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Anti-biofilm Potential and Mode of Action of Malaysian Plant Species: A Review

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ABSTRACT

Biofilm is a microbial community that attaches to a surface and is enclosed in extracellular polymeric substance (EPS) matrix. Formation of biofilm often develops resistance towards a wide spectrum of antimicrobial agents. Since the biofilm-mediated diseases are commonly difficult to treat, there is a need to find new antibiofilm agent. The studies on antibiofilm activities of plant species have received a great deal of attention over the last few decades. In Malaysia, plant species have been used as alternatives to the conventional antimicrobial therapy. Several Malaysian plant species are known to control biofilm infection by inhibition of quorum sensing pathway, disruption of EPS matrix, alteration of cell permeability and reduction in cell surface hydrophobicity. This review demonstrates that Malaysian plant species may become excellent therapeutic agents in combating the biofilm infection.

Keywords: *Malaysian plant species; Antibiofilm activity; Biofilm; Extracellular polymeric substance*

INTRODUCTION

Bacterial cells undergo two types of growth mode which are planktonic cell and sessile aggregate or biofilms [1]. The first discovery of microbial biofilms was done by Van Leeuwenhoek as he used simple microscopes to observe microorganisms on tooth surface and found out that there are biofilms [2]. Biofilm is an association of microbes in which they stick together on a surface and are enclosed in a matrix typically by an extracellular polymeric substance (EPS) produced by bacteria [3]. In the biofilm matrix, non-cellular materials such as mineral crystals, corrosion particles, clay or blood components can be found depending on the environment [4]. Biofilm can form in natural, medical and industrial settings which can give impact towards humans in many ways [5]. It can be found in both living and non-living surfaces [6].

The formation of biofilm often develops resistance towards antibiotic [7, 8]. To overcome this, various plant extracts have been tested to control the biofilm formation [9]. Plant based compounds have greater potential to be developed into new drugs and being used effectively to treat biofilm associated infections due to the perception that these drugs are safe, have less side effects and are easily available. This is proven

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by an estimation of four billion people that represent 80% of the world's population rely on herbal medicinal products as a primary source of healthcare and traditional medical practice [10]. Plant product represents an example of natural antibiofilm agents [11]. To date, the benefits of Malaysian plant species as antibiofilm agents are not well documented. Therefore, this review aimed to highlight the antibiofilm potential of various Malaysian plants species.

Biofilm

Biofilm formation is known as one of the leading causes of multidrug resistance developing bacteria. The biofilm life cycle consists of four stages, the earlier attachment of bacteria, the development of microbial colonies, bacterial growth and the generation of extracellular matrix and biofilm mature as the latest phase, followed by the dispersal of bacteria to find new niches. Biofilm begins from the initial attachment of the bacteria and then grows into a permanent attachment [12]. During these two periods, extracellular DNA, proteases, cell surface proteins or biofilm-associated proteins are involved in biofilm initiation. The substratum surface has a host polymer matrix, which consists mainly of exopolysaccharides, proteins, nucleic acids, and other substances, facilitating irreversible bacterial attachment. Conrady *et al*. [12] reported that cell surface-associated proteins such as Aap and SasG were involved in the initiating attachment of *Staphylococcus epidermidis*, and Aap protein contains G5 domain, which was responsible for bacterial intercellular cell adhesion.

Decrease in oxygen concentrations in the biofilm can result in greater programmable cell lysis (PCL) and increase in biofilm formation by *S. aureus* [13]. This development was due to SrrAB and SaeRS-dependent upregulation of AtlA murein hydrolase, accompanied by release of cytosolic DNA [14]. In addition, several genome-wide biofilm formation studies have been conducted and some genes associated with the biofilm formation have been identified, such as ClpYQ protease genes and purine biosynthesis genes [15]. After the biofilm has stabilized, the microbes can exit the biofilm and create a new connection, leading to a new biofilm existence.

