A current perspective on antibacterial and antibiofilm properties of waru (Hibiscus tiliaceus L.)

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Abstract. During the long course of evolution, disease-causing pathogenic bacteria have developed a variety of virulence mechanisms that help them establish and maintain infections. Among these mechanisms are the production of biofilm protecting the bacteria from undesirable environmental conditions and contributing to the development of new multi-drug-resistance bacterial strains. Hence, biofilm has become an attracted target for the development of a novel strategy in fighting against biofilm-forming pathogens. There has been much work to look for novel antibacterial and antibiofilm agents, including the use of plant-derived materials. Hibiscus tiliaceus is one of the widely known medicinal plant with antibacterial and antibiofilm properties. This review focuses on antibacterial and antibiofilm properties of H. tiliaceus as well as the major phytochemical constituents that might contribute to these activities.

1. Introduction
Coping with bacterial infections and pathogenicity has become increasingly more difficult due to the widespread occurrences of multi antibiotic-resistant bacteria [1, 2]. This resistance is often related to their ability to produce many extracellular compounds known as virulence factors, which are crucial for the bacterial competence to establish and maintain infections [3, 4]. Biofilm formation is one of the virulence factors that protect bacteria from host defense and provide increased resistance to antibiotics [5, 6]. The discovery that many pathogenic bacteria possess the ability to form biofilm has made biofilm as an attractive target for the design and development of new therapeutic strategies [7]. Efforts in search for novel therapeutic agents that could facilitate the eradication and attenuation of bacteria and bacterial biofilm, including anti-biofilm potential compounds, has been extensively conducted. Natural sources, such as medicinal plants, emerged as potential candidates. Several studies regarding the use of natural products to fight against pathogenic bacteria have increasingly been carried out in the last decade [8, 3]. Hibiscus tiliaceus is one of widely known medicinal plants whose chemical substances have been proved to possess excellent antimicrobial activities. Furthermore, recent studies have also indicated its potential as biofilm formation antagonist [9]; however, antibiofilm activities of H. tiliaceus has not been well documented. This review aims to give a brief account of the research reports on H. tiliaceus antimicrobial and antibiofilm activities with some description of its major chemical constituents.
2. A Brief Overview on Biofilm

2.1. Definition, Structure and Development Cycle

Biofilm is defined as an organised community of bacteria encased in a matrix of extracellular polymeric substances (EPS) that irreversibly attaches the bacterial cells to a surface [10]. It serves as a protective niche for particular pathogens from environmental threats, host defences, and antibiotics, leading to an increase in bacterial pathogenicity and antibiotic resistance [11-14]. EPS is mainly composed of exopolysaccharides, extracellular DNA, polypeptides, and biomolecules that contribute to the structure and three-dimensional architecture of biofilm as they form a highly hydrated polar mixture [15-18]. Other matrix components, such as extracellular appendages flagella, type IV pili, and cup fimbriae, are also involved in the biofilm formation as these structures contribute in adhesion process (irreversible attachment) and microcolony formation [20-24].

Biofilm development occurs in five stages. First, reversible adhesion of free-floating bacterial cells to a surface suitable for growth. Second, irreversible attachment of the bacteria as microcolonies start joining together to form EPS. Next, maturation of biofilm as EPS begins to take on a complex three-dimensional shape or mushroom-like structure. Afterwards, the biofilm fully matures developing a complex architecture. Finally, the bacteria disperse from the biofilm and re-initiate biofilm colonisation on a new surface [25]. A number of studies concerning biofilm formation and development by using *Pseudomonas aeruginosa* as the standard model elucidated that during the initial attachment, adhesion factors such as *PilA* and *PilB* found in the pili play an essential role in the bacterial attachment to the surface. In contrast, polysaccharides, such as alginate, *Pel*, and *Psl* produced by *P. aeruginosa*, take part during the maturation of the biofilm and are the primary determinant for the stability of the biofilm structure [26, 2]. Extracellular DNA, on the other hand, plays a crucial role both in the initial and early development of biofilm. It acts as a cell-to-cell interconnecting compound and helps expand the biofilm through twitching motility by maintaining coherent cell alignments [27, 28, 16]. Furthermore, extracellular DNA also constitutes a nutrient source for bacteria during starvation [29, 30].

