

COATING OF *CYANTHILLIUM CINEREUM* (POOVAMKURUNNILA) EXTRACTS ON FOLEY CATHETERS AGAINST URINARY TRACT INFECTION CAUSING ORGANISMS

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ABSTRACT: *Urinary catheters are used to manage urinary incontinence, urinary retention, and/or after prostrate or genital surgical procedures. Care should be taken to avoid contamination and subsequent infections, catheters are still susceptible to accumulation of microbes resulting in biofilm formation. In order to minimize the microbial cell attachment, Use of antimicrobial agents; antibiotic or anti-bio-filming agent is necessary. The prevalence of drug resistant pathogens has increased the risk of infection rate and the need for development of antimicrobial catheters from new sources is increased. Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources. Cyanthilliumcinereumplant extract was prepared and coated onto catheters. Antibacterial activity of the coated catheters and biocompatibility tests were evaluated. The maximum zone of inhibition for plant extract coated catheters against Staphylococcus aureus and Escherichia coli was found to be 26mm and 25mm. The zone of inhibition for herbal extract coated catheters was found to be slightly less than that of the drug-carrier coated catheters. The Chorioallantoic membrane with herbal extract coated catheter samples showed no tissue reactions like necrosis when compared to positive and negative controls. Expected mild tissue reactions like obvious edema and fibrous deposition with degenerative changes of epithelial cells was also not observed. ThusCyanthilliumcinereum(poovamkurunnila)due to its antimicrobial activity can be used as an alternative for drugs in prevention of urinary tract infection.*

Key Words: *Urinary catheters, biofilm formation, drug resistance, Cyanthilliumcinereum, Antibacterial activity, HET-CAM test*

INTRODUCTION

Urinary catheters are used to manage urinary incontinence, urinary retention, and/or after prostrate or genital surgical procedures. In simple terms, urinary catheters are used to remove urine from the body. If the body is unable to remove urine for some reason, pressure builds on the urinary bladder and as a result, kidney failure can occur. These catheters are changed daily. The intermittent or short term use catheter is used for a few weeks. This is commonly used for postoperative care when the patient is unable to urinate by themselves and need assistance. The long-term use or Foley catheters are typically used for several months at a time by patients with urine retention problems including those with spinal cord injury/disease, multiple sclerosis, enlarged prostate, or cerebrovascular accident (Gilmartin *et al.*, 2012).

However, despite the care taken to avoid contamination and subsequent infections, catheters are still susceptible to accumulation of microbes. In urinary catheters, these microbes can accumulate to form single species biofilms, which can cover even short term non-Foley catheters in a period as short as 24 hours, which can ultimately develop multispecies biofilms causing infections if not detected at an early stage. Infection occurs in 10–50% of patients undergoing non-Foley or short-term urinary catheterization (7 days) but virtually all patients undergoing Foley or long-term catheterization (>28 days) become infected (Donalan., 2001). Foley catheters are most susceptible to infection as bacteria can collect and grow rapidly over time if not identified. This infection is called catheter-associated urinary tract infection (CAUTI), an infection that has stimulated antimicrobial materials research for urinary catheters. Avoidance of CAUTI requires development of bio-film resistant catheter material (Cortese *et al.*, 2018).

Bio-film is a complex aggregation of microorganisms marked by the excretion of a protective and adhesive matrix. Bio-films are also often characterized by surface attachment, structural heterogeneity, genetic diversity, complex community interaction and an extracellular matrix of polymeric substances. In order to minimize the microbial cell attachment, Use of antimicrobial agents; antibiotic or anti-bio-filming agent is necessary. The prevalence of drug resistant pathogens has increased the risk of infection rate and the need for development of antimicrobial catheters from new sources is increased (Bendouh Z, *et al.*, 2006).

Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action may too very likely differ (Fabricant and Fansworth 2001). There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity (Prachayasittikul *et al.* 2008; Nogueira *et al.* 2008; Costa *et al.* 2008). The Indian traditional medicinal plant (poovamkurunnila) *Cyanthillium cinereum* common name little ironweed is commonly used as a poultice on cuts, wounds and skin diseases. *C. cinereum* has antioxidant protective effects against oxidative damage to biological molecules such as lipids and DNA. It also showed high efficiency in inhibiting hemolysis of erythrocytes and mild dose-dependent cytotoxic activity against human breast carcinoma. The leaves of *C. cinereum* exhibited analgesic, antipyretic and anti-inflammatory properties (Gunjan *et al.*, 2011). Thus, in the present study the herbal extracts were prepared and coated onto catheters. Antibacterial activity of the coated catheters and biocompatibility tests were evaluated.

