

Fabrication of Antibacterial Coatings: Prevention of Implant Associated Infections in Patients Indwelling Urinary Catheters

*Shethal AnilKumar¹, Geetharamani², Elayarajah, B³

¹Assistant Professor, School of Biological Sciences, CMS College of Science and Commerce, Coimbatore, Tamil Nadu, India,

²Associate Professor, Department of Microbiology, Dr. NGP College of Arts and Science, Coimbatore, Tamil Nadu, India

³Chief Scientist, RNDBio, A Biosolution Company, Coimbatore, Tamil Nadu, India,

*Corresponding Author: Shethal AnilKumar, School of Biological Sciences, CMS College of Science and Commerce, Coimbatore, Tamil Nadu, India,

Abstract: Implant-associated infection in hospitalized post-surgery patients is mainly due to microbial colonization of the catheter surface and formation of a superficial biofilm layer. Effective antibacterial modification of urinary catheter surface that can prevent device colonization was selected as a primary objective. In the present study, the antibacterial activity of urinary catheters impregnated with an herbal composite was studied under in vitro conditions. To provide sustained release of herbal constituents from the catheter surface, a drug carrier, polyvinyl alcohol was also added as a second layer. Biofilm forming abilities for the test cultures were initially determined by using a standard Exit-site challenge test. Microtitre plate assay were performed to evaluate the biofilm production using biofilm index. Qualitative and quantitative antibacterial activity for the surface modified catheters was finally determined to check the potentiality in preventing the growth of test organisms. During the in vitro conditions it was observed that the two high biofilm producers, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* and the moderate biofilm producers, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* were qualitatively and quantitatively prevented when exposed to drug-carrier coated catheters. These in vitro results suggest that the antibacterial drug-carrier coated catheters can potentially be used to combat catheter colonization and implant-associated infections.

Key words: Biofilm, herbal composite, drug carrier, *Staphylococcus epidermidis*, chi square test

I. Introduction

Implanted medical devices, like urinary catheters and more permanently surgically implanted cardiac valves, pacemakers, dental implants, cardiac implants and coronary stents suffer from recognized risks of “implant-associated” infections (Khardori and Yassien, 1995). Bacteria encounter the implant from skin or gain direct access to the implant site during device placement. Some non-pathogenic or opportunistic bacteria spontaneously alter their phenotype to become pathogenic at the implant site. To adapt themselves many pathogens, use quorum sensing mechanisms; to create protective, complex mucopolysaccharide barrier films, called ‘biofilms’. Quorum signalling mechanism in biofilm producers facilitates cell-to-cell communication, for adaptation against immune response, phenotypic alterations to promote genetic exchange and antibiotic-resistance transfer processes (March and Bentley, 2004). The complicated structure of biofilm with extracellular polymeric matrix could prevent antibiotics from reaching the bacteria (Chen *et al.*, 2013). Bacterial migration and adhesion to inserted urinary catheters are important factors in catheter associated urinary tract infections (CUTI). Urinary catheters are used for bladder drainage as a treatment option for patients with urinary retention, general surgery recovery and bladder obstruction. Millions of catheter associated urinary tract infections (CUTI) occur annually, with an average cost of US \$3000 to US \$4000 each (Cho *et al.*, 2003). Many efforts have focused on catheter surface modification in order to impede initial adhesion and biofilm formation, and reduce CUTI incidence. The approaches include small molecules and matrix-targeting enzymes, bactericidal and anti-adhesion coatings with antibiotics and other antimicrobial agents (Chen *et al.*, 2013). As these antimicrobial agents are only physically absorbed to device surfaces, this method is unlikely to load drugs for prolonged release to prevent bacterial infection over long periods: loading is low, release is rapid, and dose depletion occurs quickly.

A huge part of world’s population (80%) employs herbal medicines to deals with their daily medical issues. Twenty five percent of drugs prescribed by western pharmacists comprise of elements that are of plant origin (Karim *et al.*, 2011). Today several pharmacological classes of drugs include a natural product prototype. Aspirin, quinine, morphine, reserpine, etc., are a few examples of modern drugs, which were originally

discovered through the study of traditional cures and folk knowledge of indigenous people (Ansari and Inamdar, 2010).