Extracellular elements, including surface-exposed protein, extracellular glucan-binding protein and glycosyltransferases (GtfE, GtfG and GtfH), also play a critical role in the ability of cell adhesion [16]. Sortase A (SrtA), a transpeptidase that can attach proteins on the cell surface, often induces extracellular localization and the formation of biofilms in Gram-positive bacteria such as *Staphylococcus aureus* [17]. Inhibitors that target microbial adhesion processes have been widely developed and could potentiate good antibiofilm and antimicrobial activities [18]. Adhesive bacteria proliferate into micro colonies. As the formation of biofilms matured, complex matrix architecture is formed with water channels for nutrient flow and waste efflux [19]. According to Chung and Toh [20], extracellular matrix contains DNA, carbohydrates, proteins, TapA, fibrous protein TasA and exopolysaccharide, which are important components for biofilm development while spermidine is also required to enable the expression of these matrix components. Chemical composition of biofilm extracellular matrix is often determined by Fourier-transform infrared spectroscopy (FTIR) [21].

Antibiofilm potential of Malaysian plant species

There are about 2000 medicinal plant species are reported to possess health benefits in Malaysia [22]. The Malaysian plant species includes leaves, fruits, stems, roots and isolated compounds are extensively researched to eradicate biofilm since biofilm formation has become major issues for pharmaceutical

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industry in formulating new drugs. Thus, researchers continuously investigate about the impact of plant species against biofilm since they contain diverse nutritive values and bioactive compounds. Table 1 shows a summary of the antibiofilm potential of selected plant species found in Malaysia.

Table 1: Malaysian plant based anti-biofilm candidates.

Plant species (local name)	Part used	Type of plant extract	Pathogen used	Mode of action	References
Zingiber officinalis (Halia)	Rhizome	Ethanol	*Escherichia coli* *Salmonella typhimurium* *Pseudomonas aeruginosa* *Staphylococcus aureus* *Bacillus subtilis* *Listeria monocytogenes*	Disruption of biofilm membrane structure	[23]
		Ethanol	*Pseudomonas aeruginosa*	Disruption of extracellular DNA	[24]
		Oil	*Enterococcus faecalis*	Inhibition of cell aggregation	[25]
		Toluene	*Chromobacterium violaceum* *Pseudomonas aeruginosa*	Inhibition of quorum sensing	[26]
		Toluene	*Candida albicans*	Inhibition of cell aggregation	[27]
		Oil	*Bacillus cereus* *Staphylococcus* *Escherichia coli* *Pseudomonas aeruginosa* *Candida albicans* *Cyptococcus neoformans*	Inhibition of biofilm growth	[28]
Allium sativum (Bawang putih)	Bulb	Aqueous	*Candida albicans*	Suppression of gene expression	[29]
		Ethanol	*Candida albicans* *Candida tropicalis* *Candida krusei*	Inhibition of hyphae	[30]
		Methanol and aqueous	*Escherichia coli* *Staphylococcus aureus*	Reduction in biofilm turbidity	[31]
Allium stipitatum (Bawang putih)	Bulb	Hexane (ASHE) and dichloromethane (ASDE)	*Methicillin-sensitive Stahpylococcus aureus (MSSA),* *Methicillin-reisitance Stahpylococcus aureus (MRSA),* *Acinetobacter baumanni,*	Disrupt the matured biofilm of multi drug resistance pathogens.	[32]

