



PHYTOTHERAPY AGAINST UROPATHOGEN BIOFILMS IN CATHETER-ASSOCIATED URINARY TRACT INFECTIONS: A REVIEW

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ABSTRACT

Catheter-associated urinary tract infections are the most common healthcare-associated infections. It remains a significant clinical challenge largely due to the ability of uropathogens such as *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, to form strong biofilms on catheter surfaces. These biofilms protect bacteria from antibiotics and immune responses, contributing to persistent and recurrent infections. Current prevention measures tend to be expensive and may promote resistance development. Phytotherapy has emerged as one promising field of study. This review consolidates existing research on the antibiofilm and antimicrobial activities of various plant extracts such as *Azadirachta indica* (neem), *Piper betle* (betel), *Brassica nigra* (mustard seeds) against catheter-associated urinary tract infection pathogens, summarizing methodologies and key findings. Reported studies demonstrate that bioactive

compounds can inhibit bacterial adhesion, suppress quorum sensing and disrupt extracellular polymeric substance formation on catheters surfaces. Overall, the review supports phytotherapy as eco-friendly, and affordable way to manage catheter-associated urinary tract infections.

KEYWORDS: Catheter-associated urinary tract infections, Biofilm, Phytotherapy, Antimicrobial Resistance, Medicinal Plant Extracts, Antibiofilm Activity.

INTRODUCTION

The most prevalent bacterial illnesses affecting people worldwide are urinary tract infections (UTIs). The most common kind of healthcare-associated infections (HAIs) are catheter associated urinary tract infections (CAUTIs). Bacteria can enter the urinary tract through a urinary catheter and cause these illnesses. A urinary catheter is a small, flexible tube that is put into the body via the urethra to gather and drain urine from the bladder. These gadgets are often utilized by those who have problems urinating.^[36] The main cause of both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC), which causes 75% and 65% of these illnesses, respectively. The most frequent microbial agents in complex UTIs, particularly CAUTIs, after UPEC, include *Enterococcus* species (11%), *Klebsiella pneumoniae* (8%), *Candida species* (7%), *Staphylococcus aureus* (3%), *Pseudomonas aeruginosa* (2%), *Group B Streptococcus* (2%), *Proteus mirabilis* (2%).^[40]

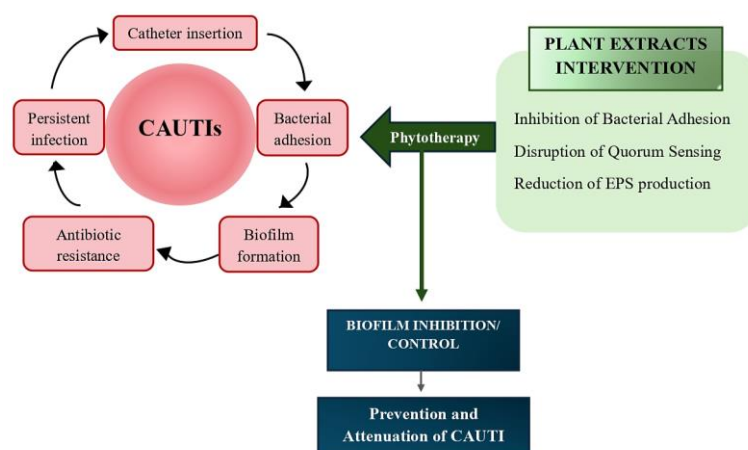


Fig. 1: schematic representation of biofilm formation in CAUTIs and phytotherapeutic intervention.

The insertion of catheter disrupts the natural host defence mechanism and introduces an artificial surface conducive to microbial adherence. When bacteria attach to the surface of catheter, it creates biofilm and catheter associated urinary tract infections (CAUTIs) start to develop. A biofilm is a group of microbes that adhere to one another and a surface, surrounded by a defensive matrix composed of extracellular polymeric substances (EPS). The process of biofilm development involves of free-swimming bacteria to bind to the surface via flagella as well as hydrophobic and electrostatic interactions. Following attachment, the bacteria divide and produce more planktonic bacteria, and start to form an extracellular matrix. Cell-to-cell communication helps in the creation of densely packed three-dimensional

