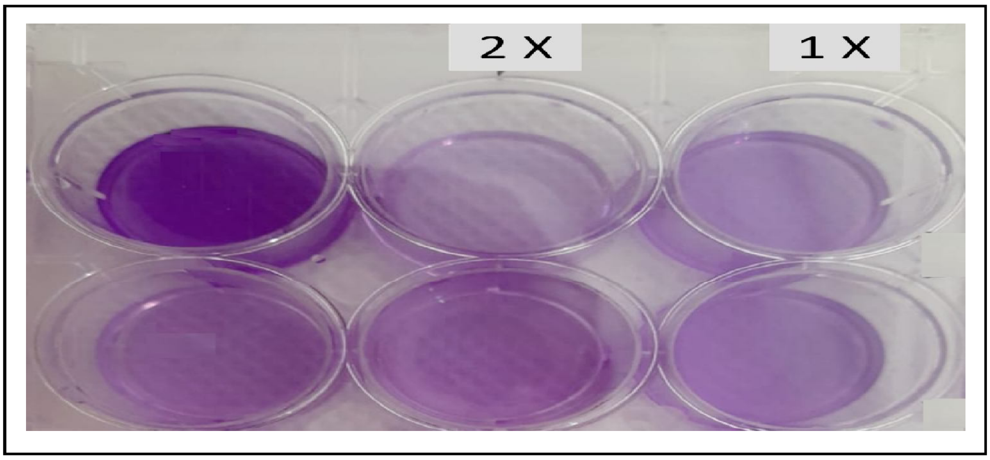

Figure 7: Visual effect of *E. indica* on *S. aureus* biofilm formation.

3.5 Antibacterial activity of *E. indica* catheter coating

The antibacterial activity of the extract of the *E. indica*-coated catheters against *S. aureus* was studied *in vitro* on catheter models as presented in Figure 8. As seen in the figure, a small sized zone of growth inhibition was noted indicating the moderate antibacterial activity for *E. indica* against *S. aureus*.

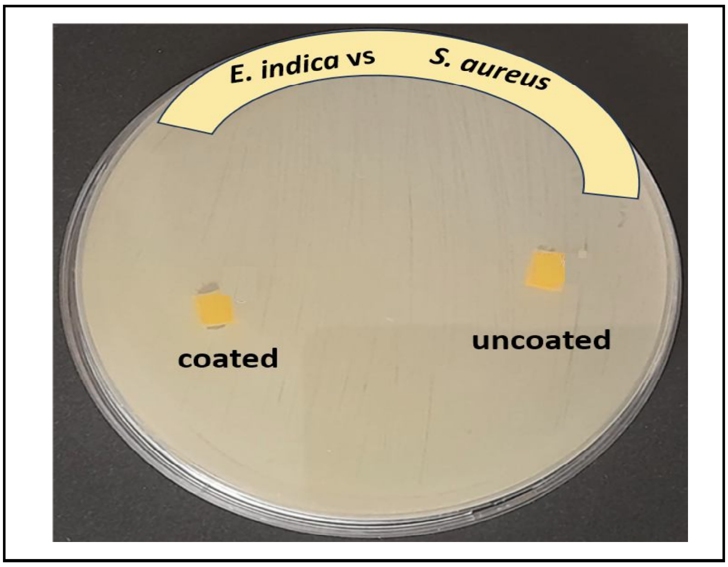

Figure 8: Catheter coating of methanolic extract *E. indica* against *S. aureus*.

4. Discussion

Even though medical devices are mainly used for saving human life by providing the health care needs (Ansari *et al*., 2020), many of them provide suitable environments for pathogens including bacteria for their nourished growth. CAUTI (Catheter-associated urinary tract infection) is one of most important of such biomedical devices-associated nosocomial infections which has serious health and socio-economic implications as it results in high hospital expenses, prolonged morbidity and a higher rate of mortality by forming antibiotic resistant biofilms (Mitchell *et al*., 2021; Smith *et al*., 2019). So, in the study, we focused on the antibacterial and antibiofilm forming activities of the methanolic extract of *E. indica* plant, which is known for their medicinal values and was investigated against *S. aureus*. Our study demonstrated the antibacterial activity of *E. indica* against *S. aureus* which is predominantly involved in CAUTI. In a similarly study, the antibacterial activity of sixteen flavonoids purified from *Erythrina variegate* were screened against methicillin resistant *S. aureus* (MRSA) by Tanaka *et al*. (2002) and the compounds were reported to have shown potential antibacterial activity against the selected MRSA. They determined the minimum inhibitory concentration as ranging from 3-6 mg/ml. Likewise, the essential oil which was purified from *Erythrina caffra* was evaluated for its antibacterial activity against *Pseudomonas aeruginosa* and *Salmonella typhimurium* (Wintola *et al*., 2021) and found that it has promising activities. Also, the methanolic extract of *Erythrina sigmoidea* showed antibacterial activity against various Gram-negative bacterial pathogens like *P. aeruginosa*, *Escherichia coli*, *Enterobacter cloacae, Klebsiella pneumonia* and *Providencia stuartii* and was concluded to be due to the presence of chemical components like bidwillon, atalantoflavone, etc. (Djeussi *et al*., 2015). Another study by Ahmed *et al*. (2020), using *Erythrina suberosa* extract concluded that the plant has potential activities against methicillin resistant *S. aureus*. In the same way, the antibacterial activity of five different medicinal plants including *Erythrina verna* was evaluated against *S. aureus, Pseudomonas aeruginosa* and *K. pneumonia* and the researcher calculated that the MIC of the *E. verna* plant against *S. aureus* was 100 mg/ml (Romha *et al.,* 2018) and it also had some activities against tuberculosis infection. Thus, these findings by various researchers underline the observations in our present investigation. Also, there were various research works to investigate the effect of different coating materials on catheters that could eradicate or prevent the biofilm formation by microorganisms. Aleksandra *et al*., 2021 found that zinc oxide nanoparticles coated catheters had antimicrobial capabilities against *S. aureus* and *E. coli*, and also the activity was seen to persist for seven days. Similarly, other works showed that the silver nanoparticle and fosfomycin coated catheter had antimicrobial activities against *Enterococcus faecalis, S. aureus* and *E. coli* when used in *in vitro* bladder models (Rahuman *et al*., 2021; Abbott *et al*., 2020). Triclosan coated catheters are also reported to have exhibited antimicrobial activity against *S. aureus* and *Enterococcus* sp. (Cadieux *et al*., 2009). Thus, the coating of catheters with substances having antibacterial activity, including that of extracts form *E. indica* needs to be studied in detail.

5. Conclusion

The antibacterial potentials of methanolic extract of *E. indica* was evaluated against the major CAUTI biofilm forming bacterial pathogen *S. aureus*. The plant extract exhibited antibacterial activity and the minimum inhibitory concentration against *S. aureus* was calculated as 4.8 mg/ml. The *E. indica* extract was also examined for its ability to prevent colony formation and the biofilm development by the bacterium and it was found that the selected medicinal plant possesses antibacterial, antibiofilm and antiadhesive properties. Thus, the authors strongly recommend that further studies be conducted to purify the compounds responsible for bioactivities in the plant *E. indica* and to develop them to make them available for clinical application.

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Conflict of interest

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

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