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Antibiofilm activity of trans-cinnamaldehyde, *p*-coumaric, and ferulic acids on uropathogenic *Escherichia coli*

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Background/aim: Biofilm on urinary catheters results in persistent infections that are resistant to antibiotics. In this study, phytochemicals were assessed as alternative antimicrobials in preventing and inactivating *E. coli* biofilm on urinary catheters.

Materials and methods: Biofilm prevention was tested using catheter fragments inoculated with *E. coli* and treated with trans-cinnamaldehyde, *p*-coumaric, and ferulic acids (0%, 0.1%, 0.25%, and 0.5%) for 0, 1, 3, and 5 days. Inactivation of *E. coli* biofilm with the same agents at concentrations of 0%, 1%, 1.25%, or 1.5% used for 0, 1, 3, or 5 days was also evaluated.

Results: All used concentrations of trans-cinnamaldehyde prevented and effectively inactivated *E. coli* biofilm formed on urinary catheter fragments. *p*-Coumaric (0.25% and 0.5%) and ferulic acids (0.5%) had preventive action on *E. coli* biofilm formation in urinary catheter fragments. The number of uropathogenic *E. coli* cells in biofilm formed in the lumen of a urinary catheter was significantly reduced in the presence of *p*-coumaric and ferulic acids, but complete inactivation of the biofilm formed was not observed, as opposed to the use of trans-cinnamaldehyde.

Conclusion: The obtained results indicate that phytochemicals may be an important source of antibiofilm agents that have preventive action on *E. coli* biofilm formation on urinary catheters.

Key words: Uropathogenic *Escherichia coli*, biofilm, cinnamic aldehyde, coumaric acid, ferulic acid

1. Introduction

Urinary tract infections (UTIs) are considered to be the most common bacterial infection in patients with a chronic indwelling bladder catheter, accounting for an estimated 150 million cases per year worldwide (1). *Escherichia coli* is the major factor in community-acquired UTIs (70%–95%) and a large part of nosocomial UTIs (50%), including cystitis, pyelonephritis, prostatitis, and asymptomatic bacteriuria (2). Uropathogenic *E. coli* (UPEC) is commonly isolated from biofilms formed on both the internal and external urinary catheter surface (3). In many cases, catheter-associated urinary tract infections (CAUTIs) are asymptomatic, but when these infections develop they are often difficult to treat and are a major reservoir of resistant pathogens (4,5). Numerous strategies to reduce the number of CAUTI cases have been investigated, including the use of antibiotic-coated or impregnated urinary catheters, but antibiotics could only prevent CAUTIs during short-term catheterization (6,7); in patients with long-term catheterization, the risk of bacteremia increases and antimicrobial treatment may lead to the emergence of more antibiotic-resistant bacteria (5). Therefore, there is a need for new strategies to prevent UPEC biofilm on urinary catheters. Plants have many chemical strategies to protect them from microbial infections and different products of plant origin are well known for their antimicrobial activities (8). Phenolic products are common plant secondary metabolites and can exhibit a wide range of biological effects, including antimicrobial, antiinflammatory, and anticarcinogenic action (9). Therefore, they are considered potential therapeutic agents against inflammatory and infectious diseases. Phenolic acids are a group of phenolic compounds biosynthesized by the shikimate pathway and include two classes: derivatives of benzoic acid (gallic acid) and derivatives of cinnamic acid, such as *p*-coumaric acid (*p*-CA) and ferulic acid (FA) (10). *p*-CA is present in large quantities in many vegetables, fruits, and graminaceous plants (11). FA is also a common phenolic compound present in cereals and is esterified to hemicelluloses

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in the cell wall, while trans-cinnamaldehyde (TC) is the principal component in cinnamon oil (12,13). The chemical structures of TC, *p*-CA, and FA are shown in Figure 1. Although the antibiofilm effect of TC on UPEC was reported earlier (14,15), the efficiency of *p*-CA and FA against UPEC biofilm formed on urinary catheters has not been determined.