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			Stenotrophomonas maltophilia		
Melastoma malabathricum (Senduduk)	Stem bark	Acetone	*Streptococcus mutans*	Suppression of *gbp*A, *brp*A, *gtf*C, and *com*DE inhibit biofilm formation	[33]
		Acetone	*Streptococcus mutans*	Disruption of biofilm membrane structure	[34]
Piper betle (Sireh)	Leaf	Aqueous	*Streptococcus mutans*	Inhibition of biofilm growth	[35]
		Ethyl acetate	*Vibrio harveyi*	Bioluminescence inhibition, initial biofilm disruption, EPS inhibition, anti-swimming efficacies	[36]
		Aqueous	*Serratia marcescens* *Proteus merabilis*	Inhibition of quorum sensing pathway	[37]
		Ethyl acetate	*Serratia marcescens*	Inhibition of quorum sensing	[38]
Andrographis paniculata (Hempedu bumi)	Stem Leaf	Aqueous Ethanol	*Staphylococcus aureus* *Pseudomonas aeruginosa*	Interference of quorum sensing	[39]
Orthosiphon stamineus (Misai kucing)	Stem Leaf	Ethanol and aqueous	*Pseudomonas aeruginosa* *Staphylococcus aureus*	Inhibition of quorum sensing	[39]
	Whole plant	Methanol and aqueous	*Staphylococcus aureus* *methicilin resistant* *Stahpylococcus aureus (MRSA) Norefrina*	Reduction in turbidity	[40]
Phaleria macrocarpa (Mahkota dewa)	Fruit Leaf Stem	Ethyl acetate Ethanol Ethyl acetate Ethyl acetate Methanol	*Streptococcus mutans*	Inhibition of bacterial adhesion by altering the cell charges and cell permeability through interaction with protein, enzyme and lipid on microbial membrane.	[41]
Mangifera indica (Mangga)	Leaf	Aqueous Ethyl acetate	*Streptococcus sanguinis* *Streptococcus mutans*	Inhibition of cell adherence by reducing hydrophobicity of cell surface	[42]
Hibiscus tiliaceus (Bebaru)	Leaves, fruits	Methanol	*Pseudomonas aeruginosa*	Disruption of extracellular polymeric	[43]

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	and twigs			substances (EPS) matrix and quorum sensing (QS) mechanism	
Dioscorea hispida (Ubi Gadong)	Starch	Sodium hydroxide	*Escherichia coli* *Staphylococcus aureus* *Pseudomonas aeruginosa* *Klebsiella pneumoniae* *Bacillus subtilis*	Disruption of quorum sensing (QS) mechanism	[44]
Euphorbia hirta (Ara Tanah)	Aerial part	Methanol	*Pseudomonas aeruginosa*	Inhibition of quorum sensing (QS) activity	[45]
Chromolaena odorata (Pokok kapal terbang)	Leaves	Ethanol	*Pseudomonas aeruginosa*	Disruption of extracellular polymeric substances (EPS) matrix	[46]

Common mechanism of antibiofilm action

There are several potential antibiofilm mechanisms including inhibition of c-di-GMP signaling system, inhibition of urease activity, suppression of gene and protein expression, reduction of polysaccharides, inhibition of quorum sensing, inhibition of curli and pili biosynthesis, inhibition of cell adherence and reduction in biofilm biomass.

Inhibition of c-di-GMP signaling system

3',5'-cyclic diguanylic acid (c-di-GMP) is a second messenger used for signal transduction by bacteria and plays a role in biofilm formation [47]. Interruption of signaling pathway of c-di-GMP in bacteria causes alteration in biofilm formation. Synthesis of c-di-GMP is mediated by the activity of diguanylate cyclase (DGC). Small molecules such as LP 3134, LP 3145, LP 4010 and LP 1062 inhibit DGC which then inhibit c-di-GMP production and hence inhibit biofilm formation in *Pseudomonas aeruginosa* [48]. C-d-GMP is produced by GGDEF domains protein and degraded by EAL domain protein or HD-GYP domain protein. A study by Kim and Park [49] reported that exposure to 1% ginger extract resulted in decrease of c-di-GMP levels that suspended biomass and biofilm formation when compared with untreated biofilm with 61% and 84% respectively (Figure 1).