MATERIALS AND METHODS

Collection of Foley catheters and processing of the samples

The entire research work was carried out from December 2018 to January 2019 in GRAM POSITIVES – Research and Development Laboratory, Coimbatore, Tamil Nadu, India. The used catheters were brought to the laboratory and decontaminated in the autoclave. The catheter was cut into required size for all testing parameters and stored at 4°C prior analysis.

Soxhlet extraction of *Cyanthillium cinereum*

Cyanthillium cinereum (poovamkurunnila) plant leaves were collected and shadow dried within a temperature range of 37-40°C. The moisture content of the herb collected was reduced to less than 14% with proper drying since most of the herb have moisture content of 60-80% and cannot be stored without drying. Proper drying has to be carried out otherwise important compounds may get contaminated. After drying, the grinding was carried out to break down the leaves of the plant into very small units ranging from coarse fragments to fine powder. Extraction refers to separating the desired material by physical or chemical means with the aid of a solvent. Antimicrobial active substances were extracted from the plant by aqueous extraction method. The powdered plant material was extracted with water by adding 20g of herbal powder in 100ml for 24h in Soxhlet extraction apparatus.

Coating of *Cyanthillium cinereum* extract on catheters (Sengodanet *et al.*, 2018)

Antibacterial coatings on the urinary catheters were carried out using a standard slurry-dipping technique. The technique started with the preparation of stable slurry with specific amount of tetracycline in the molten polyethylene glycol (PEG). Appropriate slurry temperature (37°C) was determined by an optimization process based on a trial and error approach to achieve optimum coating thickness, uniformity and stability of composite coating as well as adequate infiltration of drug particles into coating structure. PEG (2g) with a predefined molecular weight was mixed with the extract of tetracycline (0.5g) in a glass vial. The mixture was heated at the range of 60 to 70°C in a water bath to obtain homogeneous slurry.

The resulting slurry was homogenized in a magnetic stirrer for 5 to 10min. Each piece of catheter was dip coated twice with intermittent drying (suspension coating method) in the drug-PEG slurry mixture. The dip-coating procedure was carried out in sterile glass beakers on a shaker (120 rpm) for 30 mins, with a drying period of about 15 mins between the two coating procedures, followed by drying at room temperature. All coating steps were carried out under strict aseptic conditions. All samples were coated by a thickness of about 5mm of catheters outer diameters. After coating procedure, the catheter samples were stored at 4°C for upto 15 mins. In order to increase drug loading and prevent excessive increase in catheter thickness, the coating process were repeated for replicates of each sample. Subsequently, in order to slow down the release rate of drug from PEG coating and mitigate the friction effect between catheter surface and mucosa, second coating layer was formed on the catheter surface. Cyclodextrin was dissolved in DMSO to acquire a 10 w/w% solution (carrier). PEG-coated samples were submerged into cyclodextrin solution three times for 1 min each. Thereafter, these samples were stored at 0°C or in a deep freezer to implement one freeze thaw cycle and physically crosslink the samples. The coated catheters were left to dry on a clean bench for 1 week at room temperature to remove residual DMSO. The drug and carrier coated catheter

samples were thus mentioned as *drug-carrier coated (dcc)* materials; other few samples were coated with *Cyanthilliumcinereum* plant extracts (*dc*) to differentiate the antibacterial activity from *dcc* samples.

Qualitative antibacterial activity of coated catheter (Bauer *et al.*, 1996)

The method was performed for analysing the antibacterial activity of urinary catheter after slurry dip-coating with drug (tetracycline) and carriers (cyclodextrin). In this qualitative method the sterilized materials were tested from each preparation [drug-carrier coated, Herbal extract coated and uncoated catheter samples]. The materials were all rinsed twice in phosphate buffered saline (PBS) before testing to remove any surface accumulation of drug. All test materials were placed on the surface of Mueller-Hinton agar (MHA) plate which had previously been seeded with an overnight broth culture of the test organisms and incubated at 37°C for 24 to 48 hours. The experiments were carried out in triplicate. Antibacterial activity was expressed as the diameter of the zone of inhibition.