2.2. Regulation of Biofilm Formation

Biofilm formation and development (represented by *P. aeruginosa*) is regulated by several bacterial types of machinery, including quorum sensing mechanism, two-component regulatory system, and c-di-GMP dependent polysaccharides biosynthesis [6]. However, the nutritional environment, such as the availability of carbon source also influences the formation and development of biofilm [31].

Quorum sensing (QS) is defined as bacterial cell-to-cell communication involving the production and detection of the extracellular signaling molecules known as autoinducers (acyl lactones (AHLs) in Gram negative bacteria and oligopeptides in Gram-positive bacteria) that coordinate the production of virulence factors, motility and biofilm formation [32, 33]. In relation to biofilm formation studied in *P. aeruginosa*, QS can influence the biosynthesis process of EPS components by either regulating the expression of *psl* or *pel* through its two main system of *las* and *ral*, respectively. It also influences extracellular DNA release during biofilm development through its third QS system named Pseudomonas Quorum Sensing (PQS). Furthermore, QS also indirectly regulates biofilm formation by controlling the swarming and twitching motilities, as well as rhamnolipids and lectins production by *ral* system [16]. Swarming motility is implicated in the early stages of biofilm formation and defines the structure of biofilm under certain conditions, especially those related to nutritional sources availability. Under the condition that promotes bacterial swarming, for instance, growth medium is rich in glutamate or succinate as carbon source, the biofilm structure formed is flat and uniform; however, under condition that limits the swarming motility, the biofilm containing non-confluent aggregate is formed. This suggests that the contribution of quorum sensing to biofilm formation through the regulation of swarming motility is nutritionally conditional [31]. On the other hand, twitching motility is necessary for the assembly of the monolayer of *P. aeruginosa* cells into microcolony during biofilm formation [19]. Rhamnolipid was also reported to be involved in forming microcolony and facilitating three-dimensional mushroom-shaped structures [34], maintaining open channel structures [35], and
facilitating cell dispersion from biofilm during the last stage of biofilm formation cycle in *P. aeruginosa* [36,37].

Furthermore, the two-component system indirectly regulates biofilm formation through regulating QS activity responsible for biofilm formation. It involves a signalling molecule interaction with transmembrane sensor kinase and initiates a series of phosphorylation events leading to the phosphorylation of a cognate response regulator protein. This phosphorylation then allows the response regulator to bind to the promotor region of the QS-regulated target genes, including biofilm formation-associated genes (mainly genes related to EPS biosynthesis), and alter their transcription [38, 39, 16].

Biofilm formation is also controlled through the regulation of c-di-GMP. C-di-GMP plays a crucial role in the production of polysaccharides [40, 41]. High levels of c-di-GMP promote the polysaccharide (alginate and Pel) biosynthesis, while low levels of c-di-GMP promote bacterial motility by enhancing flagellar formation and bacterial dispersion, leading to the decrease of biofilm formation [41].

This knowledge concerning the global regulating system in bacterial biofilm formation has given insight to scientists on how to combat with biofilm-forming pathogenic bacteria, including the utilisation of plant-derived antibiotic agents that can interfere with biofilm regulation mechanisms [42, 3].

3. A Brief Overview on *Hibiscus tiliaceus* L.

3.1. Botany and Uses

*Hibiscus tiliaceus* L. (Malvaceae) is indigenous to the tropical shore of the Pacific and Indian oceans. However, today the plant has been widely distributed throughout the tropical and subtropical regions in the world. It thrives in a coastal and riparian environment but can also be found in inland areas and valley in its native range. It is a fast-growing evergreen tree that can grow up to 20 m tall [43]. Leaves are heart-shaped; flowers are bell-shaped with maroon-coloured heart and stigma. They are yellow in the morning, turning into orange red in the evening, and mauve the next morning [44]. Almost all parts of *H. tiliaceus* plant have been utilised for various purposes, such as ornament, fence, food, drink, and traditional medicines. In Indonesian and Malaysian folk medicines, the leaves have been used to remove phlegm, clear respiratory tract, as well as treat fever, cough, and bronchitis. In countries of Asia and Africa, its flowers are used for the treatment of ear infection, chest congestion, and in birth control. The bark has been utilised to cure dysentery in Philippine and to treat cough and bronchitis in China. In Papua Guinea, a decoction of leaves is taken for sore throat, pneumonia, cough, tuberculosis and diarrhea [43-44]. In addition, *H. tiliaceus* extracts have been reported to possess various pharmacological activities, including analgesic and immunomodulatory effects, cytotoxic, anti-inflammatory, anti-tyrosinase [44], antimutagenic [45]; antioxidant, antibacterial [46, 47, 48] and antibiofilm activities [9, 49].