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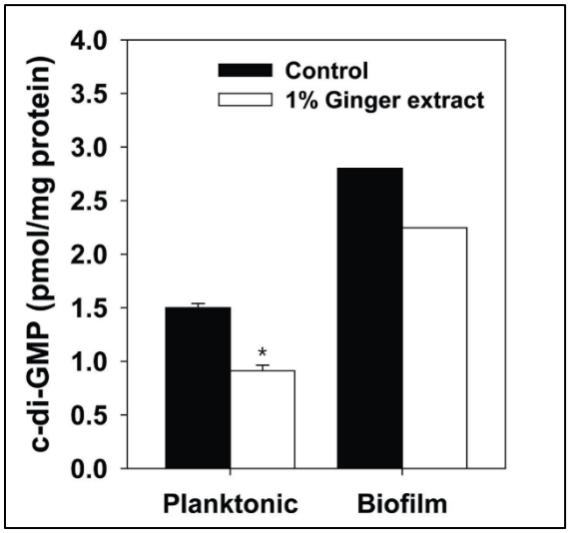

Figure 1: Concentration of c-di-GMP of planktonic and biofilm cells grown in the presence and absence of 1% ginger extract [49].

Inhibition of urease activity

Urease or also known as urea amidohydrolase is an enzyme produced by all clinical strains of the bacteria that hydrolyze urea resulting in production of ammonia and carbamate. Under an aquatic condition, the carbamate hydrolyzes into other molecules of ammonia with carbonic acid. The presence of ammonia causes an elevation in pH which makes urine become more alkaline. This causes the presence of inorganic ions in precipitate form that will be accumulated in urine which later causes crystalline bacterial biofilm development in urinary tract [50]. Due to that, antibiofilm agents from plant species such as allicin from garlic may help in solving this problem. It was reported that allicin was able to diffuse in the biofilm membrane of *Proteus mirabilis* and inhibit the urease activity [51]. As the concentration of allicin increased, the urease activity decreased as shown in Figure 2. The inhibition of urease activity may prevent the formation of biofilm. Therefore, allicin can be considered as an effective urease inhibitor.

Suppression of Gene and Protein Expression

Gene and protein expression may vary during the development of biofilm and could be suppressed by the presence of antibiofilm substances. The relative expression of genes related to virulence, toxin and efflux pump in biofilm cells is often distinct from that of free-floating cells. Eugenol, a major constituent of essential oils extracted from various plants, was able to suppress expression of biofilm- and quorum sensing-related genes (gtfB, gtfC, comDE, smu630, vicR, brpA, ftf, relA, gbpB and spaP) in *Streptococcus mutans* [52]. On the other hand, inhibition of *Salmonella typhimurium* biofilm is mediated by the suppression of many essential proteins such as outer membrane protein A, virulence transcriptional regulatory protein, trigger factor, flagellin and ABC transporter permease [53, 54].

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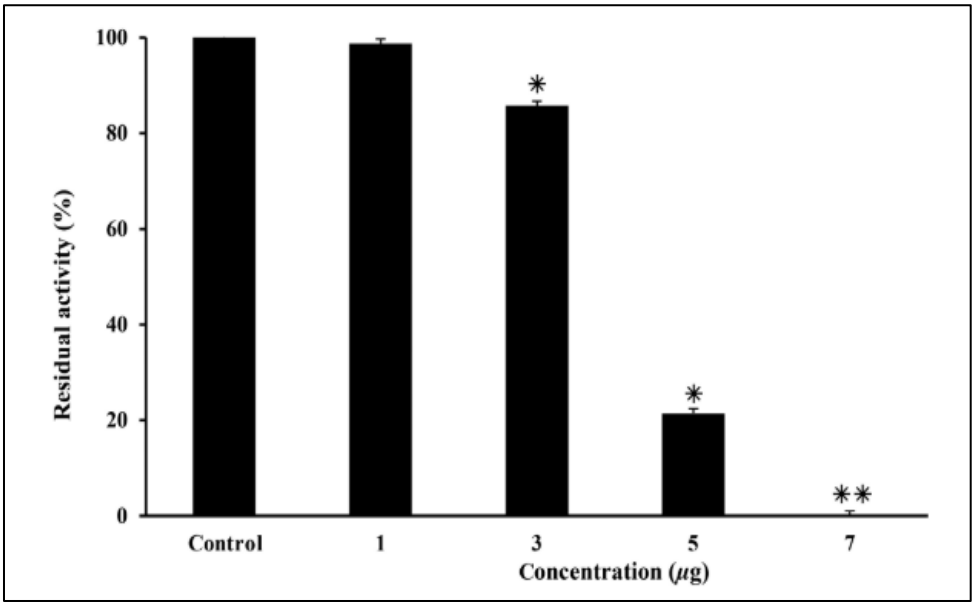

Figure 2: Inhibition of urease activity in bacterial cell using allicin [51].

