ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Inhibitory activity and degradation of curcumin as Anti-Biofilm Polymicrobial on Catheters

Hasyrul Hamzah[1], Triana Hertiani[*2], Sylvia Utami Tunjung Pratiwi[2], Titik Nuryastuti[3]

[1]Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta- 55281, Indonesia
[2]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta-55281, Indonesia
[3]Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta–55281, Indonesia

Article History:

Received on: 08.08.2019
Revised on: 12.11.2019
Accepted on: 20.11.2019

Keywords:

Biofilms, curcumin, Antibiofilms, catheters, polymicrobial

ABSTRACT

Related biofilms in urinary tract infections and cause deaths per 7500 people. Biofilms are a serious problem and are resolved with antibiotic therapy. Curcumin is a pure composition of the turmeric plant (Curcuma longa Linn.) Which has antimicrobial activity, but the activity of polymicrobial antibiofilm on the catheter has never been launched. The discovery of new antibiofilm candidates for polymicrobial biofilms on catheters is a challenge that must be overcome in preventing infections related to biofilms. This study aims to determine the effectiveness of curcumin in inhibiting and degrading polymicrobial catheters. Biofilm inhibition testing and biofilm degradation testing were determined using the microtiter broth method. The effectiveness of curcumin on biofilms was analyzed by calculating the minimum biofilm inhibitory concentration ($MBIC_{50}$) and the minimum value of biofilm eradication concentration ($MBEC_{50}$). The mechanism of action of curcumin against polymicrobial biofilms on the catheter was tested using *scanning electron microscopy* (SEM). Curcumin 1% gives 60% inhibitory activity to the formation of polymicrobial biofilms on the catheter in the middle phase by 65.05 $\pm$ 0.01 and the maturation phase by 61.23 $\pm$ 0.01, and this is better than the control of the drug nystatin in the middle phase and maturation by 57.58 $\pm$ 0.01 and 56.31 $\pm$ 0.01 and are almost equivalent to the control drug chloramphenicol. Therefore, curcumin is very potential to be developed as a candidate for new antibiofilm drugs against polymicrobial catheters.

*Corresponding Author

Name: Triana Hertiani
Phone: +62811286598
Email: hertiani@ugm.ac.id

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i1.1902

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Biofilms are collections of microbial cells that are irreversibly attached to a surface and are encased in a matrix of Extracellular Polymeric Substances (EPS) that they produce themselves and show phenotypic changes such as changes in growth rates and changes in gene transcription of planktonic cells or free cells (Bjarnsholt, 2013; Donlan, 2002; Donlan and J, 2002; Hamzah *et al.*, 2019; Hertiani *et al.*, 2010; Pratiwi *et al.*, 2015; Römling and Balsalobre, 2012). Biofilms always consist of a mixture of various types of microbes and are very resistant to antibiotics.

It is estimated that at least 80% of human microbial infections involve biofilms (Römling and Balsalobre, 2012), because biofilms are not only efficiently formed and cause infections in human tissue but also in medical devices such as urine catheters, fake heart valves, orthopedic devices, pacemakers, breast implants, and eye contact lenses. Chronic inflammation from biofilm infections can cause cancer, cardiovascular disease, dementia, and other debilitating conditions (Esser *et al.*, 2015; Sari *et al.*, 2019).

Catheter-associated urinary tract infections (CAUTIs or CAUTI) are one type of infection related to health care (*healthcare-associated infection or HAI*), which is most commonly found in hospitals. Urinary catheters are a major factor of CA-UTI, with 70-80% of infections caused by biofilms (Nicolle, 2014).

Materials used were Curcumin compound from the isolation of (*Curcuma longa* Linn.). Curcumin has been used as an essential ingredient in medicine as an anesthetic, anthelmintic, laxative, and as a medicine for liver disease (Goel *et al.*, 2008).

Until now, research on curcumin as an antibiofilm has been limited to mono-species biofilms, but research for polymicrobial biofilms on catheters has never been reported. Therefore this research focuses on the search polymicrobial catheter antibiofilm compound from curcumin compounds.

