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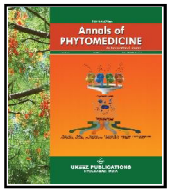

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Prevention of catheter associated urinary tract infection biofilms using *Illicium verum* Hook. f. extract coated urinary catheters

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Abstract

Biofilm is a complex organic compound made up of microorganisms that develop in colonies within an extracellular mucopolysaccharide substance. One of the most prevalent among HAIs (healthcare associated infections) is catheter-associated urinary tract infection (CA-UTI); in which 70-80% of the infections are linked to the usage of an indwelling urethral catheter. *Escherichia coli* and *Enterococcus* spp. are the most prevalent causative organisms for CA-UTI, accounting for 48% of the total infections. Even though, antimicrobial coating on the catheter surfaces is considered to be effective, development of antimicrobial resistance (AMR) among pathogens lead to undesirable impacts and development of novel antimicrobial agents from various sources become necessary. Therefore, in the present study, the biofilm inhibition potentials of *Illicium verum* seed pod extract was evaluated against major biofilm producers *E. coli* and *Enterococcus faecalis*. Antibacterial activity was evaluated by well diffusion method and the minimum inhibitory concentration was determined. Seedpod extracts were coated on urinary catheters and bacterial inhibition was evaluated. The crude extracts showed higher inhibitory zones at 500 mg/ml and 1000 mg/ml. MICs were observed to be 15.6 mg/ml and 7.8 mg/ml against *E. coli* and *E. faecalis*, respectively. The coated urinary catheters showed evident inhibitory zones demonstrating the effective biofilm prevention and elimination. From the analysis, the *I. verum* seed pod extracts can be used for development of novel antimicrobials and catheter coatings can be used for prevention of CA-UTI.

1. Introduction

UTIs or urinary tract infections are considered as the infections most frequently occur in community settings (Goda *et al*., 2022) and they contribute to 40-50% of all the hospital acquired infections (Kranz *et al*., 2020). Among patients indwelled with catheters, 15-25% are found to have developed catheter-associated urinary tract infections (CA-UTIs), which will lead to longer morbidity with increased health expenditures (Van Decker *et al*., 2021; Tedja *et al*., 2015). The urinary catheters, which are tubular devices made of silicone or latex, are easily subjected to the formation of biofilms (Goda *et al*., 2022). Bacteria normally enter the drainage system through the periurethral region or ascend the drainage tube after colonising the drainage bag (Kovach *et al*., 2017). Biofilms are thin layers of microorganisms attached irreversibly to a solid surface producing an extracellular polysaccharide coating on themselves to protect from most of the physicochemical or biological adverse conditions or antibiotics and in the case of urinary catheters, as the duration of the use of unchanged catheters increases, the extend of biofilms will also be high (Goda *et al*., 2022; Oleksy-Wawrzyniak, 2021; Liu *et al*., 2020; Stickler, 1996). Majority of the microorganisms responsible for endemic CA-UTIs are found to have originated from the perineal flora of the patient or from the hands of healthcare personnel during while caring the patient and in some exceptional cases; they have found to have originated hematogenously by pyelonephritis (Gong *et al*., 2017). Common microbial species forming biofilms in CA-UTIs are *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, Enterococcus *faecalis*, *Proteus mirabilis* and the yeast *Candida* spp., *etc.* (Rahuman *et al*., 2021; Ansari *et al*., 2020; Delcaru *et al*., 2016). The antibiotic resistance on biofilms have been identified as a serious danger to modern medicine (Raju *et al*., 2020) as infections caused by such cause higher medical costs, longer morbidity, wider health-care resources, increased patient suffering, *etc*., when compared with infections that can be treated with antibiotics (Gould *et al*., 2017) the scientific world is in search for potential; antibacterial compounds with novel modes of action (AlSheikh *et al*., 2020). The traditionally used medicinal plant *Lllicium verum* Hock . f. is a potential candidate to be screened against such drug resistant bacteria as it has already proven to possess phytocompounds which can fight a number of pathogens, and possesses wide variety of activities beneficial to human health (Abdullatif *et al*., 2022; Salem *et al*., 2021; Ângelo *et al*., 2019).