3.2. Phytochemical Constituents

Phytochemical studies on different parts of *Hibiscus tiliaceus* indicate the presence of various phytochemical constituents. Methanol extracts of mangrove-associated *H. tiliaceus* leaves, fruits, and twigs from Malaysia has been reported to contain proteins, carbohydrate, phenolics, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids. Protein was found in the leaves and fruits, while saponins and alkaloids presented only in the fruits and twigs extracts, respectively [9]. Leaves were reportedly rich in phenolics and flavonoids. A substantial amount of phenolic compounds, namely catechin (99 mg/ml), rutin hydrate (79 mg/ml), quercetin (69 mg/ml), and ellagic acid (59 mg/ml) were obtained from the ethanol leaf extract of *H. tiliaceus* growing in Bangladesh using HPLC-DAD system [46]. Other compounds such as azelaic acid, cleomiscosin C, daucosterol, frieldelin, fumaric acid, hibiscoclactone, kaempferol, scopoetin, β-sitosterol, succinic acid, syricusin A, and vanillin have also been found in the leaves and stems [50]. A continuing phytochemical study on the ethanol leaf extract of mangrove-associated *H. tiliaceus* from Vietnam has led to the investigation of a novel megastamine called tiliacic acid A, along together with other 14 known phenolic compounds of trans-tiliroside, 4’-dihydrophaseate, astragalin, blumenyl C β-D-glucopyranoside, chromone, isoquercitrin, rutin, kaempferol 3-O-rutinoside, oseoside, 3,5,7-trimethoxy-flavone, 3,7’,4-trimethylkaempferol, 7-
methylacacetin, isomericitrin, and myricitrine [47]. Triterpenoid compounds have also been identified from the leaf, bark, and stem of H. tiliaceus [44, 47], while hibiscusin (a type of coumarin) and hibiscusamide (a type of amide) were found in the stem wood of H. tiliaceus growing in Taiwan [50]. Coumarin has also been found in H. tiliaceus leaves, flowers, barks, and root for aqueous extracts collected from India, along with other major phytochemical compounds such as tannins, saponins, flavonoids, and alkaloids. Phlobatannins and cardiac glycosides were only found in the leaf extract, while quinone presented only in the bark extract. Terpenoids and anthraquinone were observed in the leaf and flower extracts, while steroids and phytosterol were noted to present in the aqueous leaf and bark extracts [51]. In addition, the stem and bark also contain friedelane-type trisperpene known as 27-oic-3-oxo-28-friedelanoic acid [44]. The presence of tannins has also been observed in the ethanol leaf and bark extracts of H. tiliaceus collected from Bangladesh, while alkalioids and reducing sugar were found only in the bark extract [52]. Phenolic compounds such as p-coumaric acid, fumaric acid, kaempferol, kaempferol 3-O-D-galactoside, quercetin, and quercetion 3-O-D-galactoside has also been identified in fruits, while anthocyanin and flavonols can be found in the flower of H. tiliaceus [44]. The differences in chemical constituents between the plants parts of H. tiliaceus or between H. tiliaceus samples collected from different locations may be a result of different factors, including geographical location, botanical sources, time of harvesting, stage of development, and method of extraction [53].