The aim of this study was to investigate TC, *p*-CA, and FA as alternative antimicrobials in preventing and inactivating UPEC biofilm on urinary catheters.

2. Materials and methods

2.1. Microorganism and growth media

E. coli clinical isolate was obtained from urine samples from women with UTIs hospitalized in the neurological unit of Wolomin District Hospital (Poland). *E. coli* UTI diagnosis was made by clinical symptoms and by the presence of $\geq 10^5$ colony-forming units (cfu) of *E. coli* per milliliter of urine sample. Urine was inoculated onto 5% sheep blood agar plate and Chrom ID CPS agar plate (bioMérieux, France) in 10 µL aliquots and incubated at 37 °C overnight. Five colonies that were coliform in morphology and were lactose-fermenting were subcultured on sheep blood agar. *E. coli* identification was performed according to standard procedures (16). Finally, the identification of the isolate was confirmed by using the ID 32 E system (bioMérieux). The *E. coli* isolate was cultured in trypticase soy broth (TSB; BBL, Becton Dickinson, USA) at 37 °C overnight. The bacterial cells were sedimented by centrifugation (3600 × *g*, 10 min), washed with sterile PBS, and finally suspended in TSB. The number of bacterial cells in suspension was determined on TSA plates (BBL, Becton Dickinson).

2.2. Prevention of *E. coli* biofilm formation in the lumen of urinary catheter

An all-silicone 20 FR Foley catheter (Romed, the Netherlands) was cut into 3 cm fragments. Under sterile conditions, each fragment was plugged with sterile pipette tips and heat-sealed at one end, filled with bacterial suspension in TSB (about 10^5 CFU/mL), and plugged with sterile pipette tips at the other end, according to the method described by Trautner et al. (17). The fragments with bacterial culture were incubated at 37 °C for 2 h. Then the cultures were removed and the catheter fragments were washed with sterile PBS and transferred to 10 mL of TSB (control) and to 10 mL of TSB containing 0.1%, 0.25%, or 0.5% TC, *p*-PCA, or FA (Sigma-Aldrich, Germany). After 0, 1, 3, and 5 days of static incubation, the catheter fragments were washed with PSB and two 1-cm segments were cut from each fragment. Each segment of catheter was transferred to 1 mL of PSB and sonicated at 40,000 Hz for 10 min in a water bath sonicator (BL3-A, China) at 37 °C. The sonication liquid was vortexed for 3 min and diluted in PSB. Subsequently, 100 µL of the sonication liquid and its dilutions were plated on MacConkey agar (BBL, Becton Dickinson) to determine the number of *E. coli* cells in the lumen of the urinary catheter fragment. Two catheter fragments were used per treatment and the experiment was repeated 3 times.

2.3. Inactivation of *E. coli* biofilm in the lumen of urinary catheter

The catheter fragments were prepared as described above. Incubation of catheter fragments with bacterial culture was carried out at 37 °C for 5 days to allow biofilm formation on the catheter luminal surface. Then the cultures were removed and the catheter fragments were washed with sterile saline. The catheter fragments were sealed at one end and filled with saline (control), or saline with 1%, 1.25%, or 1.5% TC, *p*-CA, or FA. Then the other ends were sealed and the samples were incubated at 37 °C. The number of *E. coli* cells on the catheter's luminal surface was counted after 0, 1, 3, and 5 days of static incubation. For this purpose, catheter fragments were washed with PSB and each fragment was cut into two 1-cm segments that were transferred to 1 mL PSB and sonicated as described above. After vortexing the sonication liquid and diluting it in PSB, 100 µL of the sonication liquid and its dilutions were plated on MacConkey agar to determine the number of *E. coli* cells. Two catheter fragments were used per treatment and the experiment was repeated 3 times.