MATERIALS AND METHODS

Materials Materials used were Curcumin compound from the isolation of (*Curcuma longa* Linn.). Other materials include the following: crystal violet (Merck, Germany), ethyl acetate (Merck, Germany), Brain heart infusion (Oxoid) (Merck, Germany), RPMI 1640 (Sigma-Aldrich), catheter, ethanol 95 % (Merck, Germany), nystatin, chloramphenicol (Sigma-Aldrich, Germany).

Equipment Laminar Air Flow, incubator (IF-2B) (Sakura, Japan), micropipette pipetman (Gilson, France), multichannel micropipette (Socorex, Swiss), microplate flat-bottom polystyrene 24 well (Iwaki, Japan), microtiter plate reader (Optic Ivymen System 2100-C, Spain), spectrophotometry (Genesys 10 UV Scanning, 335903) (Thermo Scientific Spectronic, USA), *autoclave* (Sakura, Japan), *incubator with orbital shaker* S1500 (Stuart, UK), analytical scales (AB204 -5, Switzerland).

Bacterial Strains

A standard strain of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), was cultured in tryptic soy broth (TSB) medium and incubated at 37°C for 72 h. *Candida albicans* (ATCC 10231) were cultured in Sabouraud Dextrose Broth (SDB) medium and incubated at 37°C with agitation at (120 rpm) for 24 h. The optical densities (OD600) of microbial cultures will be adjusted to 0.1 (equal of the 0.5 McFarland standard ~1,5 x 10^8 CFU/ml), and subsequently diluted in fresh medium to OD600 0.01 for each microbial species.

Catheter biofilm inhibition activity

Method of using (Anderson *et al.*, 2003; Hola and Ruzick, 2011; Stepanović *et al.*, 2000) with a little modification. The catheter is cut by one centimeter and then sterilized in 70% ethanol and allowed to dry. A total of 200 μL of media inserted in each microtiter plate well are then incubated at $\pm$ 37ºC for 24 and 48 hours. After the incubation phase, the plate was washed with PBS. 200 uL media contained pure isolate with concentration series (1% b/v - 0125% b/v), was added to all washed-pits. Media that contained ethanol 1% was used as solvent control, and microbe suspension was used as a negative control. A microbe suspension that was used an antifungal and anti-bacterial (chloramphenicol and nystatin 1% b/v) previously was used as a positive control, while a media with no microbial growth was used as media control. The plate was then incubated at 37°C for 24 hours for mid-phase biofilm-forming and 48 hours for the maturing phase. Then, the plate was washed with PBS. Next, 125uL crystal violet 1% solution was added to each pit, next they were incubated at room temperature for 15 minutes. After the incubation, the microplate was washed with PBS and added with 200uL of ethanol 96% in each pit to dilute the formed biofilm. An Optical Density (OD) examination was performed with a microplate reader at 595 nm wavelength.

Catheter biofilm degradation activity

This method is similar to the process of catheter biofilm inhibition activity. Still, the difference is the laying time of the testing, in the degradation of the catheter biofilm for 6 days following the method (Hamzah *et al.*, 2019; Hola and Ruzick, 2011) with a little modification. Biofilm was inoculated inside a microtiter plate in the same manner as explained above. After incubated at 370C for 48 hours, the cultures from each pit were decantated, and planktonic cells were diminished by washing it with PBS. The biofilm cells were exposed by curcumin at several concentrations, started from 1% b/v, and up to 0.125% b/v; later on, they were incubated at 37oC for 48 hours. Chloramphenicol and nystatin 1% b/v were used as a positive control. After incubated, plates were washed three times, with 200 mL of sterile PBS to diminish any attached

cells. Biofilm degradation was quantified by 125 uL of crystal violet 1% solution in each pit, then incubated at room temperature for 15 minutes. After incubation, microplates were washed with PBS, and ethanol 96% was added inside each pit to dilute the biofilm formed. An Optical Density (OD) examination was performed with a microplate reader at 595 nm wavelength.

Scanning electron microscopy

The catheter was inserted inside the microtiter plate round bottom polystyrene 24 well that contained testing suspension that had been given a similar treatment with biofilm inhibition assay. The catheter then incubated on 37 ºC for 24-48 hours, continued to the careful washing of the catheter for three-time with sterile aqua dest, then fixated with 2,5 % (v/v) glutaraldehyde inside cacodylate buffer for ± 24 hours with the aim of cell's death without changing the cell's structure that will be observed. Next, a dehydration process using methanol was done for 30 minutes to minimize the water amount so that the observing process could not be interrupted. The sample then observed under *Scanning Electron Microscopy* (SEM) with a voltage of 10 Kv (Hess *et al.*, 2012; Sofer and Denstedt, 2000).