Lllicium verum, often known as star anise, is a plant that grows in the tropics and subtropics of Asia and is used in Eastern Asian traditional medicine (Sharafan *et al*., 2022; Mohamad *et al*., 2019;

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Guo-Wei *et al*., 2011). It has a long history of application in phytotherapy, as well as in the aromatization of pharmaceuticals, foods, and cosmetics. Flatulence, spasmodic discomfort, and colics are widely treated with the fruits as a spice and pharmacological therapy. Star anise oil is used topically to treat rheumatism and otalgia, as well as being an antimicrobial (Khan *et al*., 2022; Ding, *et al*., 2020; Abeer and Mervat, 2015; Aly, *et al*., 2016). And various companies use them to produce shikimic acid, a chemical intermediary needed in the manufacturing of oseltamivir, until 2012, when they moved to a bacterial source (Brahim *et al.*, 2021). This plant is also used to treat bronchitis, asthma, and dry cough and, linalool, one of the herb's constituents, has antibacterial and antioxidant qualities; thus it may also be used as a mouthwash (de Souza *et al*., 2022; Khan *et al*., 2022; Sun *et al.*, 2016; Aly *et al*., 2016; Liang-Deng *et al*., 2016). Traditional Chinese medicine considers it a mainstay for digestive comfort, a stimulant for female reproductive systems, and beneficial for nursing mothers since it promotes milk production (Patra *et al.*, 2020). The plant is also used to treat bronchitis, asthma, and dry cough and, linalool, one of the herb's constituents, has antibacterial and antioxidant qualities; thus it may also be used as a mouthwash (Khan *et al*., 2022; de Souza *et al*., 2022; Sun *et al*., 2016; Aly *et al*., 2016; Liang-Deng *et al*., 2016). Therefore, the present study concentrates on evaluating the biofilm inhibition abilities against *E. coli* and *E. faecalis* biofilms. This is the first report on investigating the antibiofilm potential of *I. verum* seedpod extracts.

2. Materials and Methods

2.1 Processing of plant

The collected *I. verum* seedpods were collected on 15.03.2022 and dried by keeping at room temperature in shade for 6 days. The powders of seedpods were obtained by grinding and stored for extraction in sterile containers.

2.2 Extraction of bioactive compounds using Soxhlet apparatus

I. verum seedpod powder prepared was placed in a porous, cellulose-made filter paper which is in the thimble chamber of the Soxhlet apparatus. The solvent methanol was filled in the extractor and was kept for 6 h at a 60^{0}C. The extract was collected after the solvent evaporation and the dried extracts were collected at a yield of 31.2% and kept in sterile containers.

2.3 Antibacterial activity using well diffusion method

The antibacterial potentials of the extracted plant material were assessed against the isolated clinical pathogen (as per the protocol describe by Daoud *et al*. (2015) of *E.coli* and *E. faecalis*. On the sterile plates of Mueller-Hinton Agar (MHA) fresh, overnight cultures of *E. coli* and *E. faecalis* were inoculated and perfectly swabbed with a sterile cotton swab. Using 6 mm well borer, the bores were made on the agar plate 100 ml of the extract was added to each of the wells, and the plates were kept for incubation at a temperature of 37^{o}C for a period of 48 h. After the incubation, the MHA plates were observed for the formation of zones of inhibition around them and the diameters of the zones were measured in millimetre (mm) and recorded.

2.4 Evaluating theminimum inhibitory concentration of the plant extracts

The test to find out the MICs for the plant extract against both of the selected clinical pathogens was performed by dilution method. 1 ml of extract was taken and diluted into various concentrations as 1.95 mg/ml, 3.9 mg/ml, 7.8 mg/ml, 15.6 mg/ml, 31.5 mg/ml, 62.5 mg/ml, 125 mg/ml and 250 mg/ml, all in test tubes with sterile nutrient broth of 1 ml. A 100 ml of *E. faecalis* culture at 0.5 McFarland (Eucast, 2003) standard, was inoculated to the tubes. Subsequently, the same procedure was repeated for the next pathogen, *E. coli* also. The test tubes were incubated for a period of 24 h at a temperature of 37°C. after the incubation period, the tubes were observed for turbidity or growth using unaided eye (CLSI, 2012).