2.4. Statistical analysis

The data from three independent experiments were analyzed by least-squares analysis of variance using the general linear model procedure in SAS. Comparisons of the group means and identification of differences were performed using Duncan's multiple-range test and SAS software. A critical value of P ≤ 0.0001 was used for analyses.

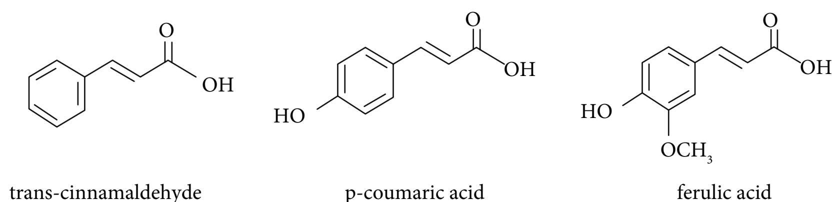

Figure 1. Chemical structure of trans-cinnamaldehyde, *p*-coumaric acid, and ferulic acid.

3. Results

The effects of TC, *p*-CA, and FA were evaluated against the *E. coli* biofilm on a urinary catheter. TC at a concentration of 0.5% was highly effective for preventing *E. coli* biofilm formation and after 1 day of incubation it completely inhibited biofilm formation, while 0.1% and 0.25% TC prevented biofilm formation after 3 days. A fully formed biofilm (about 5.7 log cfu *E. coli*) in the lumen of the control catheter with 0% TC was observed after 5 days (Figure 2A). Incubation of *E. coli* biofilm with 1.25% and 1.5% TC solution completely inactivated biofilm after 1 day, while in the case of 1% TC, the amount of bacteria in biofilm was above 2.1 log cfu. Total elimination of *E. coli* biofilm with 1% TC was observed after 3 days of incubation (Figure 2B).

p-CA at 0.5% and 0.25% prevented biofilm formation after 3 and 5 days, respectively, while 0.1% *p*-CA after 5 days of incubation reduced the number of *E. coli* cells in biofilm only to about 4.5 log cfu; the difference between this and the mean number of *E. coli* cells in the control biofilm (about 5.8 log cfu) was not statistically significant at $P \leq 0.0001$ (Figure 3A). None of the *p*-CA concentrations completely eliminated biofilm from the lumen of the urinary catheter (Figure 3B). However, the mean numbers of *E. coli* cells in the biofilm were lower (1.9–2.2 log cfu) in the presence of *p*-CA concentrations compared to the control biofilm (above 6.4 log cfu) and these differences were highly statistically significant at $P \leq 0.0001$. The mean numbers of *E. coli* cells in biofilm treated with different *p*-CA concentrations did not significantly differ at $P \leq 0.0001$.

After 5 days of incubation, 0.5% FA prevented *E. coli* formation in biofilm. At 0.1% and 0.25% FA, mean numbers of bacteria in biofilm after 1, 3, and 5 days of incubation were not significantly different ($P \leq 0.0001$) but were highly significantly lower ($P \leq 0.0001$) than in the control biofilm (Figure 4A). *E. coli* biofilm was not fully eliminated by FA (Figure 4B), but the average numbers

