Antimicrobial activity of quinine derivatives against human pathogenic bacteria

L D Antika*, D Triana and T Ernawati
Research Center for Chemistry, Indonesian Institute of Sciences (LIPI)
Kawasan PUSPIPTEK, Serpong, Tangerang Selatan, Banten 15314 Indonesia

*Corresponding author's email: lucia.dwi.antika@lipi.go.id

Abstract. Nowadays, the antimicrobial resistance is considered as one of the greatest concern facing human health, as many of bacterial strains had become resistant to available antibiotics. The misuse of antibiotics has potentially reduced the efficacy of drugs, concurrent with the increase of bacterial resistance to commercially available drugs. Therefore, the discovery of effective antimicrobial agents are desperately needed to overcome this epidemic, especially from traditional medical plants and their derivatives. Quinine is a natural alkaloid from the bark of the cinchona tree that has been used for years as an antimalarial drug. Various literatures also regarded an antibacterial effect of quinine against both Gram-positive and Gram-negative pathogenic microorganisms. With this vision, a series of some novel quinine derivatives were synthesized and their biological activities against pathogenic bacteria were assessed. This present study therefore attempted to examine the antimicrobial properties of quinine-derived compounds and their Minimal Inhibitory of Concentration (MIC) against common pathogenic bacteria strains, e.g. Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus subtilis. Disk diffusion test was performed for screening of antimicrobial activity of quinine derivatives. The results were evaluated and compared with references drug streptomycin. It was found that quinine derivatives showed moderate antimicrobial activity as compared with quinine itself on tested pathogenic bacterial strains. Ester quinine propionate was found to give the highest antibacterial activity among other derivatives, with a range in inhibition zone from 9 to 23.5 mm to bacteria strains, compared to streptomycin with a range of inhibition zone from 8 to 12 mm. Further studies are needed to assess the bactericidal mechanisms of those derivative compounds.

1. Introduction
Bacterial infections are one of a major cause of chronic infection and mortality worldwide. The concern is increasing as many of bacterial strains had become resistant to available antibiotics due to misuse of antibiotics that led to the multidrug-resistant (MDR) pathogens [1]. Antimicrobial resistance already causes 700 thousand deaths every year. If no action is taken, 10 million deaths may occur annually due to microbial resistance by 2050, overtaking cancer [2]. The discovery of effective antimicrobial agents are necessary to overcome this epidemic, therefore numerous studies have been conducted to find the potential source of antimicrobial, especially from natural sources. Group of plant-derived compounds including phenolics, flavonoids, quinolones, and alkaloids were reported to exhibit antimicrobial action. Among those compounds, alkaloids demonstrated great pharmacological activities including antimicrobial properties due to their various structural diversity [3,4,5]. In the past
decades, few novel compounds have been introduced as potentially useful for antimicrobial, and some of them are derivatives of well-known compounds.

Quinine (C_{20}H_{24}N_{2}O_{2}), an alkaloid derived isolated from the bark of the cinchona tree, has been used in medical treatment as an antimalarial drug since centuries because it exhibits specific toxicity against *Plasmodium* which has no resistance. Along with its antimalarial activity, gradually, quinine has been reported to possess other pharmaceutical properties including anti-inflammatory [6] and anticancer. Krishnaveni and Suresh (2015) [7] revealed that quinine has potent anticancer properties by effectively inhibits cell proliferation and protease’s activity induces cell death in cancer cells [7]. Besides, Ning *et al.* 2016 reported that quinine stimulates adipogenesis through ERK/S6 signaling suggesting its potency as anti-obesity [8]. Several studies also revealed that quinine also possesses antimicrobial properties. Quinine was found to be bactericidal by inhibiting *Escherichia coli*, *Klebsiella pneumonia*, and *Staphylococcus aureus* [9,10].

Several studies reported that different functional groups might improve the biological activity of a compound. Study by Završnik *et al.* (2008) reported that the substitution of chlorine at the position C of coumarine derivatives exhibited a good activity against *S. aureus*, but not *B. substilis*. Additional dimethylamino group on coumarine compound also was found to elevate bactericidal activity against *S.aureus* and *B.substilis* [11]. Another study showed that synthetic heterocyclic compounds addition of thiosemicarbazide indicated stronger antibacterial action, compared to standard chloramphenicol, against *S. aureus* [12]. Likely, the specific functional group serves different effect on its pharmacological properties.

Noticing the potential activities of quinine, a few chemical syntheses of quinine derivatives has been developed. Quinine derivatives were obtained from the chemical synthesis reaction through quinine esterification process. The esters compounds were identified by spectroscopy (UV-Vis, NMR, LCMS, and FTIR). In this present study, we synthesized several derivative compounds of quinine with specific functional group in purpose to understand how different functional groups serves different antimicrobial action. We also described the antimicrobial properties of quinine derivatives and preliminary studies of their antimicrobial properties.