Figure 1: Collection of *I. verum* seed pod.

2.5 Procurement of urinary catheters and coating

The urinary catheters (Figure 2) were collected from an authorized medical supplier. The dip and dry method were used to coat the extract of *I. verum*. The extract was prepared and the catheters were dipped for 2 min and kept for drying at room temperature. The technique was repeated twice to obtain the effective coating. The coated catheters were used for further studies.

Figure 2: Procurement of urinary catheters.

2.6 Biofilm inhibition assay

Using polystyrene tube assay which is based on the crystal violet staining method, the biofilm inhibition ability of extracts was determined. For the studies, 96 well titter plates were used. 10 µl of fresh pathogen (OD 0.4) is inoculated in the well and various concentration of extracts (10 µl, 20 µl, 30 µl and 40 µl) were inoculated and fresh MHB is inoculated making up to 200 µl the plates were incubated for a period of 48 h at a temperature of 37^{0}C. after the incubation period, the liquid medium part was discarded, and cells which are adherent were rinsed with PBS (phosphate-buffered saline) for two times. It was then stained with 0.5% of crystal violet for a period of 30 min after that, vertexing for 5 min in using an ethanol solvent, the stain was eluted from the adherent cells. Absorbance at 590 nm using an ELISA reader (Shishin, SH-U830, Taipei, Taiwan, ROC). Using fresh samples, the assay was repeated for thrice.

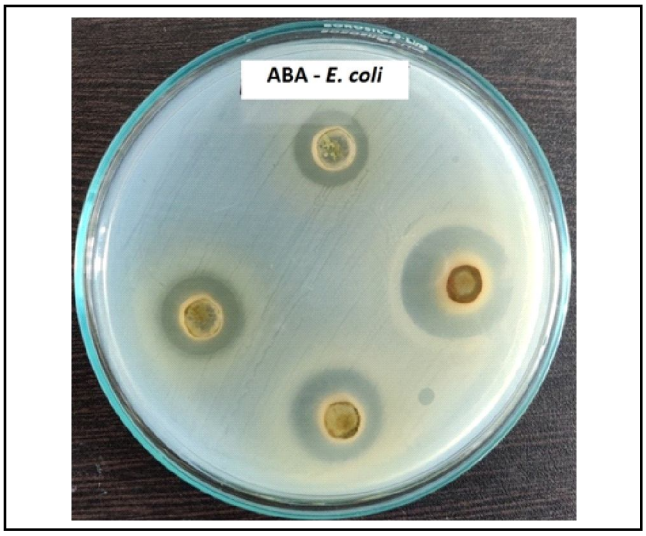

Figure 3: Antibacterial activity of the *I. verum* seedpod extracts against *E. coli.*

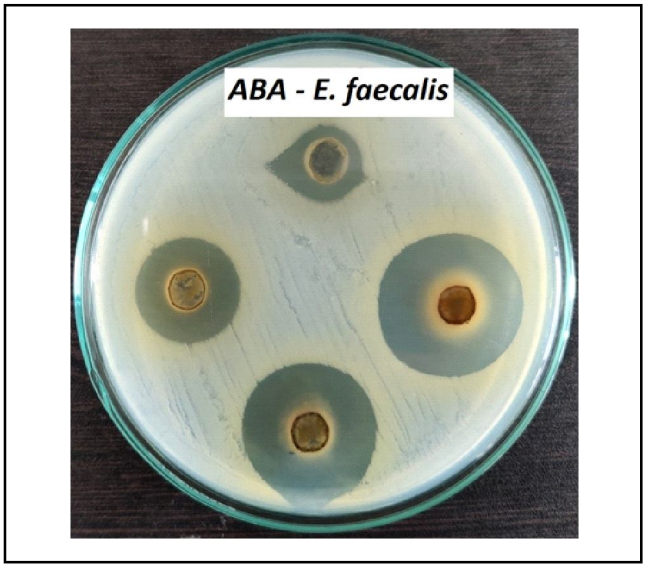

Figure 4: Antibacterial activity of the *I. verum* seedpod extracts against *E. faecalis.*