In recent years, the emergence of biofilm infections have generated an urgent alarm in research and development field in seeks for novel antimicrobials from ethnomedicinal plant. Three such plants with anti-biofilm properties were selected in the present research. As a novel approach, the extracts were prepared as herbal composite with the aid of a drug-releasing carrier, polyvinyl alcohol (PVA) and coated aseptically on the commercial urinary catheter surface. The approach is to modify the catheter surface for an effective antibacterial coating to prevent implant-associated infections. *Andrographis paniculata* (Murugan *et al.*, 2013), *Euphorbia hirta* (Shanmugapriya and Roziahaman, 2013) and *Terminalia chebula* (Malckzadeh *et al.*, 2001) leaf extracts were made into a composite under a controlled condition. Initiation and development of a bacterial infection in an animal requires a three dimensional interaction between bacterial agents, environment and the immune status of the animal (Dhama *et al.*, 2014). The urinary catheter samples were coated on their surface with the prepared herbal composites using a standard slurry-dipping technique. Qualitative and quantitative antibacterial activity against the biofilm producing bacterial cultures were tested and statistically calculated.

II. Materials And Methods

About 5 different bacterial test cultures isolated from the biological specimens were procured from a diagnostic laboratory at Coimbatore, Tamil Nadu, India. Three different medicinal herbs, *Andrographis paniculata*, *Euphorbia hirta* and *Terminalia chebula* leaf powders were commercially procured after authentication from Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. All the chemicals, media used in the research were commercially procured from HiMedia, Mumbai, India. The entire research work was carried out from November 2013 to October 2014.

Determining the surface colonizing capability of test bacteria on urinary catheter (UC) materials using Preliminary Exit-site challenge test (Bayston *et al.*, 2009)

Exit-site challenge test was performed as the preliminary test. This test was used to identify the ability of specific test organism to grow on a type of biomedical materials used in the study. In this method, three-quarter strength of Iso-sensitest semi solid Agar was poured into a sterile boiling tube and allowed to solidify. The surface of the agar was then inoculated with 10 μ l of 18 hours test cultures (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The pre-measured size (length - 15mm) of UCs were cut, sterilized and partially inserted into the Iso-sensitest semi-solid medium through the inoculated area and incubated at 37°C. Migrating ability of the test bacteria from the exit site down the material track i.e., outside of the materials were assessed visually up to 24-48 hours.

Assessing the biofilm forming capability of test bacteria on urinary catheter using standard Microtitre plate biofilm assay (Christensen *et al.*, 1985)

Bacterial attachment to an abiotic surface is assessed by measuring the stain taken up by adherent biomass in a 96-well plate format by means of microtitre biofilm assay. The test organisms were grown in 96-well microtitre plate for 48 hours. The wells were washed to remove any unbound test bacteria. Cells remaining adhered to the wells were subsequently stained with a dye that allowed visualization of the attachment pattern. Each of the test organisms were inoculated in a 5 ml culture broth and grown to stationary phase. Cultures were diluted at 1:100. Following this, 100 μ l of each diluted cultures were pipetted into eight wells in a fresh microtitre plate. The plate were covered and incubated at optimal growth temperature for 24-48 hours. Four small trays were set up in a series and 1 to 2 inches of tap water were added to the last three. The first tray was used to collect waste, while the others were used to wash the assay plates. Unbound bacteria if any were removed from each microtitre dish by briskly shaking the dish out over the waste tray. About 125 μ l of 0.1% crystal violet solution was added to each well. Staining was done for 10 min at room temperature. The crystal violet solution was removed by shaking each microtitre dish out over the waste tray. The dishes were washed successively in each of the next two water trays and as much liquid as possible was shaken out after each wash. To remove any excess liquid, each microtitre dish was inverted and vigorously tapped on paper towels. The plates were allowed to air-dry. Added 200 μ l of 95% ethanol to each stained well. The plates were covered to allow solubilization by incubating for 10 to 15 min at room temperature. The contents of each well were briefly mixed by pipetting. Following this, 125 μ l of the crystal violet-ethanol solution was transferred from each well to a separate well in an optically clear flat-bottom 96-well plate. The optical density (OD) of each of these 125 μ l samples was measured at a wavelength of 500 to 600 nm. Optical density (OD) of stained adherent bacteria was determined with a micro ELISA auto reader (Braun, Germany). Based on the OD value, the adherence of organism in the silicate tubes and titre plates were classified as mentioned in Table 1.