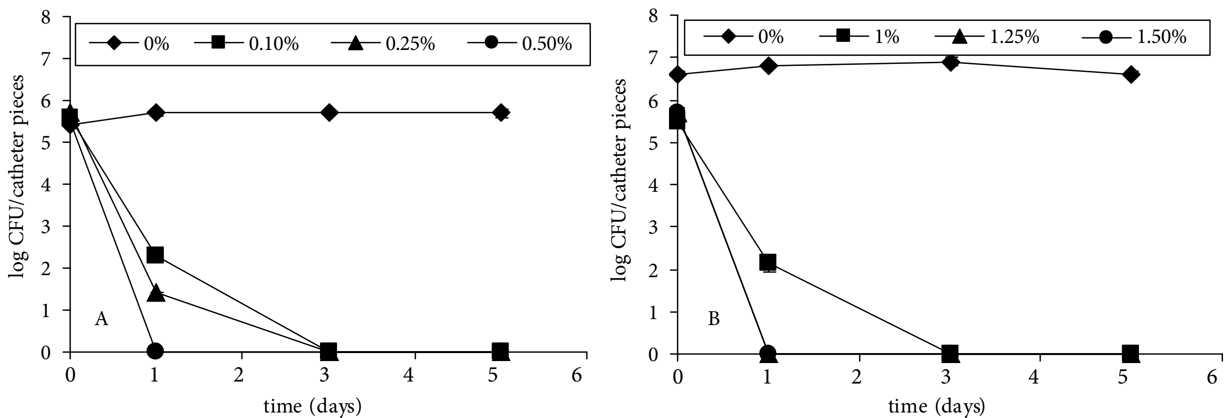

Figure 2. *E. coli* biofilm prevention (A) and inactivation (B) in the lumen of a urinary catheter by trans-cinnamaldehyde (TC). Biofilm formation prevention with 0% (diamonds), 0.1% (squares), 0.25% (triangles), and 0.5% (circles) TC (A); inactivation of biofilm treated with 0% (diamonds), 1% (squares), 1.25% (triangles), and 1.5% (circles) TC solution in 0, 1, 3, and 5 days (B).

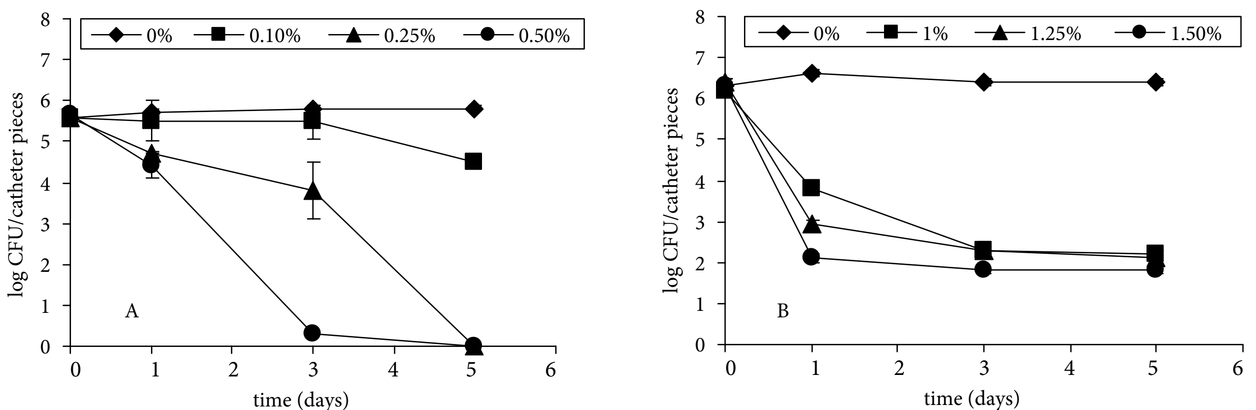

Figure 3. *E. coli* biofilm prevention (A) and inactivation (B) in the lumen of a urinary catheter by *p*-coumaric acid (*p*-CA). Biofilm formation prevention with 0% (diamonds), 0.1% (squares), 0.25% (triangles), and 0.5% (circles) *p*-CA (A); inactivation of biofilm treated with 0% (diamonds), 1% (squares), 1.25% (triangles), and 1.5% (circles) *p*-CA solution in 0, 1, 3, and 5 days (B).