3. Results

3.1 Antibacterial activity of the *I. verum* extracts

Methanolic extracts of the *I. verum* seedpods were examined against *E. coli* and *E. faecalis*. Four concentrations of the extracts were used (125 mg/ml, 250 mg/ml, 500 mg/ml and 1000 mg/ml). Methanolic seedpod extracts showed 11.66 ± 0.57 mm at 125 mg/ml, 14.33 ± 1 mm at 250 mg/ml, 18.33 ± 0.57 mm at 500 mg/ml, 23.66 ± 0.57 mm at 1000 mg/ml, respectively, against *E. coli* (Figure 3). Similarly, plant extracts at 125 mg/ml, 250 mg/ml, 500 mg/ml and 1000 mg/ml showed 14.33 ± 0.57 mm, 17.33 ± 1.52 mm, 22.66 ± 0.57 mm and 27.33 ± 1 mm, respectively, against *E. faecalis* (Figure 4). Figure 5 shows the graphical representation of inhibitory zones.

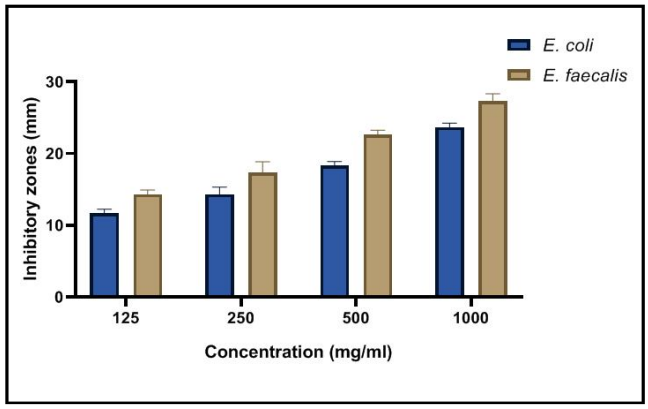

Figure 5: Graphical representation of antibacterial activity of *I. verum* seedpod extracts.

3.2 Minimum inhibitory concentration (MIC) analysis

The least concentration required to inhibit the test pathogen is defined as the minimum inhibitory concentration. Eight different concentrations were utilised to evaluate the MIC of the *I. verum* extracts. From the analysis, the MIC against *E. coli* and *E. faecalis* were observed to be 15.6 mg/ml (Figure 6) and 7.8 mg/ml (Figure 7).