structures. This is accompanied with fluid channels to enable nutrient and waste exchange. The cycle is completed by the separation of individual organisms from the biofilm, which may cause the discharge of pathogens into the urine.^[16,36] The extracellular polymeric substances (EPS) matrix is composed of polysaccharides, proteins, lipids, extracellular DNA, which provides mechanical stability and protection against environmental stressors. The biofilm serves as a physical barrier to the biofilm-associated bacterial cells, providing resistance to antibiotics, antimicrobial treatment, phagocytosis, and host immune responses. This contributes to persistent infections. In vitro experiments conducted using biofilm models have revealed that those bacteria protected by biofilms have survival rates that are hundreds or even thousands of times greater than those of planktonic bacteria when exposed to antibiotics.^[19] Additionally, close cellular proximity of microbes belonging to one or more species within a biofilm facilitates horizontal gene transfer of antimicrobial resistance genes.^[39]

Some of the most common hospital-acquired infections (HCAs) are Urinary tract infections (UTIs), representing 40% or more of all reported cases. They often extend hospital stays worldwide.^[10] UTIs impact around 150 million people each year across the globe, being one of the most prevalent bacterial illnesses.^[40] In developing countries, UTIs account for 24% of hospital-acquired infections.^[35] 20 to 49% of all hospital acquired infections are catheter-associated urinary tract infections (CAUTIs). And about 90% of these infections are linked to the use of catheters. These infections are associated with extended hospital stays, higher healthcare expenses, and excessive antibiotic use.^[40] Catheter-associated urinary tract infections (CAUTIs) are more prevalent in elderly patients, with the highest incidence reported in individuals aged 60 years and above. The susceptibility increases in advanced ages due to prolonged catheter use, comorbidities, and reduced immune function.^[18] Despite advances, CAUTIs causes significant clinical and economic implications. Complications include infection, tissue irritation, and encrustation. These infections often show symptoms like blood in the urine (haematuria), fever, pain in the lower abdomen, visible biofilm, or acute confusion.^[24,30] CAUTIs therefore contributing substantially to morbidity and mortality in hospitalized populations.^[5] The chance of contracting a CAUTI rises by 3–7% for every day the catheter stays in place.^[22] CAUTIs is defined as infection in patients with catheters left in place for 48 hours or longer as per the Centres for Disease Control and Prevention.^[14] CAUTIs can result in serious complications like prostatitis, epididymitis, orchitis, cystitis, pyelonephritis, gram-negative bacteraemia, endocarditis, vertebral osteomyelitis, septic

arthritis, endophthalmitis, and meningitis. This can lead to pain, extended hospital stays, higher expenses, and increased death rates. An indwelling urinary catheter if it is used for less than 30 days, is typically considered to be short-term and long-term or chronic if it is used for longer, more than 30 days.^[30] The World Health Organization (WHO) considers antibiotic resistance to be one of the greatest risks to public health worldwide and warns antimicrobial resistance could cause 10 million deaths per year by 2050. The use of antibiotics is one of the main causes of this increasing issue. The use of antibiotics may also have unintended consequences, like upsetting the balance of the beneficial gut microbiome. This may cause an excess of dangerous germs and the release of toxins into the body. Due to these concerns, alternatives to traditional antibiotics are urgently needed. As a result, there has been more curiosity in nonantibiotic strategies for tackling catheter associated urinary tract infections (CAUTIs).^[40]

Current research highlights combining improved catheter materials, strict aseptic protocols, and innovative anti-biofilm strategies, including natural extracts, to reduce CAUTI occurrence and improve patient outcomes.^[1,9] A significant amount of study is currently focused on developing alternative methods for managing biofilms produced by bacteria that cause urinary tract infections. In this context, natural plant-derived compounds have attracted considerable research interest. This is because medicinal plants have been used in traditional healthcare systems for centuries and are considered as potential antimicrobial and antibiofilm agents due to the presence of bioactive phytochemicals with therapeutic potential. The mechanisms underlying CAUTI development and phytotherapeutic intervention are illustrated in Fig. 1. Extracts from neem (*Azadirachta indica*), betel (*Piper betle*), and mustard seeds (*Brassica nigra*) contain phytochemicals like flavonoids, alkaloids, phenolics, terpenoids, and essential oils, demonstrating antimicrobial and antibiofilm potential.^[20,32,41] Traditional plants have documented antimicrobial and antioxidant effects.^[2] Studies suggest that these extracts may have the ability to inhibit biofilm formation and act against multidrug-resistant pathogens.^[27] Other reviews support that natural products from plants can stop biofilm formation and even break up existing biofilms produced by pathogens such as *K. pneumoniae*.^[11,31] Natural plant compounds may cause microbial membrane disruption, enzyme activity inhibition, interfere with quorum sensing, and reduce EPS production. This results in impairing biofilm formation and stability.^[19,41]