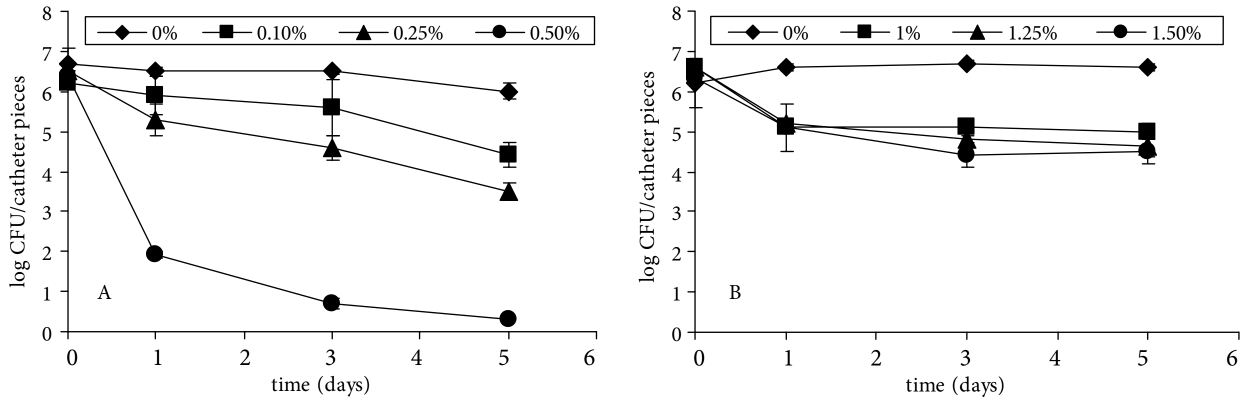

Figure 4. *E. coli* biofilm prevention (A) and inactivation (B) in the lumen of a urinary catheter by ferulic acid (FA). Biofilm formation prevention with 0% (diamonds), 0.1% (squares), 0.25% (triangles), and 0.5% (circles) (FA) (A); inactivation of biofilm treated with 0% (diamonds), 1% (squares), 1.25% (triangles), and 1.5% (circles) FA solution in 0, 1, 3, and 5 days (B).

of *E. coli* cells that formed in biofilm in the presence of different FA concentrations were highly significantly lower ($P \leq 0.0001$) than the average number in the control biofilm (above 6.5 log cfu). The differences in numbers of cells in the biofilm formed in the presence of different FA concentrations were not significant at $P \leq 0.0001$.

4. Discussion

Urinary catheterization is frequently used in the process of treating of many diseases. CAUTI is the most common infection acquired in hospitals and nursing homes worldwide (5). The morbidity of the symptomatic bacterial infection was found in 100% of patients after 30 days of urinary catheterization (18). Biofilm formation on the inner or outer surface of the urinary catheter plays a main role in the pathogenesis of CAUTI and is a major reservoir of resistant pathogens (6).

Different methods of CAUTI prevention have been evaluated, including the use of catheters coated with various materials targeted against the pathogenesis of biofilms (19,20) or bladder and catheter irrigation with antimicrobial solutions (1,14,21). Treatment with prophylactic antibiotics is not recommended because it is not always effective in preventing CAUTI and the effect is only temporary. Moreover, continuous use of antibiotics leads to the selection of strains that are resistant to antibiotics (1). Effective alternative strategies to decrease UPEC infections include the use of plant-derived molecules that contain different functional groups in their structure, because bacteria develop resistance to these antimicrobials less frequently (22).

In this study, the activity of TC and phenolic acids against UPEC biofilm on a urinary catheter was evaluated. All used concentrations of TC prevented development of UPEC biofilm on catheter fragments. Moreover, TC used as an antimicrobial constituent in a catheter lock solution effectively inactivated already-formed *E. coli* biofilm. This observation confirms the previous report by Amalaradjou et al. (14), who showed that TC was effective against UPEC biofilm on polystyrene or latex, and that the expression of *E. coli* genes encoding attachment and invasion of bladder cells was significantly decreased by TC (15). In our study, *p*-CA had high activity in UPEC biofilm prevention. The results showed that two concentrations (0.25% and 0.5%) were effective in preventing *E. coli* biofilm formation on the internal urinary catheter surface. The number of UPEC cells in biofilm was also significantly reduced in the presence of *p*-CA, but we did not observe complete inactivation of the biofilm similar to the use of TC. Lou et al. (23) reported that *p*-CA effectively inhibited the growth of *E. coli* and other gram-negative bacteria by disrupting bacterial cell membranes. *p*-CA also has the capacity to bind bacterial genomic DNA, leading to cell death (23). Alves et al. (24) demonstrated the antibacterial activity of *p*-CA against fluoroquinolone-resistant *E. coli* isolated from urine. However, the activity of *p*-CA against *E. coli* biofilm, as far as we know, has not been investigated.