Hen's Egg Test on the Chorioallantoic Membrane – CAM test

To study the allergic reactions or biocompatibility of drug-carrier coated catheter was placed on the surface of chorio-allantoic membrane (CAM) of embryonated chick eggs. A standard HET-CAM protocol was followed to detect the inflammatory reactions. HET-CAM (Hen's egg test-chorioallantoic membrane) method uses the vascular fetal membrane of chicken embryos. It is assumed that acute effects induced by a test substance and the small blood vessels and proteins of this soft tissue membrane are similar to effects induced by the same test substance in the skin of a treated rabbit. The membrane was evaluated for the development of irritant endpoints (vascular lysis, haemorrhage and coagulation) and qualitative assessments of the irritation potential of test substances are made.

Test substance preparation

Test samples were prepared carefully with one negative control (0.9% NaCl) and one positive control (0.1N NaOH).

Table 1: Sample numbers and its designation

| S. No. | Sample number | Designation |
|--------|---------------|-------------------------------|
| 1 | Sample-1 | Negative control (0.9% NaCl) |
| 2 | Sample-2 | Positive control (0.1N NaOH) |
| 3 | Sample-3 | Drug-carrier coated catheters |

Preparation of Chorioallantoic membrane for experiment

Eggs from single hen was collected and observed for the presence of embryo using candle method. Defective eggs without embryo or cracks in the shell can be identified by this method. Eggs with good hard shell and developed embryo was selected and incubated in rotary egg incubator at $38.3 \pm 0.2^\circ\text{C}$. After 9th day of incubation, the eggs were used for the experiment. About one square inch window was marked over the shell (above the air sac). The shell was carefully drilled and the window was removed without damaging the chorioallantoic membrane.

Treating the CAM using the samples Treatment of Eggs with Test Substances

Catheter samples and filter paper discs impregnated in sodium chloride solution (negative control) and sodium hydroxide solution (positive control) was used in the experiment. The samples were kept over the developing chorioallantoic membrane and observed for the irritation endpoints to develop with in 300seconds.

Observations

During the incubation time (300 seconds), the morphological change over the CAM was recorded. Three types of endpoints were noted viz., haemorrhage, coagulation and vascular lysis.

Irritation Score (IS) calculation – IS [B] analysis method

The time taken for the development of each endpoints, hyperemia, hemorrhage and coagulation was substituted in the standard formula as per IS [B] analysis method. The time values assigned/obtained to each endpoint were totalled to give an overall IS value for the test substance. An IS score could be calculated using the following general formula,

$$\left(\left(\frac{(301 - \text{Hemorrhage time})}{300} \right) \times 5 \right) + \left(\left(\frac{(301 - \text{Lysis time})}{300} \right) \times 7 \right) + \left(\left(\frac{(301 - \text{Coagulation time})}{300} \right) \times 9 \right)$$

Where, *Hemorrhage time* = time (in seconds) of the first appearance of blood hemorrhages, *Lysis time* = time (in seconds) of the first appearance of vessel lysis

Coagulation time = time (in seconds) of first appearance of protein coagulation

In Table 2 the final IS value ranged from 0 (for test substances that do not induce development of any of the observed endpoints) to 21 (for test substances that induce development of all three endpoints within 5min of application of the test substance) was presented. The relationship between scores and category of irritation was tabulated below.