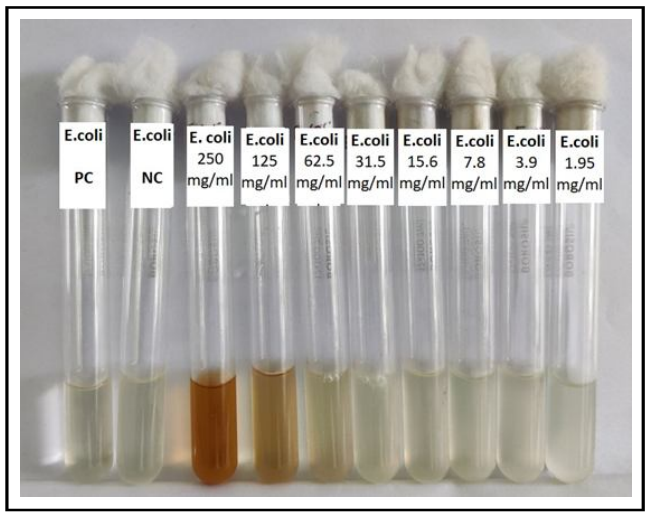

Figure 6: MIC evaluation of *I. verum* seedpod extracts against *E. coli.*

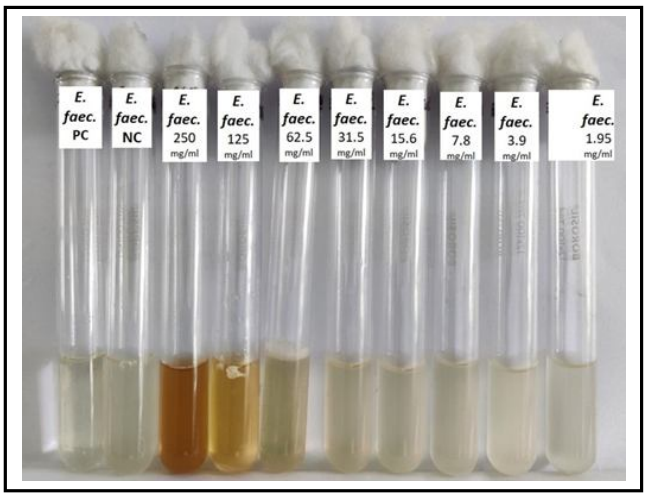

Figure 7: MIC evaluation of *I. verum* seedpod extracts against *E. faecalis*.

3.3 Biofilm inhibition studies

The biofilm inhibiting potential of the *I. verum* extracts was evaluated by crystal violet staining in 96 well MTP. Figure 8 shows the staining of biofilms produced by both the pathogens and treatment with various plant extracts. Treatment with 7.8 mg/ml of plant extract showed 90.5% and 71.7% of biofilm inhibition against *E. coli* and *E. faecalis*, respectively. 100% biofilm inhibition was observed at 31.5 mg/ml against *E. coli* and 125 mg/ml against *E. faecalis*. Figure 9 shows the graphical representation of biofilm inhibition potential of *I. verum* seedpod extracts.

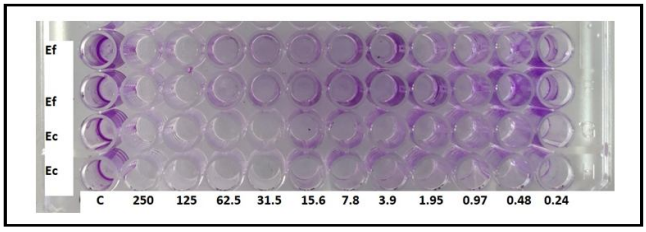

Figure 8: Biofilm inhibition assay.

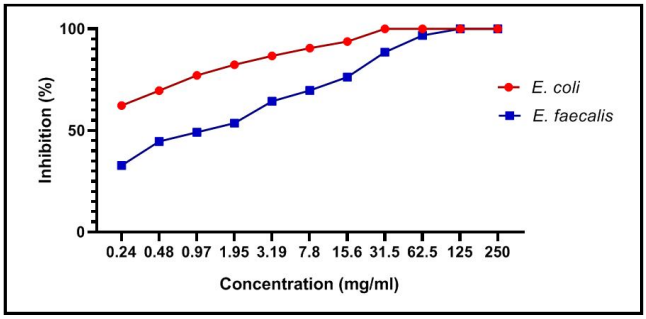

Figure 9: Biofilm inhibition potential of *I. verum* seedpod extracts.

3.4 Coating of catheters and antibacterial activity

Dip and dry coating method was adopted for effective coating of seedpod extracts on the surface of the catheters (Figure 10). The coated catheters were evaluated for antibacterial activity post coating. The coated catheters showed 9 mm against *E. coli* and 13 mm against *E. faecalis*. Figures 11 and 12 show the antibacterial activity of the coated and uncoated catheters against test pathogens.

Figure 10: Coating of catheters.

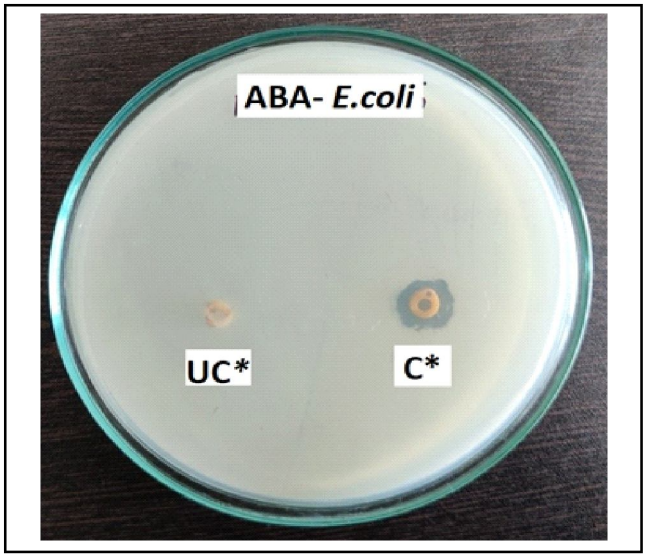

Figure 11: Antibacterial activity of the coated catheters against *E. coli*.