RESULTS AND DISCUSSION

Curcumin Effect on Mid-phase (24 h) Polymicrobial biofilm on catheter

In this study, we evaluated the potential of curcumin antibiofilm against catheter polymicrobial inhibition of catheters. These results indicate that curcumin can inhibit 50% of polymicrobial biofilm formation on the catheter Figure 1.

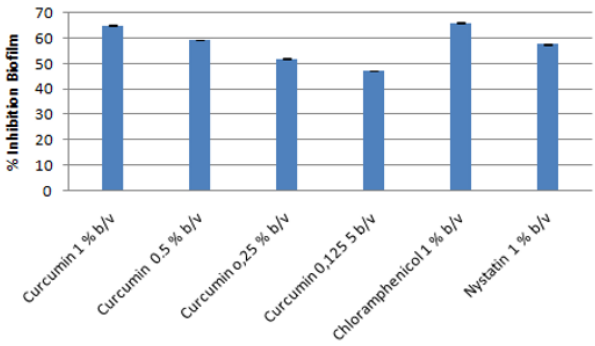

Figure 1: CurcuminEffect on Mid-phase (24 h) Polymicrobial biofilm on the catheter

Inhibition of curcumin against polymicrobial biofilms in the mid-phase catheter was 65.05 ± 0.01 and had almost the same activity as the control drug chloramphenicol 66.05 ± 0.01 and was more effective than the control drug nystatin 57.58 ± 0.01 at the content of 1% b/v. $MBIC_{50}$ activity of curcumin is at the level of 0.25% b/v. This proves that the activity of curcumin compounds can inhibit the formation of biofilms so that biofilms cannot be formed perfectly to make the EPS matrix biofilm structure more complex. The incomplete nutrition between microbial cells in this phase results in the growth of microbes in forming EPS that is not formed perfectly, this results in curcumin compounds capable of damaging the process of microbial travel to form biofilms. These results indicate that the potential curcumin compound to be developed as a candidate for polymicrobial antibiofilm on the catheter. This result is in accordance with the statement (Pratiwi *et al.*, 2015) that the process of inhibition of test compounds against the growth of biofilm phase by inhibiting the attachment of microbes to the surface so that the development of biofilms is disrupted when the development of biofilms is disrupted, this will affect the structure of the biofilm to increase its defense against antimicrobials.

The formation of biofilms also depends on the concentration of nutrients available and regulated by a chemical substance that is released by cells as communication between cells. For example, when living free, *P. aeruginosa* produces low levels of signal molecules. When *P. aeruginosa* cells form biofilms, the concentration of signaling molecules will increase and cause changes in the activity of genes. One of them is a gene that regulates the synthesis of alginates for the formation of extracellular matrix (Costerton *et al.*, 1995).

Curcumin Effect on Maturation-phase (48 h) Polymicrobial biofilm on catheter

Biofilm maturation is another important step in the biofilm life cycle. The development of microcolonies into mature biofilms produces persistent and recalcitrant serious infection. It is therefore recommended that there are agents that interfere with the structure of biofilms having great potential in the control and prevention of infections caused by biofilms (Săndulescu, 2016).

In the ripening phase, the curcumin compound can still provide activity above 50% and is better than the activity given the control of chloramphenicol and nystatin with the same level of 1% b/v. MBIC50 value of curcumin compound in this phase is at 0.5% b/v Figure 2.

These results also reported that there was a decrease in activity given curcumin compounds and drug control in the maturation phase, this is because in the maturation phase the structure of the biofilm structure in the catheter is structured to produce a matrix that is quite complex so that antimicrobial

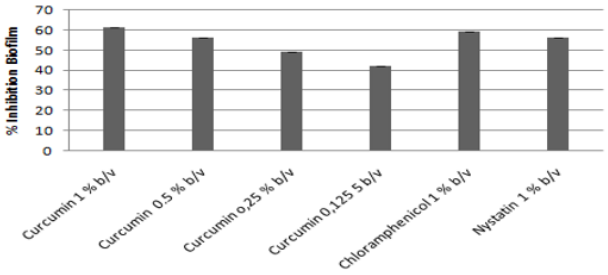

Figure 2: CurcuminEffect on Maturation-phase (48 h) Polymicrobial biofilm on catheter

agents are more difficult to penetrate cells wrapped by biofilm.