Table 2: Relationship of scores with category of irritation

| Scores on HET-CAM | Category of irritation |
|-------------------|-----------------------------|
| 0 – 0.9 | No irritation |
| 1 – 4.9 | Weak or slight irritation |
| 5 – 8.9 | Moderate irritation |
| 9 – 21 | Strong or severe irritation |

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RESULTS AND DISCUSSION

Qualitative antibacterial activity of coated catheter

The diffusing ability of the herbal extract coated materials to retard the growth of test bacteria seeded on MHA plate was calculated based on the zone of inhibition. The zone of inhibition measured in millimetres was calculated. In Table 3, the antibacterial activity of *drug-carrier* coated catheters and herbal extract coated catheters for all the test organisms was presented. No inhibitory zones were observed for all uncoated materials. In contrast, the *drug-carrier* coated catheter sample materials showed significant inhibitory zones ranged from 19mm to 28mm against the high biofilm producers. The maximum zone of inhibition for plant extract coated catheters against *Staphylococcus aureus* and *Escherichia coli* was found to be 26mm and 25mm. The zone of inhibition for herbal extract coated catheters was found to be slightly less than that of the drug-carrier coated catheters. Figure 1 represents the antibacterial activity of plant extract coated catheters.

In qualitative antibacterial activity, the herbal extract coated catheters recorded strongest inhibition against both Gram-Negative and Gram-Positive organisms. The different cell wall susceptibility amongst bacteria may be the key contributor to various inhibitory concentrations of drugs. Gram-Positive bacteria are often found to be more susceptible to antibacterial compounds than the Gram negative bacteria. It is well known that the outer membrane present only in the Gram negative bacteria play an important role as an effective barrier. Although Gram positive bacteria lack of outer membrane, the thicker cell wall consist of few peptidoglycan layers could act as functional barrier thus hinder the penetration of antimicrobial compound into the bacterial cell (Tian *et al.*, 2004). The bioactive compounds present in the herbal extract were responsible for antibacterial activity.

Hen's Egg Test on the Chorioallantoic Membrane: Histological Evaluation of CAM

The test sample (Herbal extract coated) does not develop any irritant end points, revealing that they are biocompatible. When compared with the negative control (sodium chloride) samples, no irritation points were observed. And the positive control (NaOH) samples revealed different irritant end points as described earlier. Irritation score was evaluated by the time in seconds consumed for the endpoints developed. Using the standard formula mentioned above (methods) end points was calculated. The mean value of time for the development of haemorrhage, hyperemia and coagulation were identified as 5.2, 5.8 and 7.4 respectively (Table-4).

The naked eye observations of three endpoints like haemorrhage hyperemia and coagulation was presented in Figure 2. The CAM exposed with Negative control (0.9 % NaCl) samples showed well developed blood vessels and nucleated epithelial cells without any irritation endpoints; in Figure 3 these observations were clearly evident. Irritation end points for test material implanted CAM samples were compared with the interpretations of positive and negative control samples. The Chorioallantoic membrane with herbal extract coated catheter samples showed no tissue reactions like necrosis when compared to positive and negative controls (Figure 4). Expected mild tissue reactions like obvious edema and fibrous deposition with degenerative changes of epithelial cells was also not observed.

Phytochemical analysis of petroleum ether, ethanol and aqueous extracts of *C. cinereum* revealed that it contains several phytochemicals such as alkaloids, phenols, tannins, saponins, steroids, glycosides, flavonoids, carbohydrates, phlorotannins and terpenoids[18]. These phytochemicals and secondary metabolites have been documented to exhibit antimicrobial activities

DISCUSSION

Owing to the development of antibiotic resistance strains among the urinary tract disease causing microbial strains (WHO., 2002), we focused our efforts in exploring the use of medicinal plant to inhibit biofilm formation on catheter. In this study, the antibacterial activity of herbal extract coated catheters was evaluated against the UTI pathogens. Phytochemical analysis of petroleum ether, ethanol and aqueous extracts of *C. cinereum* revealed that it contains several phytochemicals such as alkaloids, phenols, tannins, saponins, steroids, glycosides, flavonoids, carbohydrates, phlorotannins and terpenoids (Varsha et al., 2015). These phytochemicals and secondary metabolites have been documented to exhibit antimicrobial activities. The results from this study therefore support the *Cyanthilliumcinereum* extracts as suitable alternatives to antibiotics in urinary catheters. There have been no reports of attempts to use *Cyanthilliumcinereum* extracts in the control of urinary catheter infections to date and we report for first time.