Solvent extraction of medicinal herbs

About 5 to 10g of *Andrographis paniculata*, *Terminalia chebula* and *Euphorbia hirta* plant leaves were collected and mixed into 50ml of acetone (80%). All the prepared solvent extracts were incubated for 12 hours at room temperature. This process was carried out in a controlled condition for proper extraction of bioactive compounds from each herb into the solvent. After incubation period, the extracts were filtered through whatmann No. 1 filter paper. The collected filtrate was evaporated in a room temperature under dark storage condition aseptically for the period of 12 hours. The obtained herbal concentrates were then subjected to form a herbal-composite mixture.

Preparation of antibacterial herbal composite and antibacterial coating of urinary catheters (Boccaccini et al., 2003)

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 herbal composite in the molten polyethylene glycol (PEG). Appropriate slurry temperature (40°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 *Andrographis paniculata* (0.5g) in a glass vial. The mixture was heated at the range of 40 to 45°C in a water bath to obtain homogeneous slurry. The resulting slurry was homogenized in a magnetic stirrer for 5 to 10min. Under this stirring condition, the extracts of *Euphorbia hirta* and *Terminalia chebula* were added slowly to obtain herbal composite slurry mixture.

Each piece of catheter (length - 6mm) was dip coated twice with intermittent drying (suspension coating method) in the herbal-composite 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 herbal drug from PEG coating and mitigate the friction effect between catheter surface and mucosa, second coating layer was formed on the catheter surface. Polyvinyl alcohol (PVA) was dissolved in DMSO to acquire a 10 w/w% solution. PEG-coated samples were submerged into PVA 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 herbal composite and carrier coated catheter samples were thus mentioned as *drug-carrier coated (dcc)* materials; other few samples were also coated with carrier alone and herbal-composite alone separately to differentiate the antibacterial activity among them.

Assessing the qualitative antibacterial activity of dip-coated UC materials (El-rehewy et al., 2009)

The method was performed for analysing the antibacterial activity of urinary catheter after slurry dip-coating with herbal composite and carriers (PVA). In this qualitative method the pre-measured size (length-6mm) of all sterilized materials were tested from each preparation [drug-carrier coated, carrier coated, herbal composite 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.

Quantitative antibacterial activity of coated materials using the standard bacterial adherence test (El-Rehewy et al., 2009)

Anti-adherent/antibiofilm activity of coated and uncoated materials was quantitatively measured using standard bacterial adherence test. The drug-carrier coated (*dcc*) materials were placed separately in a tube with 5 ml of each of the test bacteria and incubated at 37 °C for 18 hours. During the incubation period the bacterial cells adhere on the surface. After incubation, the numbers of viable adherent cells were determined as follow: Catheter materials were collected aseptically and washed in sterile normal saline twice to remove the non-adherent cells. The washed pieces were sonicated for 30 seconds to dislodge the sessile adherent. After sonication, serial dilution of the sonicated saline was made and the number of sessile bacteria was detected to determine the degree of adherence by viable count technique. Similar experimental set up was run in parallel for carrier-alone coated (*cc*) and uncoated (*uc*) materials. The difference in number of adhered cells on *dcc* and *cc*

catheter materials was determined statistically using chi square analysis. The percentage reduction of adhered organisms on the coated materials was determined using a standard percentage reduction formula.

$$\text{Bacterial reduction (\%)} = A - B/A \times 100$$

Where,

A = number of adhered organisms (in CFU) obtained from the uncoated materials

B = number of adhered organisms (in CFU) obtained from the coated materials

Statistical analysis of total viable bacteria on coated materials

Chi-square non parametric test using SPSS-9 for Windows 7 was used as a statistical tool to determine the effect of antibacterial herbal composite on bacterial adherence. The hypothesis selected (H₀) was that "There is significant effect of antibacterial herbal composite on the test organisms". The difference in the bacterial reduction percentage between the *dcc* and *cc* catheter materials were statistically calculated with P<0.05 considered significant.