These results are consistent with the statement (Kannappan *et al.*, 2017) Biofilms in the maturation phase are more difficult to penetrate than biofilms in the middle phase. The above statement is also in accordance with research (Hamzah *et al.*, 2018), which states that in the maturation phase, antimicrobial agents will have more difficulty penetrating the defenses of biofilms. Inter-species interactions cause colonization and infection dynamics, as well as a number of other responses (Donlan, 2002; Harriott and Noverr, 2011).

Curcumin Effect on degradation- Polymicrobial biofilm on catheter

In biofilms, the degradation phase is long in biofilm growth so that the EPS matrix of biofilms that are formed is thicker and thicker, and the biofilm microbial defenses of antimicrobial compounds are very strong and difficult to penetrate. We report that 1% curcumin compound can degrade biofilms by 50% with inhibitory activity of 51.49 $\pm$ 0.01 and is almost equivalent to the inhibitory activity given by the drug control chloramphenicol and nystatin 1% b/v Figure 3.

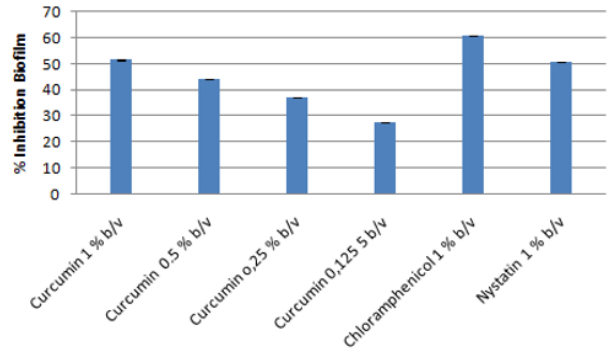

Figure 3: CurcuminEffect on degradation-Polymicrobial biofilm on catheter

Microbes in the degradation phase form a highly structured cell communication with each other. They synergize between microbes in forming biofilms to produce EPS compositions and nutrients that are complete and thick. Matrisk EPS can be seen from microbial biofilm mucus tested at the catheter, where the mucus produced in the degradation phase is very thick and dense. Therefore, antimicrobial compounds are very difficult to destroy biofilms in this phase compared to other phases.

This is consistent with the statement (Costerton *et al.*, 1995) Antibiotic therapy can eliminate or reduce planktonic bacteria, but bacteria in biofilms persist when given antibiotics. When treatment with antibiotics is complete, then biofilms will form more planktonic cells, which result in acute infection.

C. Albicans and *S. aureus* microbes work together to form complex polymicrobial biofilms in serum. Although S. aureus forms a small amount of serum monospecies, *S. aureus* can form polymicrobial biofilms quite significant in the presence of *C. Albicans* (Harriott and Noverr, 2011).

The Result of Scanning Electron Microscopy (SEM) Polymicrobial Biofilm on the catheter with No Treatment

The results of *scanning electron microscopy* (SEM) polymicrobial biofilms on the catheter without administration of the test compound Figure 4A show that the density of the microbes is quite dense, structured, and protected by the EPS matrix. These results indicate that microbes enhance cell communication between them, informing the community and structure of biofilms so that antimicrobial compounds are difficult to penetrate their defenses. The distribution of nutrients in their environment causes more and more powerful biofilms to be formed.