4. Antibacterial Activity of Hibiscus tiliaceus L.
The biopotency of Hibiscus tiliaceus phytochemical constituents as antibacterial and antibiofilm potential has been extensively studied in the last decade. Almost each part of the plant from various geographical locations have been evaluated as an effort in search for a new antibacterial and antibiofilm agent. The literature review on its antibacterial and anti-biofilm activities is summarised in the Table 1. The methanol extract of H. tiliaceus leaves collected from Bandar Sunway, Malaysia, has been reported to exhibit antibacterial activity against Gram-positive bacteria of Staphylococcus aureus, Micrococcus luteus and Bacillus cereus with a minimum inhibitory dose of 0.25, 0.5, and 1 mg/disc, respectively. At the concentration of 1 mg and 2 mg extract/disc, the inhibitory effect was moderate on S. aureus and weak on M. luteus and B. cereus when compared to that of the positive control of Streptomycin (concentration 10 µg/disc). No inhibition was observed for Gram-negative bacteria of Pseudomonas aeruginosa, Salmonella choleraesuis and Escherichia coli [54]. Other methanol leaf extract from coastal area in Malaysia (University Malaysia Terengganu), as well as its fractions (hexane, dichloromethane, and ethyl acetate) showed positive inhibition towards both Gram-positive (S. aureus and Bacillus subtilis cereus) and Gram-negative (Klebsiella pneumonia and E. coli) bacteria. However, the water fraction was only active against the Gram-positive bacteria [48]. The ethanol leaf extract from Bangladesh was also declared to positively inhibit Gram-positive bacteria of S. aureus as well as Gram-negative bacteria of Salmonella paratyphi and E. coli at the concentration of 250 µl/disc and 500 µl/disc. The inhibitory effect on both Gram-positive and Gram-negative bacteria was weak at dose of 250 µl/disc and moderate at dose of 500 µl/disc, when compared to that of Kanamycin (30 µl/disc) as positive control [55]. A contrary finding has been reported for the ethanol leaf extract which was also obtained from Bangladesh. In this study, the extract did not show any antibacterial activity towards all the twelve tested bacteria (both Gram-positive and Gram-negative) at the concentration of 10 µg/µl; however, the ethanol bark extract showed positive inhibition against S. aureus and S. epidermidis (Gram-positive) at the same extract concentration [52]. The lack of antibacterial effect of the leaf extract is possibly due to the absence of most of the main phytochemical compounds in the sample evaluated in this study. Among the six phytochemical substances screened (alkaloids, flavonoids, saponins, reducing sugar, tannins, and gums), tannins were the only chemical component presented in the leaf extract. A broad spectrum of antibacterial activity has been reported for Indonesian (North Sumatra) mangrove-associated H. tiliaceus chloroform-methanol leaf extract containing high polyisoprenoid (polyprenoyl) content. The polyisoprenoid substance isolated in this study showed moderate inhibition against S. aureus (diameter of inhibition zone 11.57 mm) and E. coli (10.52 mm) at the concentration of 100 mg/ml [56]. Remarkable antibacterial properties were also exhibited by H. tiliaceus TLC-based methanol fractions
of leaves, barks, fruits, and roots against Gram-positive (Staphylococcus aureus, Streptococcus pyogenes, Streptococcus faecalis) and Gram-negative (Salmonella typhi, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa) bacteria with diameter of inhibitory zone ranged from 10-18 mm. The best inhibitory activity was shown by TLC bands of leaves. The TLC band of bark showed positive inhibition only on Gram-positive bacteria, while the TLC band of flower and root were only active against Gram-negative bacteria. Based on the FTIR spectrum analysis, all of the H. tiliaceus plant parts evaluated in this study were discovered to be rich in phenolics, alkaloids, flavonoids, and terpenoids which may contribute to their antibacterial activities [51]. Furthermore, n-hexane bark extract of H. tiliaceus and its purified triterpenoid constituents were also evidenced to moderately inhibited E. coli. The diameter of inhibitory zone produced by the n-hexane crude extract and its partitioned triterpenoid were 6.92 and 5.77 mm (the average result of the three active triterpenoid fractions against E.coli), respectively. In addition, the best inhibitory action was demonstrated by triterpenoid substance present in fraction 1 with the MIC value of 0.2 mg/ml [47]. Other investigation regarding the antibacterial and antibiofilm potential of methanolic extract and fractions (chloroform, ethyl acetate, methanol) of Malaysian mangrove-associated H. tiliaceus flowers, leaves, and twigs revealed that 9 samples, including the methanol crude of leaves and fruits, chloroform fraction of fruits and leaves, ethyl acetate fraction of leaves, fruits and twigs, as well as methanol fraction of leaves and twigs showed antibacterial activity against Pseudomonas aeruginosa at the concentration of 30 mg/ml. The assay was performed by microdilution method in 96-well microtiter plate. The strongest inhibitory effect was produced by the chloroform fraction of fruits (87.2% inhibition), while the weakest was exhibited by the ethyl acetate fraction of twigs (0.3% inhibition). The other mentioned extracts and fractions revealed weak antibacterial activity; however, despite their slight antibacterial effect, all of these extracts and fractions, except the ethyl acetate fraction of leaves, have also been reported to exhibit antibiofilm activity against P. aeruginosa.