III. Results

Determining the surface colonizing capability of test bacteria on urinary catheter (UC) materials using Preliminary Exit-site challenge test

In this present study the surface colonizing ability of test bacteria on the UC sample materials was investigated using exit-site challenge test. All the test organisms used in the research colonized the material surfaces between 24 to 48 hours. Among the test organisms *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* colonized with in 24hours; *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* colonized the catheter surface after 48 hours.

Assessing the biofilm forming capability of test bacteria on urinary catheter using standard Microtitre plate biofilm assay

The observations of MTP biofilm assay was recorded in Table 2. The optical density (OD) values and biofilm index of the test organisms were tabulated based on the biofilm classification described by Christensen *et al.*, (1985). The test organisms considered as strong biofilm producers in MTP assay were *S. epidermidis* (0.248) and *Pseudomonas aeruginosa* (0.242) (Fig. 1). These organisms showed OD values >0.240. Moderate biofilm formation was observed during the MTP assay for *Staphylococcus aureus* (0.186), *Klebsiella pneumoniae* (0.192) and *Escherichia coli* (0.184). The high and moderate biofilm producers were identified by the colour intensities formed in the microtitre plates.

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

The diffusing ability of the antibacterial herbal composite from the *drug-carrier* 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 for each drug-carrier combinations (tested in triplicates) was calculated to obtain the mean value. In Table 3, revealed the antibacterial activity of *drug-carrier* coated materials for all the test organisms. No inhibitory zones were observed for all uncoated materials. In contrast, all the *drug-carrier* coated UC sample materials showed significant inhibitory zones ranged from 41.9mm to 47.6mm against all the test organisms. Modified urinary catheter samples showed maximum inhibitory zones of 47.6mm and 43.3mm against high biofilm producers, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* respectively. Other moderate biofilm producers, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* also showed significant inhibitory zones measuring 42.6mm, 41.6mm and 41.9mm respectively. The antibacterial activity with the clear inhibitory zones around the *drug-carrier* coated materials against the biofilm producing test cultures was presented. Simultaneously, carrier alone coated and herbal composite alone coated materials showing significant inhibitory zones less than the *drug-carrier* coated materials were also observed.

Quantitative antibacterial activity of coated materials using the standard bacterial Adherence test

Anti-adherent activity for each coated catheter sample materials were analysed using bacterial adherence test. The anti-adherent/antibiofilm activity was calculated by bacterial reduction percentage. The anti-adherent activity of modified urinary catheter materials against the test organisms was concentration dependent as the reductive effect of drugs and carriers was in the range of 91.0% to 96.5% (Table 4). Bacterial reduction percentage calculated from the colony forming unit (CFU) of the antibacterial drug coated materials against the test organisms was measured. Maximum bacterial reduction percentage was observed for the high biofilm producer, *S. epidermidis* (96.5%). Other biofilm producers also showed significant reduction percentages of 92.0%, 91.0%, 94.5% and 96.0% for *S. aureus*, *P. aeruginosa*, *K. pneumoniae* and *E. coli* respectively.

Statistical analysis of total viable bacteria on coated materials

Using chi-square statistical analysis, the effect of antibacterial drug on bacterial adherence was determined. The difference in bacterial reduction percentage of *drug-carrier coated (dcc)* and *carrier coated (cc)* materials were taken as the experimental design. The hypothesis selected was "There is significant effect of antibacterial drug on the test organisms". The difference in the bacterial reduction percentage between the *dcc* materials and the *cc* materials were statistically calculated with $P < 0.05$ considering significant. For all the data, the calculated value was less than the table value. In Table-4, the calculated value of each *dcc* materials tested against all the test bacteria was presented. Since the calculated value was less than the table value, the assigned hypothesis could be accepted. The *dcc* materials showed more bacterial reduction percentage than the *cc* materials. The statistical survey of the research proved the quantitative antibacterial activity of the *dcc* materials.