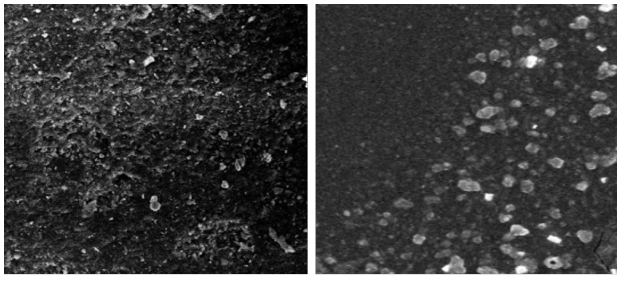

Figure 4: polymicrobial biofilm S.aureus - P. aureginosa - E.coli and C. Albicans on thecatheter is taken using Scanning Electron Microscopy with enlargement x1000. (A) before giving curcumin, (B) after administration of curcumincompounds

According to (Trautner and Darouiche, 2004) about, 20 - 34% of patients will immediately be colonized at the time of catheter placement, when bacteria ascend to the catheter lumen through reflux of urine from contaminated urine sacs (intraluminal route) or through the urethra along the external surface of the catheter urine The risk of bacteremia increases 3-10% every day after catheter placement. The SEM results are also supported from the statement of This finding supports the theory from (Pierce *et al.*, 2017), that stated the biofilm matrix acted as the connecting of the protective binding and cohesive interaction that provided a mechanic stability of the biofilm, controlling cell dispersion from the biofilm, and acted as the nutrition provider for cell communication.

The Result of Scanning Electron Microscopy Polymicrobial Biofilm on the catheter with the administration of curcumin compounds 0,5 % b/v

Our results show that the curcumin compound can damage the polymicrobial biofilm defense of the catheter, this is evidenced by the destruction of the EPS polymicrobial biofilm matrix after administration of 0.5% b/v curcumin compound. In addition, the administration of curcumin is also able to change the morphological structure of the polymicrobial biofilm on the catheter and break the structure of the biofilm cell layer Figure 4B.

This is in accordance with the statement of (Batoni *et al.*, 2016)that one of the biofilm inhibitory mechanisms is by inhibiting and degrading the EPS biofilm matrix, this mechanism causes the microorganisms that form the biofilm not to last longer so that it cannot help the formation of microconsortium with various species of organisms. This result is also strengthened from the statement stated (Burhan, 2017) that the concentration of the test compound affects the biofilm inhibition; the higher the concentration of the test compound, the greater the inhibitory effect given. According to (Gomes *et al.*, 2015) that antibiotics can affect bacteria by means of bactericides, such as inducing morphological changes.

CONCLUSIONS

Curcumin compounds have activity as polymicrobial antibiofilm on the catheter in the middle phase, maturation and degradation. Based on the results of the Scanning Electron Microscopy (SEM), curcumin can damage the polymicrobial EPS biofilm matrix in the catheter. Therefore curcumin is very potential to be developed as a polymicrobial antibiofilm candidate on a catheter.

FUNDING SUPPORT

Authors gratefully acknowledge research funding from UGM through RTA research Grant 2019 Nr. 2127/UN1/DITLIT/DIT-LIT/LT/2019

REFERENCES

Anderson, G. G., Palermo, J. J., Schilling, J. D., Roth, R., Heuser, J., Hultgren, S. J. 2003. Intracellular bacterial biofilm-like pods in urinary tract infections. *Science*, 301:105–107.

Batoni, G., Maisetta, G., Esin, S. 2016. Antimicrobial peptides and their interaction with biofilms of medically relevant bacteria. *Biochim. Biophys. Acta BBA - Biomembr*, 1858.

Bjarnsholt, T. 2013. The role of bacterial biofilms in chronic infections.APMIS. *APMIS*. Suppl. 1-51.

Burhan, F. 2017. Mekanisme Aksi AntiJamur Minyak Masoyi Terhadap Membran Sel Candida Albicans. *Yogyak. Univ. Gadjah Mada*.

Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R., Lappin-Scott, H. M. 1995. Microbial biofilms. *Annu. Rev. Microbiol*, 49:711–745.

Donlan, R. M. 2002. Biofilms: Microbial Life on Surfaces.Emerg. Infect. Dis. . *Emerg. Infect. Dis.* , 8:881–890.

Donlan, R. M., J, C. 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev*, 15(2):167–193.

Esser, N., Paquot, N., Scheen, A. J. 2015. Anti-inflammatory agents to treat or prevent type 2 diabetes, metabolic syndrome and cardiovascular disease. *Expert Opin. Investig. Drugs*, 24:283–307.

Goel, A., Kunnumakkara, A. B., Aggarwal, B. B. 2008. Curcumin as "Curcumin": from the kitchen to the clinic. *Biochem. Pharmacol*, 75:787–809.