CONCLUSION

The drug carrier coated catheter sample materials showed significant inhibitory zones ranged from 19mm to 28mm against the high biofilm producers. The maximum zone of inhibition for plant extract coated catheters against *Staphylococcus aureus* and *Escherichia coli* was found to be 26mm and 25mm. The zone of inhibition for herbal extract coated catheters was found to be slightly less than that of the drug-carrier coated catheters. The Chorioallantoic membrane with herbal extract coated catheter samples showed no tissue reactions like necrosis when compared to positive and negative controls. Expected mild tissue reactions like obvious edema and fibrous deposition with degenerative changes of epithelial cells was also not observed. Thus *Cyanthilliumcinereum* (poovamkurunnila) due to its antimicrobial activity can be used as an alternative for drug coating in prevention of urinary tract infection.

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Figure 1: Qualitative antibacterial activity of coated catheter



Staphylococcus aureus



Escherichia coli



Figure 2: Positive control CAM sample

All three irritation types like lysis of blood vessels, haemorrhage and coagulation on the chorioallantoic membrane of chick embryo was observed for the positive control (NaOH) coated sample

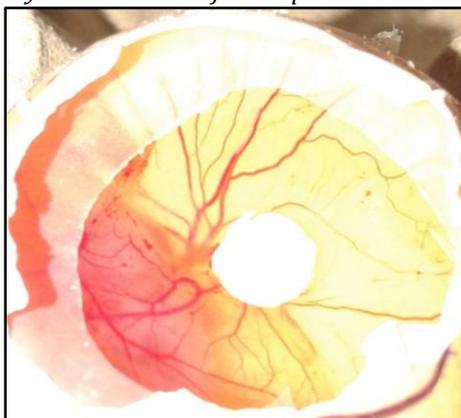


Figure 3: Negative control CAM sample

No Irritation types on the membrane of chick embryo was observed for the sample coated with negative control Sodium chloride

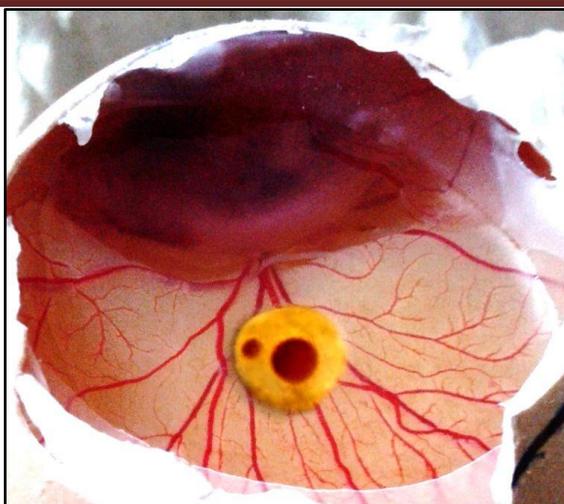


Figure 4: CAM after exposed coated catheter samples

No Irritant endpoints were observed for coated catheter samples thus indicating the biocompatible properties of the herbal extract used in the research.

Table 3: Assessing the qualitative antibacterial activity of dip-coated urinary catheter materials

| S. No. | Organisms | Zone of inhibition (mm) | | |
|--------|-------------------------------|-------------------------|----|-----|
| | | UC | DC | DCC |
| 1 | <i>Staphylococcus aureus</i> | 0 | 26 | 28 |
| 2 | <i>Escherichia coli</i> | 0 | 25 | 27 |
| 3 | <i>Enterobacter sp</i> | 0 | 23 | 25 |
| 4 | <i>Pseudomonas aeruginosa</i> | 0 | 21 | 22 |
| 5 | <i>K. pneumoniae</i> | 0 | 17 | 19 |

UC: Uncoated DC: *Cyanthilliumcinereum* coated DCC: Drug-carrier coated

Table 4: Comparative evaluation of irritation scores for test materials, negative control and positive control by HET-CAM test

| Materials on CAM | Endpoint development | | | Irritation score ¹ |
|---|----------------------|-----------|-------------|-------------------------------|
| | Haemorrhage | Hyperemia | Coagulation | |
| Sample-1 (Negative control) | 0 | 0 | 0 | 0 |
| Sample-2 (Positive control) | 5.2 | 5.8 | 7.4 | 18.4 ² |
| Sample-3 (<i>Cyanthilliumcinereum</i> coated) | 0 | 0 | 0 | 0 |

¹Irritation score calculated as described by IS [B] analysis, ²Irritation category – Severe irritation