Reduction in Polysaccharide

Among the components of the extracellular matrix, polysaccharide plays a major role in biofilm formation. It provide many diverse benefits to the cells in the biofilm structure, adhesion to surface and protection against a wide range of stresses, such as desiccation, immune effectors, and predators such as phagocytic cells and amoebae. The fresh extract from *Allium sattivum* (garlic) was proved to inhibit the biofilm activity in *Escherichia coli* through FTIR analysis [55]. The biofilm attachment was inhibited by the garlic through the reduction of carbohydrate content in the biofilm. Moreover, according to Huang and Stewart [56], the total polysaccharide content in the biofilm treated with bismuth dimercaprol (BisBAL) was lowered as compared to the control.

Quorum Sensing Inhibition

Quorum sensing (QS) is the regulation of gene expression in response to fluctuations in cell-population density. Bacterial quorum sensing produces and releases chemical signal molecules called autoinducers that increase in concentration as a function of biofilm cell density. Inactivation of the pathogen's QS mechanism may result in a significant decrease in the output of the virulence factor [57]. The QS cycle may be disturbed by a number of mechanisms: (i) reducing the function of N-Acyl homoserine lactone (AHL) cognate receptor protein or AHL synthesis; (ii) inhibiting the generation of QS signal molecules; (iii) degradation of AHL; and (iv) The imitation of primary signal molecules by the use of chemical compounds as analogs of signal molecules (AHLs) has been most appreciated and applied. The detection of QS-related pathogenicity is one of the most important prerequisites for circumventing it [58].

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Inhibition of Curli and Pilli Biosynthesis

Curli are active extracellular amyloid fibres formed by uropathogenic *Escherichia coli* (UPEC) and other Enterobacteriaceae. They serve to promote adhesion to surface, cell aggregation, biofilm formation and host cell invasion. Curli gene expression is known to be responsive to many environmental factors such as temperature, nutrient limitation and oxygen tension. Ring-fused 2-pyridones, such as FN075 and BibC6, blocks UPEC curli biogenesis and stops the *in vitro* polymerization of the main CsgA curli subunit [59]. Curlicides FN075 and BibC6 share a common molecular heritage with other ring-fused 2-pyridones referred to as pilicides. Curlicides maintains pilicide activity and inhibits both curli-dependent and type 1 pili-dependent biofilms. Curli and Type 1 pili demonstrated exclusive and distinct functions in the promotion of UPEC biofilms. Thus, the ability of FN075 to block the biogenesis of both curli and Type 1 pili endows it with unique antibiofilm and antivirulence activities. Figure 3 shows the chemical structures that are responsible for curli inhibition and the representative high-resolution EM images of UTI89 prepared.