Given the clinical significance of biofilm-associated infections and the lack of existing treatments, identifying effective plant extracts could help reduce CAUTIs thereby leading to short term hospital stays, reduced healthcare expenses, and fewer side effects associated with long-term antibiotic use.^[29,35] From a public health view, such strategies may decrease reliance on antibiotics and slow the spread of AMR.^[37] Economically, plant-based treatments are ideal for healthcare systems in low and middle-income countries as these treatments are affordable, widely available, and eco-friendly.^[29] Consequently, this review may contribute to the development of novel strategies for managing CAUTIs and may greatly impact microbiological research, clinical practices, and global efforts to mitigate the effects of antimicrobial resistance.

PHYTOCHEMICAL EXTRACTION AND ANTIMICROBIAL POTENTIAL OF PLANT EXTRACTS

The reviewed studies describe various approaches for the preparation and characterization of plant extracts. Plant materials were typically dried, powdered, and subjected to solvent extraction. Aqueous or organic solvents were used to isolate bioactive phytoconstituents. Maceration and solvent extraction were commonly employed for recovering antibacterial compounds, particularly for medicinal plants and spices.^[4,21] Phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, and tannins associated with antibacterial and antimicrobial activity.^[23,34] Medicinal plants including neem and other botanical extracts have been evaluated for their antimicrobial and antibiofilm activity against clinically relevant bacteria. This demonstrated inhibition of microbial growth and colonization.^[20,32] Betel leaf extracts have similarly assessed for phytochemical and antimicrobial activity, confirming inhibitory potential against pathogens.^[25,26] A study on spices, including mustard seeds, have also demonstrated the antimicrobial and antibiofilm potential.^[6] Broader studies highlight solvent selection and extraction conditions as key determinants of antibacterial efficacy.^[11,33]

Plant extraction was carried out using alcoholic solvents such as methanol and ethanol.^[6,20,32] The reviewed studies revealed that plant-derived extracts exhibit antimicrobial activity due to the presence of bioactive phytochemicals such as phenolics, flavonoids, alkaloids, and tannins.^[4,21,34] These compounds have the ability of membrane disruption, enzyme inhibition, and interference with microbial metabolic processes. Studies revealed that extraction solvent greatly influences the recovery of phytochemicals and its antimicrobial activity. However,

ethanolic and methanolic extracts showed stronger inhibition compared to aqueous extracts.^[11,33] Neem extracts have shown bacterial adhesion inhibition and reduce biofilm development, thereby indicating their role in controlling surface colonization.^[20,32] Betel leaf extracts revealed antibacterial activity that helps against biofilm formation.^[25,26] Studies investigating medicinal spices, including mustard-containing preparations, showed antimicrobial and biofilm-modulating effects.^[6] Overall, plant extracts possess inhibitory activity against pathogenic bacteria, though its efficacy varies with extraction conditions, solvent type, and concentration.^[11,12,33] These findings point out phytochemicals as potential alternatives or adjuncts to conventional antimicrobials.

BIOFILM FORMATION AND SURFACE COLONIZATION MODELS AND DYNAMICS

The studies reveal that biofilm formation assays frequently use clinically relevant bacterial species that are associated with urinary tract and device-related infections. Under controlled conditions, biofilms are developed using laboratory strains or clinical isolates. These controlled conditions allow adhesion and maturation on inert or biomaterial surfaces. A major contributor to infection persistence and antimicrobial resistance (AMR) is the biofilm formation on catheter substrates.^[13,19,36] Test-tube, microplate, glass-slide, and petri dish systems are the experimental models used for comparative evaluation of biofilm development.^[8] Studies involving biofilm growth on surfaces such as silicone and catheter materials further highlight the role of host–surface interactions in microbial colonization.^[15,38] These *in vitro* systems provide reproducible frameworks for evaluating antimicrobial potential against biofilm establishment.