5. Antibiofilm Activity of Hibiscus tiliaceus L.
As mentioned earlier, in addition to its antibacterial activity, H. tiliaceus is also endowed with potent antibiofilm property. Of the total 12 extracts and fractions assessed for the antibiofilm activity from the leaves, flowers, and twigs of coastal-associated H. tiliaceus, 10 samples were reported to possess either antibiofilm or both antibacterial and antibiofilm activity against P. aeruginosa at the concentration of 30 mg/ml by using 96-well microplate assay. Among them with only antibiofilm effect were methanol crude of flowers, methanol fraction of flowers, and chloroform fraction of twigs, while those with both antibacterial and antibiofilm activities were methanol crude of twigs, methanol fraction of leaves and twigs, chloroform fraction of fruits, leaves, and twigs, as well as ethyl acetate fraction of fruits and twigs. The highest antibiofilm activity was showed by the chloroform fraction of fruits with 94.7% inhibition, while the lowest was exhibited by the methanol fraction of leaves (53. 8%). Similarly, the methanol crude of fruits and twigs, methanol fraction of fruits and twigs, chloroform fraction of twigs, and ethyl acetate fraction of twigs also exhibited strong antibiofilm activity against P. aeruginosa with the percentage of inhibition comprised between 80%- 86%, while the ethyl acetate fraction of fruits as well as chloroform fraction of leaves showed moderate inhibition (66% - 69.5%) as compared to the positive control (50% DMSO) [9].

Ethanol extract of H. tiliaceus leaves growing in Aceh region, Indonesia, was also reported to possess potential antibiofilm activity against Vibrio alginolyticus at various extract concentrations (2%, 4%, 8%, and 10%). The strongest antibiofilm effect was recorded at the extract concentration of 10% with the approximate percentage of biofilm formation inhibition of 75%, whereas the lowest effect was obtained at the extract concentration of 2% (inhibitory percentage around 21%). Thses results suggest that antibiofilm activity of the ethanol leaf extract assessed in this study was dose-dependent [49].
| Plant part | Extract/fraction | Phytochemical compounds | Activity | Inhibition against | Bioassay method | References |
|------------|------------------|------------------------|----------|--------------------|----------------|------------|
| Leaf       | Methanol extract | Phenolics, anthocyanins | Anti-bacterial | *B. cereus* | Disc diffusion method | [54] |
| Leaf       | Ethanol extract  | Phenolics, flavonoids   | Anti-bacterial | *S. aureus* | Disc diffusion method | [55] |
| Bark       | Ethanol extract  | Alkaloids, Tannins, reducing sugar, gums | Anti-bacterial | *S. aureus* | Agar diffusion method | [52] |
| Leaf, flower, twig | Methanol extract/fractions (chloroform, ethyl acetate, methanol) | Phenolics, tannins, flavonoids, glycosides, steroids, terpenoids, saponins, alkaloids, protein, carbohydrates | Anti-bacterial | *P. aeruginosa* | Microdilution method (96-well microplate assay) | [9] |
| Leaf       | Ethanol extract  | Phenolics, tannins, flavonoids, terpenoids, steroids, saponins | Anti-bacterial; Anti-biofilm | *V. alginolyticus* | 96-well microplate assay | [49] |
| Leaf       | Chloroform-methanol extract | Polyisoprenoids (polyprenol) compund | Anti-bacterial | *S. aureus* | Agar diffusion method | [56] |
| Leaf       | Methanol extract/fractions (Hexane, dichloro methane (DCM), ethyl acetate, water) | Phenolics, tannins, flavonoids, glycosides, steroids, terpenoids, proteins, carbohydrates | Anti-bacterial | *S. aureus* | Agar and disc diffusion method | [46] |
| Leaf, bark, flower, root | TLC-based fractions of methanol extract | Alcohols, phenols, alkanes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds, amines | Anti-bacterial | *S. aureus* | Agar cup method | [51] |
| Bark       | N-hexane extract | Triterpenoid compounds | Anti-bacterial | *E. coli* | Broth dilution and disc diffusion method | [47] |
6. Results and Discussion