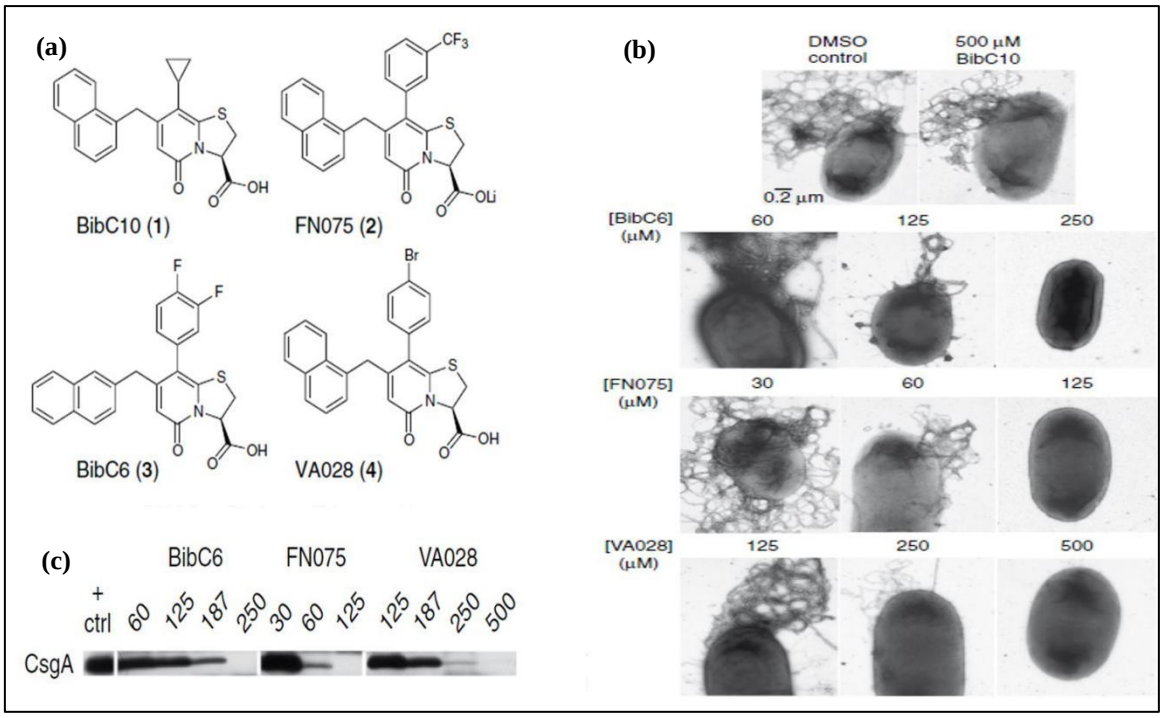

Figure 3: (a) BibC10 (1), FN075 (2), BibC6 (3) and VA028 (4) are ring-fused 2-pyridones that differ in their phenyl ring modifications (b) Representative high-resolution EM images of UTI89 prepared as in (c) Titratable reductions in bacterial curliation were observed for cells grown in the presence of curlicides. The scale bar in the first electron micrograph represents 0.2 µm and applies to all images [59].

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Inhibition of cell adherence

Cell adherence is dependent on cell membrane and is often influenced by temperature, surface hydrophobicity and medium composition. There is also a situation where extracellular molecules of a bacterial species increase the adherence of another bacterial species. Some of antimicrobial peptide kill the bacteria by direct interaction with nuclei acids without causing permeabilization of cell membrane such as Buforin II [60]. Cao *et al*. [61] used macrocyclic peptides (cyclotides) derived from plants such as Violaceae, Rubiaceae, and Cucurbitaceae families to modify stainless steel surfaces and demonstrated inhibiton of bacterial adherence to metal surfaces. Figure 4 shows the inhibited cell adherence that result in impaired biofilm formation.