Gomes, L. C., Silva, L. N., Simões, M., Melo, L. F., Mergulhão, F. J. 2015. Escherichia coli adhesion, biofilm development, and antibiotic susceptibility on biomedical materials. *J. Biomed. Mater. Res. A*, 103:1414–1423.

Hamzah, H., Hertiani, T., Pratiwi, S. U. T., Nuryastuti, T. 2019. The Inhibition Activity of Tannin on the Formation of Mono-Species and Polymicrobial Biofilm Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Candida albicans. *Majalah Obat Tradisional*, 24(2):110–110.

Hamzah, H., Pratiwi, S. U. T., Hertiani, T. 2018. Efficacy of Thymol and Eugenol Against Polymicrobial Biofilm. *Efficacy of Thymol and Eugenol Against Polymicrobial Biofilm. Indonesian Journal of Pharmacy*, 29(4).

Harriott, M. M., Noverr, M. C. 2011. Importance of Candida-bacterial polymicrobial biofilms in disease. *Trends Microbiol*, 19:557–563.

Hertiani, T., Edrada-Ebel, R., Ortlepp, S., Soest, R. W. M. V., Voogd, N. J. D., Wray, V., Hentschel, U., Kozytska, S., Müller, W. E. G., Proksch, P. 2010. From anti-fouling to biofilm inhibition: New cytotoxic secondary metabolites from two Indonesian Agelas sponges. *Bioorg. Med. Chem*, 18:1297–1311.

Hess, D. J., Henry-Stanley, M. J., Barnes, A. M. T., Dunny, G. M., Wells, C. L. 2012. Ultrastructure of a Novel Bacterial Form Located in Staphylococcus aureus In Vitro and In Vivo Catheter-Associated Biofilms. *J. Histochem. Cytochem*, 60:770–776.

Hola, V., Ruzick, F. 2011. The Formation of Poly-Microbial Biofilms on Urinary Catheters. *Urinary Tract Infections*.

Kannappan, A., Sivaranjani, M., Srinivasan, R., Rathna, J., Pandian, S. K., Ravi, A. V. 2017. Inhibitory efficacy of geraniol on biofilm formation and development of adaptive resistance in Staphylococcus epidermidis RP62A. *J. Med. Microbiol*, 66:1506–1515.

Nicolle, L. E. 2014. Catheter-associated urinary tract infections.Antimicrob. Resist. Infect. Control. *Antimicrob. Resist. Infect. Control*, 3.

Pierce, C. G., Vila, T., Romo, J. A., Montelongo-Jauregui, D., Wall, G., Ramasubramanian, A., Lopez-Ribot, J. L. 2017. The Candida albicans Biofilm Matrix: Composition, Structure and Function. *J. Fungi Basel Switz*.

Pratiwi, S. U. T., Lagendijk, E. L., Hertiani, T., Weert, S., De, M., Hondel, C. A., Den, J. J. V. 2015. Antimicrobial effects of Indonesian medicinal plants extracts on planktonic and biofilm growth of pseudomonas aeruginosa and staphylococcus aureus. *SE-Original Article(s)*, 7:183–191.

Römling, U., Balsalobre, C. 2012. Biofilm infections, their resilience to therapy and innovative treatment strategies. *J. Intern. Med*, 272:541–561.

Săndulescu, O. 2016. Managing sticky situations – anti-biofilm agents. *Managing sticky situations - anti-biofilm agents. Germs 6, 49*.

Sari, S., Andayani, T. M., Endarti, D., Widayati, K. 2019. Health-related quality of life in non-small cell lung cancer (NSCLC) patients with epidermal growth factor receptor (EGFR) mutation with tyrosine kinase inhibitors treatment: a systematic review. *Int. J. Res. Pharm. Sci*, 10:2898–2906.

Sofer, M., Denstedt 2000. The encrustation of biomaterials in the urinary tract. *Curr Opin Urol*, 10(6):563–572.

Stepanović, S., Vuković, D., Dakić, I., Savić, B., Švabić Vlahović, M. 2000. Methods 40.J. Microbiol. Methods. *J. Microbiol. Methods*, 40:122–128.

Trautner, B. W., Darouiche, R. O. 2004. Role of biofilm in catheter-associated urinary tract infection☆. *Am. J. Infect. Control*, 32:177–183.