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Microbial resistance to most commonly antibiotics is increasing and therefore requiring continuous development of new anti-microbial agents. Moreover, recent research has revealed that microbial biofilms causing elevated resistance to both most anti-microbial drugs and the host defense systems, which often results in persistent and difficult-to-treat infections. The discovery of anti-infective agents which are active against planktonic and biofilm microorganisms are therefore urgently required to deal with these biofilm-mediated infections.

Recently, scientists have focused on the anti-pathogenic potential of natural products. Plants are an interesting and important source for finding novel anti-biofilm compounds. They are a rich source of new molecules with pharmacological properties for the development of new drugs. Indonesia is one of the countries which has a very diverse flora and a rich tradition in the use of medicinal plants. Since several Indonesian medicinal plants contain anti-microbial compounds it was considered conceivable that they might also be a source of new anti-biofilm compounds. Therefore the research present in this thesis has been focused on the screening and identification of mixtures with anti-microbial and anti-biofilm activity, which may contain candidate compounds for developing new anti-biofilm drugs.

In Chapter 1 a description is given about microbial biofilms which colonize polymer surfaces and forms a multilayered cell cluster. The biofilms are difficult to control due to their recurrence and high inherent resistance to anti-microbial agents and host immune responses. Quorum sensing, a cell-to-cell communication between microorganisms, is responsible for biofilm formation by microorganisms and plays an important key in microbial pathogenicity and antibiotic resistance. Infections due to the presence of microbial biofilms are major clinical concerns since they have the ability to adhere to and colonize surfaces of medical devices like e.g. implants. In many cases, this device has to be removed in order to cure the infections. These medical issues can be prevented if novel anti-microbials with high activity against microbial biofilms are discovered and identified. Medicinal plants are considered as an interesting source for novel anti-biofilm compounds. Plant-derived compounds have gained widespread interest in the search of drugs from natural sources. The compounds are widely accepted because of the perception that they are safe and have a long
history of use in folk medicine or treatment and prevention of the diseases and infections. In this chapter, we also discussed *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* as model microorganisms which are capable to form biofilm, and their pathogenicity.

**Chapter 2** describes the screening of 54 ethanol extracts of different Indonesian medicinal plants for the presence of anti-microbial and anti-biofilm compounds against *P. aeruginosa* PAO1 and *Staphylococcus aureus* Cowan I. The extracts showed an inhibitory effect on planktonic grown bacteria but even more interestingly also on the formation of biofilm structures. At a concentration as low as 0.12 mg/mL, biofilm formation of *P. aeruginosa* PAO1 and *S. aureus* Cowan I is inhibited by 5 plant ethanol extracts: *Kaempferia rotunda* L., *Caesalpinia sappan* L., *Cinnamomum burmanii* Nees ex Bl., *C. sintoc* L., and *Nymphaea nouchali* Burm.f. The same extracts showed activity in degradation of the established biofilm of bacterial strains tested with higher concentration. Limited bacteriostatic activity was evident. This study demonstrated the effectiveness of *Kaempferia rotunda* L., *C. sappan* L., *C. burmanii* Nees ex Bl., *C. sintoc* L., and *N. a nouchali* Burm.f extract towards *P. aeruginosa* PAO1 and *S. aureus* Cowan I biofilm. This property can be applied clinically to treat infectious biofilm along with conventional antibiotics, or applied industrially e.g. to remove biofilms from water pipes (Kim and Park, 2013).

In **Chapter 3 and 4** we report screening for anti-biofilm compounds in 29 essential oils from Indonesian medicinal plants against *P. aeruginosa* PAO1, *S. aureus* Cowan I, and towards *C. albicans* ATCC 10231 biofilms. *Candida* cells, like many other microorganisms, are able to adhere to and colonize surfaces of medical devices, resulting in development of a biofilm. The anti-biofilm activity of essential oils was confirmed by confocal laser scanning microscope analysis, along with LIVE/DEAD staining for the monitoring of live/dead cells. Essential oil from *C. burmanii* and *M. aromatica* showed a 50% inhibition of *P. aeruginosa* PAO1 and *S. aureus* Cowan I planktonic growth (PMIC50) at concentration of 0.25 % v/v. Essential oil from *C. burmanii* and *M. aromatica* have been demonstrated to exhibit 50% (MBIC50) of *P. aeruginosa* PAO1 and *S. aureus* Cowan I biofilm formation at concentration of 0.03 % v/v, whereas higher concentration (0.12 % v/v) was needed by both oils to disrupt 50% of *P. aeruginosa* PAO1 and *S. aureus* Cowan I established biofilms.