Almost all parts of *H. tiliaceus*, including leaf, flower, bark, twig, and root, have been intensively assayed for their antibacterial activities. The leaf, flower, and twig, indeed, have also been evaluated for their antibiofilm property in the last three years. Among *H. tiliaceus* plant parts, leaf has been the most common part assessed and reported for its antibacterial and antibiofilm potential; however previous study found that the antibacterial activities produced by leaves were generally weak and moderate against the standard tested bacteria. No strong inhibitory effects were observed for *H. tiliaceus* leaf samples on bacterial cell growth of either Gram-positive or Gram negative bacteria during the period covered in this review. A strong antibacterial property was reported to be exhibited by the fruit chloroform fraction of *H. tiliaceus* growing in Malaysia [9]. On the other hands, different findings were noted for *H. tiliaceus* antibiofilm activity, that the leaf extracts and fractions collected from Malaysia showed slight inhibition on *Pseudomonas aeruginosa* biofilm formation [9], whereas the ethanol leaf extract obtained from Indonesia could possess strong antibiofilm property against *Vibrio alginolyticus* in a dose-dependent manner [49]. The strong antibiofilm activity was obtained from the extracts and fractions of *H. tiliaceus* fruit and bark [9]. This difference in biological activity may be due to the difference of the geographical location and the ecosystem of *H. tiliaceus* samples taken or due the difference in sample preparation methods applied, where all of these factors has direct bearings on the phytochemical compounds and biological activities [53, 9].

Antibacterial and antibiofilm activities of *H. tiliaceus* are strongly attributed to its phytochemical constituents, mainly phenolics, present in the plant parts. It has been evidenced that the ethanol and methanol extracts of leaves containing phenolic compounds were able to inhibit the growth of Gram-positive and Gram-negative bacteria [54, 55, 9, 48]. TLC-based methanol fractions of *H. tiliaceus* leaf, bark, flower, and root containing high level of phenolic substances were also discovered to exhibit a potent broad-spectrum antibacterial activity [51]. Furthermore, methanol, ethyl acetate, and fractions of leaf, flower, and twig were discovered to possess not only antibacterial properties, but also a promising antibiofilm activity [9]. Ethanol leaf extract collected from Aceh, Indonesia, with the presence of phenolic contents also demonstrated positive inhibition against aquatic-associated *Vibrio alginolyticus* in a dose-dependent manner [49]. In addition, many other phytochemical preparations containing phenolics or polyphenols, including flavonoids (quercetin, kaempferol, rutin), anthocyanins, coumarins, and tannins (catechin, gallic acid, ellagic acid), which also present in various plant parts of *H. tiliaceus* [46], have been reported as the main phytochemical constituents responsible for strong antibacterial [57] and antibiofilm activities [58, 59, 3].

Phenolics contribute to the eradication of bacteria by altering bacterial cell permeability which permits the loss of macromolecules from the cell interior. They have also been suggested to interfere with membrane function and interact with membrane protein, resulting in structural and functional deformation of the cell [53, 59]. Moreover, the presence of the number of hydroxyl groups in the phenolic ring would increase hydroxylation. Hydroxylation, which is determined by the sites and the number of hydroxyl groups, has been thought to be related to phenols toxicity to microorganisms. Thus, the increase in hydroxylation will result in the increase of antimicrobial effect of these compounds [60].