IV. Discussion

Plants are the main source of drugs that being used from the ancient times as a herbal remedies for the health care, prevention and cure of various diseases and ailments (Archana *et al.*, 2011). Herbal composite used in the present research contains three medicinal herbs. *Andrographis paniculata* leaf extracts possesses anti-inflammatory, antiviral, immune-stimulatory, hypoglycemic, hypotensive and anticancer properties (Murugan *et al.*, 2013). Pitts *et al.*, (2003) graded that a drug which could control 40% and above can be considered as a potent biofilm eliminator. This is the first report to provide evidence that the leaf extract of *A. paniculata* have antibiofilm activity. *Euphorbia hirta* another significant medicinal herb in the composite plays an important role for the evident antibacterial and anti-biofilm activity. Terpenoids present in solvent extracts of *E. hirta* were found most likely to be involved in the detachment of planktonic cells from the biofilm (Shanmugapriya and Roziahaman, 2013). The third herb, *Terminalia chebula* in the composite was proved to exhibit antibacterial activity against a number of both Gram-positive and Gram-negative human pathogenic bacteria (Malckzadeh *et al.*, 2001). Phenolic compound in the plant extract showed strong antibacterial activity against multidrug-resistant uropathogenic *Escherichia coli* (Bag *et al.*, 2011). There are a lot of herbal plants that have been used both in primary forms or combined in to mixtures as traditional medicines. Traditional medicines have been used as folk medicines as they are assumed to be non-toxic due to their origin from natural sources (Hanapi *et al.*, 2010). Our present research investigates the possibility of exploiting the bioactive leaf extracts of three herbs in a composite form to coat urinary catheter.

Biofilm is a microbial lifestyle, in which the microbes attach to surfaces, allowing this community to survive in hostile environments. The predominant mode of microbial life is biofilm and they can be harmful to both human life and industrial processes (Murugan *et al.*, 2011). Biofilms are the leading example of physiological adaptation and are one of the most important sources of bacterial resistance to antimicrobial products (Simo~es *et al.*, 2010). Biofilms produced by urease-positive bacteria such as *Proteus mirabilis*, pose particular threats to the health of catheterized patients. As urease generates ammonia and creates alkaline conditions under which crystalline biofilms develop rapidly and block the urine flow from bladder resulting in urinary retention, painful distension of bladder, reflux of infected urine to kidneys, pyelonephritis and septicaemia (Elayarajah *et al.*, 2011). Urinary catheter and stent-associated infections are difficult to be treated with antibiotics and there is a need to change catheters (Quesada and Light, 1993). Catheters are manufactured from silicone or from latex; these materials provide attractive, unprotected sites for bacterial attachment. In addition, irregular surfaces left by the manufacturing process, particularly around eye-holes, can trap cells from an infected urine flows through the catheter (Stickler *et al.*, 2003).

The simplest way to prevent biofilm formation is to impregnate catheters with a broad-spectrum antimicrobial agent that elutes into the surrounding environment and attack plank tonic bacteria in the vicinity of the device before they colonize the surface and adopt biofilm-resistant phenotype (Danese, 2002). The antibacterial agents used in the present study were chosen according to several criteria. The first was that they should be capable of molecular migration through cross-linked silicone elastomer, an index of this being solubility in solvent (Matl *et al.*, 2008). The second criterion was that they should be active against most strains of urinary tract pathogens, and the third was that they should have been administered systemically in humans without known significant risk of hypersensitivity or toxicity (Bayston and Barsham, 1998). The fourth criterion was that they should be sufficiently stable to allow sterilisation. The fifth was that they showed good synergistic activity to inhibit the growth of biofilm producers at greater level compared when the antibacterial agents where used alone. When each herbal extract is used alone therapeutically, resistance develops rapidly and this is the main reason for combining three herbs to form as composite. Many antimicrobials were excluded on one or more of these backgrounds.

In preliminary exit-site challenge test, the migration or growth of the test organism around the materials after incubation was indicated by tracking of bacteria along the abluminal surface. The inoculated site was considered to be as skin exit-site and migration and growth of the organisms along the media surface was considered to be as the tissue tunnel and tissue surroundings. Bayston *et al.*, (2009) reported that the most

frequent routes of catheter associated infection are from the skin exit site, the tissue tunnel associated with the catheter and the catheter lumen. Similar exit-site challenge model under *in vitro* condition used by Bayston *et al.*, (2009) showed surface colonization of methicillin resistant *Staphylococcus aureus* (MRSA) on the CSF silicone shunts surface. Two significant biofilm producing organisms, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* produced biofilm within 24 hours in the present study. The reason may be the expression of *icaR* gene encoding a transcriptional repressor involved in environmental regulation of the *ica* operon expression and biofilm formation in *S. epidermidis* (Conlon *et al.*, 2002). Murugan *et al.*, (2011) detected that *cupA* genes were responsible for biofilm formation in *P. aeruginosa*. The obtained results were thus considered as the preliminary test to determine the surface colonizing ability of the test organisms.