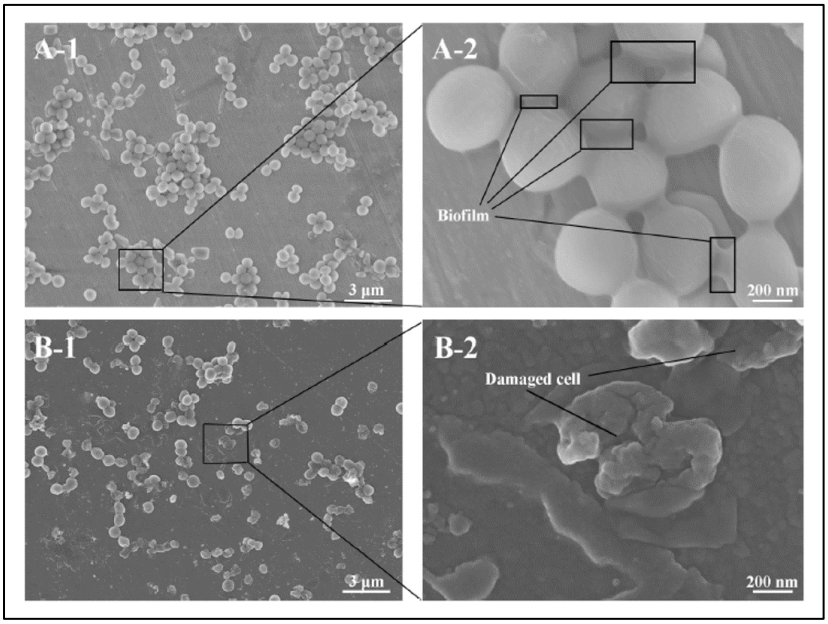

Figure 4: Field emission scanning electron microscopy (FESEM) images of bacterial Adherence of untreated (A) and cyclotides (Viphi G) treated samples (B) [61].

Reduction in biofilm biomass

An increase in biofilm biomass may dilute the metabolically active cells which lead to a decrease in metabolic activity. This is because the total biofilm biomass often contains high proportion of extracellular matrix which can diminish the concentration of metabolically active cells, weaken calorimetric signal and cause lower level of metabolic activities. Therefore, metabolic assay may not be suitable for quantifying biofilm biomass. According to [62], the growth of *P. aeruginosa* biofilm in the presence of herbs extract of *H. patriniae* was lower than that without the extract at irreversible attachment stages and mature stages (Figure 5). The reduction in biofilm biomass by *H. patriniae* extract was due to inhibition of the genes associated with biofilm formation namely *algU, pslM, pelA, algA, ppyR,* and *bdlA*. On the other hand, a decrease in *P. aeruginosa* biofilm biomass following treatment with *C. odorata* extracts [46] is associated with differental proteome expression [63].

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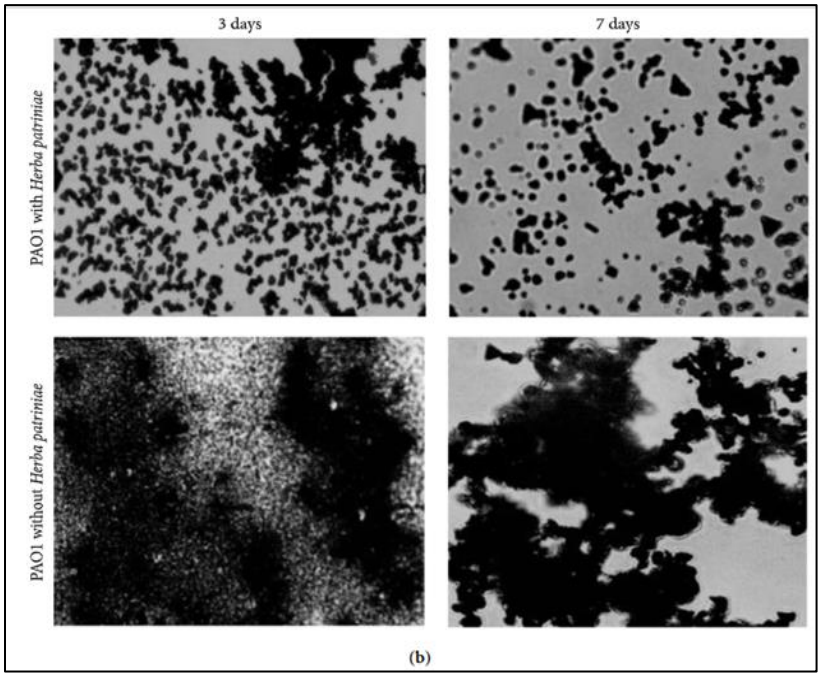

Figure 5: Micrographs of biofilms formed with and without *H. patriniae* [62].

CONCLUSION

As a conclusion, a large group of Malaysian plant species possess promising antibiofilm potential. Their mechanism of action against biofilms have also been elucidated. This review suggests that further research need to be performed on these plant species to control a wide range of biofilm-mediated infections**.**

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