In relation to its antibiofilm properties, phenolic compounds may interrupt with biofilm formation by influencing the expression of biofilm-formation-associated genes. A phenolic extract from olive plant water was reported to inhibit biofilm formation of *E. coli* K12 by affecting the regulation of QS-associated gene expression and the synthesis of fimbriae, curli, and exopolysaccharides, which affected the bacterial adhesion to the surface [61]. At subinhibitory concentration, the leaf extracts of *Olea europaea* and *Camellia sinensis* that are rich in phenolic compounds were also reported to inhibited biofilm formation and virulence factor production as well as downregulate *lasI, lasR, rhlI, rhlR* expression without affecting *P. aeruginosa* cell growth [62]. Their phenolic extracts were also reported to inhibit the expression of gene associated with flagellar synthesis and flagellar rotation. Other possible mode of action of plant-derived antibiofilm agent in decreasing the biofilm formation is by inhibitory action on sucrose-dependent bacterial adherence, glycolytic acid production, and acid tolerance [3].
Tannins, which represent the most prominent groups of plant polyphenols, have also been reported to play a significant role in fighting against pathogenic bacteria. Various extracts and fractions of various *H. tiliaceus* plant parts, such as leaves, barks, flowers, and twigs containing tannins have been evidenced to possess antibacterial property [9, 52, 56]. The efficacy of tannins as plant-derived antimicrobial agents may be related to their ability to form a complex with bacterial polysaccharides, as well as inactivate their adhesins, enzyme, and cell envelope transport protein [63]. Deprivation of the substrate required for bacterial growth as well as disrupting bacterial metabolism through the inhibition of oxidative phosphorylation have also been related to its antimicrobial mode of actions [64]. Concerning its antibiofilm activity, tannins was able to eradicate the extracellular polymeric substance (EPS) matrix of *P. aeruginosa*, *E. coli*, *S. aureus*, and polymicrobial biofilm [65]. Catechin has been reported to be the most abundant tannin compounds found in the ethanol extract of *H. tiliaceus* leaves, followed by rutin hydrate, quercetin, and ellagic acid [46]. Catechin such as EGCg has been reported to reduce slime production in *S. aureus* at subinhibitory concentration, while ellagic acid form pomegranate methanol extract was noted to inhibit biofilm formation of *S. aureus*, MRSA, and *E. coli* through the eradication of bacterial cell membrane [59]. Gallic acid, on the other hand, interferes with *S. aureus* biofilm formation by affecting its adhesion properties [66].

Flavonoids are chemical compounds that could be linked to antibacterial and antibiofilm potential of *H. tiliaceus*. Potent antibacterial activities against *S. aureus*, *E. coli*, and *S. paratyphi* have been shown by *H. tiliaceus* leaf extract containing flavonoids [55]. Furthermore, other chemical preparation of *H. tiliaceus* from different plant parts that contained flavonoids have also been reported to possess antibacterial effect against Gram-positive and Gram-negative bacteria [9]. Many other chemical substances of plant origin containing high flavonoid substances have also been reported to exhibit antibacterial property [67, 68]. Antibacterial property of flavonoids is mainly related to their structure, which can form a combined complex with bacterial cell walls and increase hydroxylation. [60]. Furthermore, flavonoids can also inhibit the bacterial nucleic acid synthesis and energy metabolism [59]. Several flavonoids such as quercetin, kaempferol, fisetin, apigenin, chrysin, luteion contained in *Vitis vinifera* were observed to be effectively inhibit *S. aureus* biofilm formation [69]. Quercetin found in the extract of *Alnus japonica* has been reported to inhibit *S. aureus* biofilm formation by suppressing the expression of biofilm formation-associated genes of icaA and icaD, quorum-sensing gene of agrA, and two virulence-regulatory genes of sigB and sarA [57].

Antibacterial activity of *H. tiliaceus* has also been assumed to be influenced by triterpenoid substance. Triterpenoid compound isolated from N-hexane extract of *H. tiliaceus* bark was recorded to possess antibacterial activity against *E. coli* [47]. Triterpenoid, one of terpenoid derivates, has been announced for its capability of hindering bacterial growth [70].