Microtitre plate (MTP) biofilm assay confirmed the strong biofilm index for *S. epidermidis* and *Pseudomonas aeruginosa* after expressing the growth within 24 hours during exit-site challenge test. The differences in OD values were due to the amount of crystal-violet (dye) absorbed by the test organisms in the microtitre well. This method was found to be most sensitive, accurate and reproducible screening method for the detection of biofilm formation. The method has the advantage of being a quantitative model to study the adherence of organism on biomedical devices. In this test, even though some of the test organisms were proved to as weak or moderate biofilm producers, still due to their clinical complications and pathogenicity in the medical sciences, it made curious to proceed for further analysis.

To begin our investigation on antibacterial activities, the growth inhibition effect of three herbs in the composite were determined first on test cultures of resistant clinical isolates. In the present study, the herbal composites were prepared using solvent extract (acetone). In general, the solvent extracts of herbal compounds were found to exhibit the highest inhibitory activity compared to the others and the standard antibiotics. The antibacterial and antibiofilm properties of the *A. paniculata*, *E. hirta* and *T. chebula* extracts were extensively reported in the literature with the aid of GC-MS analysis. The GC-MS analysis of *A. paniculata* (Murugan *et al.*, 2011) and *E. hirta* (Shanmugapriya and Roziahaman, 2013) revealed the presence of over 34 and 19 bioactive components respectively. Terpenoids of *E. hirta* influences the membrane integrity in all organisms and helped to eradicate most biofilm cells. Mode of action of terpenes is membrane disruption, inhibiting respiration and ion transport mechanism in biofilm producing cells. Another significant factor is to influence the fatty acid composition of the cell membrane leading to hydrophobicity, thus eradication of the biofilm (Mastelic *et al.*, 2005).

In both qualitative and quantitative antibacterial activity, the herbal composite 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 herbal extracts. Gram positive bacteria are often found to be more susceptible to plant extracts than the Gram negative bacteria (Fennel *et al.*, 2009). 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).

However in this study, the prominent sensitivity of both Gram-negative and Gram-positive organisms may possibly due to the membrane permeability. The permeability of membrane is mainly due to the synergistic action of three herbs in the composite. The prepared herbal composite in the study displayed a distinct bactericidal mode of action against all the bacteria tested. Thus in the present study, the expression of synergy, or additive effects among the major phytochemicals found in the crude extract of herbal composite was found to be rationale for the apparent anti-biofilm activity.

Acknowledgment

The authors would like to thank The Director and the doctoral committee members, Centre for Research and Development, Bharathiar University, Coimbatore-46 for helpful comments and valuable discussions during the tenure of the work.

References

- [1]. Ansari, J.A. and N.N. Inamdar, 2010. The promise of traditional medicines. *Int. J. Pharmacol.*, 6(6): 808-812.
- [2]. Archana, S.J., R. Paul and A. Tiwari, 2011. Indian medicinal plants: A rich source of natural immune-modulator. *Int. J. Pharmacol.*, 7: 198-205.
- [3]. Bag, A., S.K. Bhattacharyya, N.K. Pal and R.R. Chattopadhyay, 2011. Synergistic effect of *Terminalia chebula* against multidrug-resistant uropathogenic *Escherichia coli*. *Med. Aromat. Plant Sci. Biotechnol.*, 5(1): 70-73.
- [4]. Bayston, R., and S. Barsham, 1998. Catheter colonisation: A laboratory model suitable for aetiological, therapeutic and preventive studies. *Med. Lab. Sci.*, 45: 235-239.
- [5]. Chen, M., Q. Yu and H. Sun, 2013. Novel strategies for the prevention and treatment of biofilm related infections. *Int. J. Mol. Sci.*, 14, 18488-18501.
- [6]. Cho, Y.W., J.H. Park, S.H. Kim, Y.H. Cho, J.M. Choi, H.J. Shin, Y.H. Bae, et al., 2003. Gentamicin-releasing urethral catheter for short-term catheterization. *J. Biomater. Sci. Polym. Ed.*, 14(9): 963-972.