The essential oil of *C. burmanii*, *M. aromatica*, *O. basillicum* and *L. cubeba* (seeds part) showed an evident antifungal activity against planktonic growth of *C. albicans* and inhibited the formation of *C. albicans* biofilm at three different stages of development at a sub-PMIC concentration. The initial biofilm formation inhibition by plant essential oils was found to be concentration dependent. In the
presence of the essential oils, a significant decrease of biofilm biomass compared to negative control (biofilm cells without addition of plant essential oil) was evident. We also analyzed major components contained in Cinnamomum oil and Massoia oil by GC-MS. Cinnamic aldehyde (92.02 %) was found to be the major component of C. burmanii essential oil, and massoia lactone (92.05 %) is the main constituent of M. aromatica essential oil. The results obtained in this study indicate that the oil of C. burmanii and M. aromatica is an interesting source for anti-biofilm agents in the development of new strategies to treat infections caused by P. aeruginosa, S. aureus and C. albicans biofilm.

Chapter 5 reported the screening of the 54 ethanol extracts of Indonesian medicinal plants and 29 essential oils for their capability in inhibiting microbial quorum sensing mechanism using quorum sensing biosensor Chromobacterium violaceum ATCC 31532 (wild-type strain) and C. violaceum CV026 mutants strain. We also investigated the effect of the extracts and essential oils on the motility of pathogen Pseudomonas aeruginosa PAO1 strain, since bacterial motility has been shown to be associated with its virulence. Of the 54 plant extracts and 29 plants essential oils screened, Nymphaea nouchali ethanol extract, Syzygium aromaticum essential oil and Massoia aromatica essential oils demonstrated varying level of inhibition in violacein production of the reporter strains. A significant reduction in quorum sensing related motility of P. aeruginosa PAO1 has also been observed compare to the control. These plants extracts and essential oils may be selected for activity guided fractionation to identify and characterize the active principle.

Based on research described in Chapter 3, 4 and 5 which showed the activity of M. aromatica essential oil to inhibit biofilm formation of P. aeruginosa, S. aureus and C. albicans, and also violacein production of C. violaceum and P. aeruginosa motility related quorum sensing activity, the isolation of the active compound from this plant sample has been described in Chapter 6. Preparative thin layer chromatography along with GC-MS and ¹H-NMR elucidation were used to isolate and identify the active compound. Result from bioautography analysis revealed that massoia lactone (synonym: 5-Hydroxy-2-Decenoic Acid Lactone; 2H-Pyrane-2-one, 5,6-dihydro-6-pentyl-) is the active compound of massoia oil. The microtiter broth method was performed to detect the antifungal and antibacterial activity, as well as anti-biofilm activity of massoia lactone, alone and in combinations with antibacterial and antifungal agents. Massoia lactone found to exhibit anti-biofilm activity against C. albicans ATCC 10231 at concentration of 25 µg/mL. Biofilm formation of P. aeruginosa PAO1 and S. aureus Cowan I are also partially hindered by massoia lactone at concentration of 50 and 100 µg/mL. The anti-biofilm activity of massoia lactone also found to be
three to four times greater in combinations with antifungal/antibacterial drug. We performed initial toxicity study of massoia lactone by hemolysis assay using human red blood cells. At the highest concentration tested (100 µg/mL), massoia lactone showed 12.4±0.9 % human red blood cells hemolysis compare to Triton X-100 which gave 100% hemolysis. This result indicates that at the range concentration tested, massoia lactone is less toxic to the human erythrocytes. The result obtained in this thesis indicated that massoia lactone displayed potent activity against microbial biofilms in vitro and therefore has potential therapeutic implication for biofilm-associated microbial infections.

In Chapter 7 a brief discussion is given about the results described in this thesis