Generally, there have been a linear correlation between the ability to inhibit biofilm formation and the ability to inhibit bacterial growth. Biofilm formation, in most cases, is reduced due to the reduction of viable bacteria [59, 9]. As an example, the chloroform fraction of *H. tiliaceus* flowers that showed strong antibiofilm activity was also recorded to possess strong antibacterial activity. Conversely, the sample of *H. tiliaceus* with the weakest antibiofilm effect (methanol fraction of leaves) also exhibited inadequate antibacterial activity [9]. This results might suggest that the bioactive constituents present in the leaves and flowers of *H. tiliaceus* which act as antibiofilm antagonist, which is usually attributed to phenolic compounds, are presumably inhibit the biofilm formation by inhibiting the bacterial cell growth. It has been reported that the inhibition of biofilm formation by phenolic compounds was generally observed at the concentrations that suppresses bacterial growth, suggesting that the suppression of biofilm formation probably due to the inhibition of bacterial growth, rather than the specific effects of the phenolic substances themselves on the inhibition of biofilm formation. In fact, at subinhibitory or weakly bacterial growth-suppressing concentration, certain phenolic compounds such as gallic, 4-hydroxybenzoic, and cinnamic phenolic acid have been reported to induce biofilm formation in *Pseudomonas aeruginosa PAO1* by affecting AHLs synthesis via the interaction with the bacterial metabolism [71].
Despite their destructive activity on bacteria that ultimately leads to the inhibition of biofilm formation, other certain antibiofilm substances of plant origin have also been reported to be able to inhibit biofilm formation without affecting the bacterial growth [59]. For instance, as previously mentioned, some samples of *H. tiliaceus* (methanol crude and methanol fraction of flowers, chloroform fraction of twigs) were noted to inhibit biofilm formation without affecting bacterial growth, or those with strong antibiofilm activities (all extract and fractions of twigs) were observed to possess very weak antibacterial activities [9]. These phenomenon suggest that the chemical compounds with antibiofilm properties present in the flowers and twigs of *H. tiliaceus* might probably suppress the biofilm formation through different mechanisms, such as by disrupting the extracellular polysaccharide (EPS) matrix, disturbing the quorum-sensing mechanisms, or contaminating the nutrient source required for biofilm formation [9, 59]. In this case, flavonoid constituents, such as quercetin, are presumably the main bioactive compounds contributing to the antibiofilm effects of *H. tiliaceus* flowers and twigs.

A review of several research reports concerning Malaysian plant-origin biofilm antagonist [72] suggested that plant-derived antibiofilm substances act in some potential mechanisms. This includes (1) inhibition of c-di-GMP signaling system; (2) inhibition of urease activity; (3) suppression of gene and protein expression; (4) reduction in polysaccharide; (5) quorum sensing inhibition; (6) inhibition of curli and pili biosynthesis; (7) inhibition of cell adherence; and (8) reduction in biofilm biomass.

Almost all of the phytochemical components attributed to antibiofilm properties have been reported to present in *H. tiliaceus* [46], suggesting that *H. tiliaceus* could be a promising source of a potential antibiofilm agent that might inhibit biofilm formation through one or more previously elucidated mechanism of actions.

7. Conclusion

Almost all parts of *Hibiscus tiliaceus* were reported to possess remarkable antimicrobial and antibiofilm properties, highlighting its potential application as novel plant-derived antibacterial and antibiofilm agents. The best antibacterial property of *H. tiliaceus* was found in the phytochemical compounds of flowers, while the best antibiofilm activity was presented by the chemical constituents of the flowers and twig. This antibacterial and antibiofilm property of *H. tiliaceus* are strongly linked to various phytochemical constituents present in the plant, mainly phenolics. The antibiofilm substance found *H. tiliaceus* may possibly work in two modes of action mechanism including by affecting bacterial cell growth which ultimately affects the biofilm formation or by interfering with the biofilm formation regulation system without affecting the bacterial cell growth. The later mechanism may include the interruption of biofilm formation-associated quorum sensing system, suppression of biofilm-formation-associated gene expressions, or contamination of the nutrient source required for biofilm formation. Finally, the ability of *H. tiliaceus* chemical compounds to suppress biofilm formation suggest that *H. tiliaceus* could also be a potential candidate for the source of quorum sensing inhibition agents. Hence, further investigations on its bio-therapeutic property against pathogenic bacteria, such as its anti-quorum sensing potential is worth conducted.

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