Experimental studies demonstrated successful biofilm establishment across multiple laboratory systems such as test tubes, microplate, glass-slide models showing variations in biofilm density.^[8] The studies involving biofilm formation on catheter and silicone materials showed stable colonization and enhanced persistence, highlighting their clinical relevance.^[15,38] These findings emphasize the importance of surface-targeted antimicrobial treatments as surface-associated growth provides greater resistance compared to planktonic states and contributes to chronic infection risk.^[13,36] Also, laboratory adhesion studies reveal reproducible colonization under controlled conditions.^[3]

QUANTITATIVE ASSESSMENT OF BIOFILM DEVELOPMENT

Quantitative and semi-quantitative techniques for the evaluation of biofilm formation were widely reported across the reviewed studies. Crystal violet staining is widely used for the estimation of total biomass. It is simple and reproducible.^[17] Alternative methods such as XTT assays is used to assess metabolic activity and viable cells within biofilms. This demonstrates methodological variability in outcome interpretation.^[7] To ensure reliable quantification, optical and spectrophotometric measurements are frequently used.^[17] These analytical techniques enable assessment of antibiofilm activity of plant extracts targeting microbial adhesion and matrix stability.^[31,42]

Crystal violet staining was commonly reported as the primary method for quantifying total biofilm biomass due to its simplicity and reproducibility.^[17] Complementary XTT assays provided differences between biomass presence and cellular viability.^[7] The comparative interpretation of antibiofilm effects is demonstrated by integration of optical and spectrophotometric measurements.^[17] These assays revealed that antimicrobial agents, including plant extracts, can reduce biofilm formation and metabolic activity.^[31,42] However, direct comparison of quantitative outcomes narrow downs, due to methodological variability across studies. This emphasizes the need for standardized evaluation frameworks.^[7,17,31]

PHYTOTHERAPY AS A STRATEGY TO COMBAT BIOFILM-MEDIATED ANTIMICROBIAL RESISTANCE

The reviewed literature for the antimicrobial assessment commonly includes diffusion assays, minimum inhibitory concentration determination, and evaluation of extract-mediated biofilm disruption. Plant-derived compounds have been tested against opportunistic and uropathogenic bacteria to evaluate inhibitory potential and resistance-modulating effects.^[12,41] These studies help in understanding alternative therapeutic strategies addressing rising antibiotic resistance and biofilm persistence.^[29,37] Studies focusing on catheter-associated urinary infections emphasizes the disruption of biofilm formation as a key intervention strategy for the prevention and control of infection.^[28,40]

Susceptibility and diffusion-based assays showed inhibitory potential of plant-derived compounds.^[12,41] These findings are relevant to the increasing antimicrobial resistance and associated treatment challenges, thereby highlighting the need for alternative strategies.^[29,37] Research focusing on catheter-associated urinary infections emphasizes that preventing biofilm establishment is more effective than attempting eradication of biofilm after

maturation.^[28,40] Despite encouraging outcomes, variability in experimental conditions across studies emphasizes the need for standardized protocols and deeper mechanistic studies so as to improve reproducibility and advancing in plant-based antibiofilm strategies.^[11,17,29]

CONCLUSION

Catheter-associated urinary tract infections remain a major clinical problem due to the biofilm-mediated persistence and increasing antimicrobial resistance. This review shows that plant-derived extracts such as neem, betel, and mustard possess antimicrobial and antibiofilm potential which interfere with bacterial adhesion and biofilm stability. Across the reviewed studies, alcoholic solvents (methanol and ethanol) demonstrated superior extraction efficiency compared to aqueous extracts. While findings show phytotherapy as an affordable and eco-friendly alternative strategy for CAUTI management, differences in extraction methods, assay systems, and experimental conditions limits direct comparison across studies. Standardized methodologies along with in vivo and clinical examinations are therefore necessary to validate their therapeutic potential in infection-control applications.

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