- [7]. Conlon, K.M., H. Humphreys, J. P. O’Gara, 2002. *icaR* encodes a transcriptional repressor involved in environmental regulation of *ica* operon expression and biofilm formation in *Staphylococcus epidermidis*. *J. Bacteriol.*, 184(16): 4400-4408.
- [8]. Dhama, K., R. Tiwari, S. Chakraborty, M. Saminathan, A. Kumar, K. Karthick, M.Y. Wani, et al., 2014. Evidence based antibacterial potentials of medicinal plants and herbs countering bacterial pathogens especially in the era of emerging drug resistance: An integrated update. *Int. J. Pharmacol.*, 10(1): 1-43.
- [9]. Danese, P.N., 2002. Antibiofilm approaches: Prevention of catheter colonization. *Chem. Biol.*, 9: 873-880.
- [10]. Fennel, C.W., K.L. Lindsey, L.J. McGaw, S.G. Sprag, G.I. Staffort, E.E. Elgorashi, O.M. Grace and J.V. Staden, 2004. Assessing African medicinal plants for efficacy and safety: Pharmacological screening and toxicity. *J. Ethnopharmacol.*, 94:205-217.
- [11]. Hanapi, N.A., J. Azizi, S. Ismail, S.M. Mansor, 2010. Evaluation of selected Malaysian medicinal plants on phase I drug metabolizing enzymes, CYP2C9, CYP2D6 and CYP3A4 activities in vitro [RID B-7741- 2011]. *Int. J. Pharmacol.*, 6: 494-499.
- [12]. Karim, A., M. Nouman, S. Munir and S. Sattar, 2011. Pharmacology and phytochemistry of Pakistani herbs and herbal drugs used for treatment of diabetes. *Int. J. Pharmacol.*, 7(4): 419-439.
- [13]. Khardori, N. and M. Yassien, 1995. Biofilms in device-related infections. *J. Ind. Microbiol.*, 15(3): 141-147.
- [14]. Malckzadeh, F., H. Ehsanifar, N. Shahamat, M. Levin and R.R. Colwell, 2001. Antibacterial activity of black myrobalan (*Terminalia chebula* Retz.) against *Helicobacter pylori*. *Int. J. Antimicrob. Agents*, 18(1): 85-88.
- [15]. March, J.C. and W.E. Bentley, 2004. Quorum sensing and bacterial cross-talk in biotechnology. *Curr. Opin. Biotechnol.*, 15(5):495-502.
- [16]. Mastelic, J., O. Politeo, I. Jerkovic and N. Radosevic, 2005. Composition and antimicrobial activity of *Helichrysum italicum* essential oil and its terpene and terpenoid fractions. *Chem. Nat. Comp.*, 41: 35-40.
- [17]. Matl, F.D., A. Obermeier, S. Repmann, W. Friess, A. Stemberger and K.D. Kuehn, 2008. New anti-infective coatings of medical implants. *Antimicrob. Agents. Chemother.*, 52(6): 1957-1963.
- [18]. Murugan, K., S. Krishnasamy, V.B. Kalyanasundaram, and S. Al-Sohaibani, 2013. Nanotechnological approach for exploring the antibiofilm potential of an ethanomedicinal herb *Andrographis paniculata* for controlling lung infection causing *Pseudomonas aeruginosa*. *J. Nanomat. Biostruct.*, 8(1): 117-126.
- [19]. Murugan, K., K. Selvanayagi and S. Al-Sohaibani, 2011. Antibiofilm activity of *Andrographis paniculata* against cystic fibrosis clinical isolate *Pseudomonas aeruginosa*. *World. J. Microbiol. Biotechnol.*, 27: 1661-1668.
- [20]. Pitts, B., M.A. Hamilton, N. Zelver, P.S. Stewart, 2003. A microtiter plate method for biofilm disinfection and removal. *J. Microbiol. Methods.*, 54: 269-276. doi:10.1016/S0167-7012(03)00034-4.
- [21]. Quesada, E.T. and J.K. Light, 1993. The AMS 700 inflatable penile prosthesis: long-term experience with the controlled expansion cylinders. *J. Urol.*, 149: 46-48.
- [22]. Shanmugapriya, P. and M. Roziathanim, 2013. Chemical analysis, inhibition of biofilm formation and biofilm eradication potential of *Euphorbia hirta* L. against clinical isolates and standard strains, *BMC Complement Altern. Med.*, 13: 346-353.
- [23]. Simões, M., L.C. Simões, M.J. Vieira, 2010. A review of current and emergent biofilm control strategies. *LWT Food Sci. Technol.*, 43: 573-583. doi:10.1016/j.lwt.2009.12.008.
- [24]. Stickler, D., R. Young and G. Jones, 2003. Why are Foley catheters so vulnerable to encrustation and blockage by crystalline bacterial biofilm? *Urol. Res.*, 31: 306-311.
- [25]. Tian F., B. Li and B. Ji, 2009. Antioxidant and antimicrobial activities of consecutive extracts from *Galla chinensis*; the polarity affects the bioactivities. *Food Chem.*, 113: 173–179.