In this study we also evaluated the antibiofilm activity of FA. Effective *E. coli* biofilm prevention was observed only at the highest concentration (0.5%). When biofilm formed in the presence of lower FA concentrations, *E. coli*'s ability to form a biofilm decreased compared with the control (in the absence of FA), but complete inhibition of biofilm formation was not observed. The activity of FA against *E. coli* biofilm formation on a microtiter plate was evaluated by Borges et al. (25), who showed that FA had no preventive effects on the biofilm formation ability of *E. coli;* reduction of biofilm biomass in the presence of FA was only 13% compared with the control.

We also evaluated the potential of FA to inactivate existing biofilm on catheter fragments. FA showed high potential to reduce the number of *E. coli* cells in biofilm

formed on the internal urinary catheter surface, but total biofilm removal was not achieved. Borges et al. (25) observed a high reduction in *E. coli* biomass in biofilm on microtiter plates with FA, but, similar to our results, total elimination of biofilm was not obtained. It was found that FA led to irreversible changes in membrane properties that resulted in leakage of intracellular constituent (26).

Our results revealed that TC and two phenolic acids had preventive action on biofilm formation on an internal urinary catheter surface. TC at all concentrations effectively prevented *E. coli* biofilm, while preventive activity of phenolic acids depended on the concentration. The effective preventive activity of *p*-CA was not observed at the lowest concentration, whereas FA only blocked *E. coli* formation at the highest concentration.

Among the applied antimicrobial agents, TC had the highest potential to reduce the number of *E. coli* cells in biofilm and completely removed biofilm from the urinary catheter fragment. On the other hand, *p*-CA and FA were effective in reducing the biofilm formed by *E. coli,* but total elimination of biofilm was not observed. Different activities of the evaluated phytochemicals may result from their different chemical structures. The presence of a CHO group in TC determines its high antibiofilm activity. On the other hand, *p*-CA and FA are carboxylic acids with a free OH group. The antibiofilm activity of these acids was similar but lower compared to TC. This study demonstrated the potential of the plant-derived molecules to prevent and control UPEC biofilm on urinary catheter. TC is the principal component of cinnamon oil and is an effective antimicrobial product to prevent and inactivate biofilm on catheters. *p*-CA and FA showed the potential to prevent *E. coli* biofilm and seem to be promising as antibacterial agents that may be used as catheter surface coatings or as ingredients in catheter lock solutions to prevent UTIs. However, research into the cytotoxic effects of these phytochemicals on human bladder epithelial cells at the tested concentrations is needed.