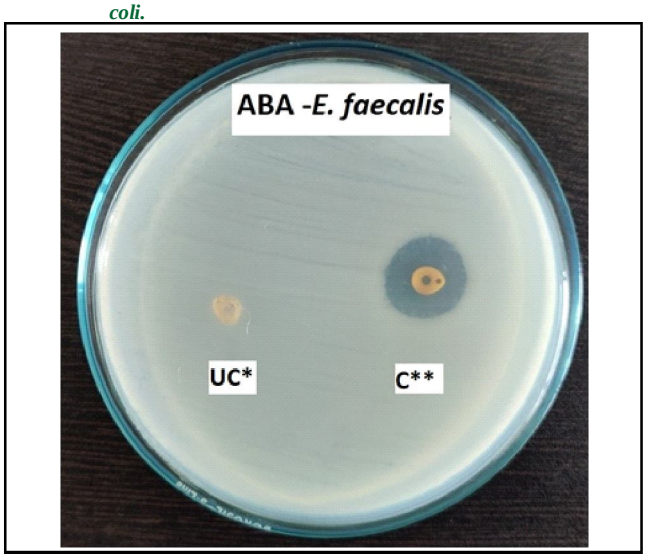

Figure 12: Antibacterial activity of the coated catheters against *E. faecalis*.

4. Discussion

The biofilm formation by multidrug resistant bacterial pathogens on urinary catheters is a challenge in modern medicine and a number of studies are underway to find a permanent solution to control this menace. The present study is one of such trials in which we use the antibiofilm potentials of *I. verum* seedpod extracts against two major CA-UTI biofilm forming bacteria, *viz.*, *E. coli* and *E. faecalis*.

The plant has widely been studied for its physicochemical parameters and antibacterial, antifungal, antiviral (Liu *et al*., 2020) and anti-cancerous (Muhammed *et al*., 2016) activities. In *in vitro* a research work performed by Salem *et al*. (2021), the team concluded that, the aq. methanolic plant extract had a potential prevention as well as detachment activity against biofilms formed by MDR strains of methicillin-resistant *S. aureus* (USA300) and *Acinetobacter baumannii* (AB5057). Another study conducted by Huma *et al*. (2020) concluded that the organic solvent extracts from *I. verum* possessed potential antioxidant and antibacterial activities including against *E. coli* and gram-positive bacterial pathogens. Alhajj *et al*. (2019) also concluded that both gram-positive and gram-negative pathogens like *E. coli* and *S. aureus* were sensitive to the bioactive compounds of this plant. Ebani *et al*. (2018), it has been found that Enterococci from urinary tract infections were resistant to oil from *I. verum*, to which many of the antibiotic resistant bacteria are sensitive. Another investigation conducted by Yang *et al*. (2021) using the leaves and twigs of *I. verum* suggested that the plant parts had the compounds with potentials to be developed as novel antibiotic against *Acinetobacter baumannii*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Findings in all these studies, in principle, are in correlation with the conclusions we made. At the same time, there are no reported data on the activity of *I. verum* seed extract coated materials against bacteria and this study proved that such a coating is effective against both the major CA-UTI biofilm causing bacteria, *viz*., *E. coli* and *E. faecalis*.

5. Conclusion

The antibacterial potential of *I. verum* seedpod extracts was evaluated against major biofilm producers *E. coli* and *E. faecalis*. The seedpod extracts showed significant inhibitory zones and MIC was determined. The coated urinary catheters showed evident inhibitory zones demonstrating the effective biofilm prevention and elimination. Thus, the developed *I. verum* seedpod extracts coated catheters can be used for prevention and eradication of CA-UTI.

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Conflict of interest

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

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