Fig. 1: Microtitre plate biofilm assay

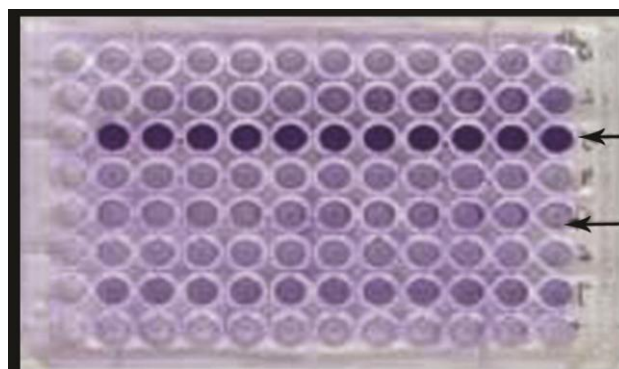


Table 1: Classification of biofilm formation

Mean OD values	Biofilm formation	Biofilm index
<0.120	Nil	Non / weak
0.120-0.240	Moderately	Moderate
>0.240	Strong	High

Table adapted from Mathur *et al.*, (2006)

Table 2: Assessing the biofilm forming capability of test bacteria on urinary catheter using standard microtitre plate biofilm assay

Test bacteria	Biofilm formation (OD 570nm)	Biofilm index
<i>S. epidermidis</i>	0.248	High
<i>S. aureus</i>	0.186	Moderate
<i>P. aeruginosa</i>	0.242	High
<i>K. pneumoniae</i>	0.192	Moderate
<i>E. coli</i>	0.184	Moderate

Biofilm Index - <0.120: Weak, 0.120-0.240: Moderate, >0.240: High

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

Sample	Organisms	Zone of inhibition (mm)	
		<i>dcc</i> urinary catheters*	<i>uc</i> urinary catheters
Urinary catheter	<i>S. epidermidis</i>	43.3	0
	<i>S. aureus</i>	42.6	0
	<i>P. aeruginosa</i>	47.6	0
	<i>K. pneumoniae</i>	42.3	0
	<i>E. coli</i>	41.9	0

*Mean value, *dcc*: Drug-carrier coated catheter samples, *uc*: Uncoated catheter samples

Table 4: Quantitative antibacterial activity of coated materials using the standard bacterial adherence test

Sample	Organisms	Bacterial reduction (%)		Chi square test
		<i>dcc</i> urinary catheters	<i>cc</i> urinary catheters	
Urinary catheter	<i>S. epidermidis</i>	96.5	41.0	P < 0.05
	<i>S. aureus</i>	92.0	39.5	P < 0.05
	<i>P. aeruginosa</i>	91.0	42.5	P < 0.05
	<i>K. pneumoniae</i>	94.5	38.5	P < 0.05
	<i>E. coli</i>	96.0	39.0	P < 0.05

Significant difference in the reduction percentage between *Drug-carrier coated (dcc)* materials and the *carrier coated (cc)* materials were statistically observed (P < 0.05)