References

1. Jacobsen SM, Stickler DJ, Mobley HLT, Shirtliff ME. Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. Clin Microbiol Rev 2008; 21: 26–59.
2. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Dis Mon 2003; 49: 53–70.
3. Nicolle LE. Catheter-related urinary tract infection. Drugs Aging 2005; 22: 627–639.
4. Trautner BW, Darouiche RO, Hull RA, Hull S, Thornby JI. Pre-inoculation of urinary catheters with *Escherichia coli* 83972 inhibits catheter colonization by *Enterococcus faecalis*. J Urol 2002; 167: 375–379.
5. Tambyah PA. Catheter-associated urinary tract infections: diagnosis and prophylaxis. Int J Antimicrob Agents 2004; 24S: 44–48.
6. Ha US, Cho YH. Catheter-associated urinary tract infections: new aspects of novel urinary catheters. Int J Antimicrob Agents 2006; 28: 458–490.
7. Desai DG, Liao KS, Cevallos ME, Trautner BW. Silver or nitrofurazone impregnation of urinary catheters has a minimal effect on uropathogen adherence. J Urol 2010; 184: 2565–2571.
8. Dixon RA. Natural products and plant disease resistance. Nature 2001; 411: 843–847.
9. Russell W, Duthie G. Plant secondary metabolites and gut health: the case for phenolic acids. Proc Nutr Soc 2011; 70: 389–396.
10. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 2010; 15: 7313–7352.
11. Mussatto SI, Dragone G, Roberto IC. Ferulic and *p*-coumaric acids extraction by alkaline hydrolysis of brewer's spent grain. Ind Crop Prod 2007; 25: 231–237.
12. Burt S. Essential oils: their antibacterial properties and potential application in foods–a review. Int J Food Microbiol 2004; 94: 223–253.
13. D'Archivio M, Filesi C, Di Benedetto R, Gargiulo R, Giovannini C, Masella R. Polyphenols, dietary sources and bioavailability. Ann Ist Super Sanita 2007; 43: 348–361.
14. Amalaradjou MAR, Narayanan A, Baskaran SA, Venkitanarayanan K. Antibiofilm effect of trans-cinnamaldehyde on uropathogenic *Escherichia coli*. J Urol 2010; 184: 358–363.
15. Amalaradjou MAR, Narayanan A, Venkitanarayanan K. Trans-cinnamaldehyde decreases attachment and invasion of uropathogenic *Escherichia coli* in urinary tract epithelial cells by modulating virulence gene expression. J Urol 2011; 185: 1526–1531.
16. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC. Color Atlas and Textbook of Diagnostic Microbiology. 5th ed. Philadelphia, PA, USA: Lippincott-Raven; 1997.
17. Trautner BW, Hull RA, Darouiche RO. *Escherichia coli* 83972 inhibits catheter adherence by a broad spectrum of uropathogenes. Urology 2003; 61: 1059–1062.
18. Dellimore KH, Helyer AR, Franklin SE. A scoping review of important urinary catheter induced complications. J Mater Sci: Mater Med 2013; 24: 1825–1835.

19. Lee SJ, Kim SW, Cho YH, Shin WS, Lee SE, Kim CS, Hong SJ, Chung BH, Kim JJ, Yoon MS. A comparative multicentre study on the incidence of catheter-associated urinary tract infection between nitrofurazone-coated and silicone catheters. Int J Antimicrob Agents 2004; 24S: 65–69.

20. Regev-Shoshani G, Ko M, Crowe A, Av-Gay Y. Comparative efficacy of commercially available and emerging antimicrobial urinary catheters against bacteriuria caused by *Escherichia coli* in vitro. Urology 2011; 78: 334–339.

21. Trautner BW, Darouiche RO. Role of biofilm in catheter-associated urinary tract infection. Am J Infect Control 2004; 32: 177–183.

22. Domadia P, Swarup S, Bhunia A, Sivaraman J, Dasgupta D. Inhibition of bacterial cell division protein FtsZ by cinnamaldehyde. Biochem Pharmacol 2007; 832: 831–840.

23. Lou Z, Wang H, Rao S, Sun J, Ma C, Li J. *p*-Coumaric acid kills bacteria through dual damage mechanisms. Food Control 2012; 25: 550–554.

24. Alves MJ, Ferreira ICFR, Froufe HJC, Abreu RMV, Martins A, Pintado M. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. J Appl Microbiol 2013; 115: 346–357.

25. Borges A, Saavedra MJ, Simões M. The activity of ferulic and gallic acids in biofilm prevention and control of pathogenic bacteria. Biofouling 2012; 28: 755–767.

26. Borges A, Ferreira C, Saavedra MJ, Simões M. Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. Microb Drug Resist 2013; 19: 256–265.