2. Materials and methods

2.1. Chemicals

Quinine was isolated from the bark of *Cinchona* sp. provided by PT. SIL Lembang. An amount of 50 mg of *Cinchona* bark powder was treated with ammonia water and acidified with 1% HCl. After that, the solution was evaporated and transferred into a soxhlet. Sample were soxhlet extracted with toluene for 6 h and the extract was diluted with sulfuric acid. Acidic solvent was separated and the precipitate was neutralized until the alkaloid (quinine, cinchonine, and cinchonidine) crystals were obtained [13]. Quinine derivatives were synthesized through esterification process and identified by spectroscopy (UV-Vis, NMR, LCMS, and FTIR). The chemical structure of quinine and its derivatives were shown in figure 1.

2.2. Bacterial Strains

Numerous pathogenic bacterial strains were employed for antimicrobial activity test of quinine-derived compounds. All strains were provided by the Indonesian Culture Collection (InaCC), Research Center for Biology, LIPI. *Escherichia coli* (InaCC-B5), *Staphylococcus aureus* (InaCC-B4), *Pseudomonas aeruginosa* (InaCC-B290), and *Bacillus substilis* (InaCC-B334) were chosen based on their clinical and pharmacological importance.
2.3. Preparation of Standard Inoculum

0.5 McFarland turbidity standard was used to prepare the standard inoculum for antimicrobial susceptibility test. McFarland standard reagent was made by mixing 1% sulfuric acid (Merck, Darmstadt) and 1% barium chloride (Sigma-Aldrich, Missouri, USA) to get specific optical density (OD) solution. Bacteria stains were cultured in nutrient agar (HiMedia, Mumbai, India) and incubated at 37°C for 24 h. Pure colonies were picked up and diluted in a sterile saline water. Afterwards, its absorbance were adjusted to 580 nm. The turbidity was matched to 0.5% McFarland standard equivalent to 1x10^8 CFU/ml.

2.4. Antimicrobial Activity using Disk Diffusion Method

Antimicrobial activity of quinine-derived compounds were determined with the disk diffusion method [14]. Quinine-derived compounds were dissolved in DMSO in a serial dilution starting from 10% (w/v), 5% (w/v), 2.5% (w/v), 1% (w/v), and 0.5% (w/v), then loaded over sterile filter paper disks with a diameter of 6 mm on the surface of nutrient agar (HiMedia, Mumbai, India) plate. These compounds were tested in vitro against various Gram-negative (E. coli, P. aeruginosa) and Gram-positive (S. aureus, B. substilis) bacterial strains with concentration of 1x10^6 CFU/ml. Commercially available references drug streptomycin (Merck, Darmstadt, Germany) was served as positive control. The plate was incubated at 37°C overnight and the zone of inhibition was measured. Antibacterial activity was expressed as the mean zone of inhibition diameter (millimeter) by quinine-derived compounds against pathogenic microorganisms.

2.5. Determination of Minimum Inhibitory Concentration (MIC)

The bacterial suspension was diluted in 0.9% saline buffer and was matched to give the turbidity equivalent to the 0.5 McFarland standard (approx. 1x10^8 CFU/ml). This could be done by visually comparing the appearance of the suspension or photometrically using 625 nm wavelength. Serial dilution of samples was prepared and a standard inoculum of bacteria strain was added to each concentration of samples. A medium inoculated with bacteria strain without antimicrobial agent was served as a growth control. A loopful inoculum from each of sample was cultured to NA agar plated that were divided into six sections. The plates were incubated at 37°C for 24 hours. The lowest concentration of quinine derivatives that suppress invisible growth of microorganism was considered.
the minimum inhibitory concentration (MIC). Minimum lethal concentration (MLC) is the lowest dose of sample required to kill microorganisms.

2.6. Statistical analysis
Data obtained separated experiments are expressed as the mean ± SEM. Statistical analysis were performed using SPSS software version 16.0. The difference between two values was determined by one-way ANOVA followed by Duncan’s test for multiple comparisons. Differences were statistically considered significant at P < 0.05.

3. Results and discussions
A clear inhibition zone was observed around each sample compounds (figure 2). In this present study, quinine-derived compounds were tested against common human pathogenic Gram-negative (E. coli, P. aeruginosa) and Gram-positive (S. aureus, B. substilis) bacterial strain by disk-diffusion agar method. The results of the zone of inhibition were summarized in table 1 and their graphical representation was shown in figure 3, respectively. The activity of quinine-derived compounds was comparable to standard drug streptomycin against the tested bacterial strains.

Figure 2. Quinine (left) and ester quinine propionate (right) inhibited growth of E. coli 24-h post inoculation. The width of the zone of inhibition is indicated by the red dotted line.

Several studies showed that different functional groups can improve the biological activities of compounds. It was found that DMSO extract of quinine derivatives remarked moderate antimicrobial activity as compared with quinine itself on pathogenic bacterial strains (table 1). In this present study, all the tested pathogenic bacteria were highly sensitive to quinine. The result was similar by those of Kharal (2009) who revealed that quinine inhibited pathogenic organism including S. aureus, P. aeruginosa, E. coli, Proteus mirabilis, Salmonella thypi, and Streptococcus pyogenes [12]. Quinine derivatives tend to have quite good activity against gram-negative bacteria strains such as E. coli and P. aeruginosa in the dose of more than 5%, however gram-positive bacteria (S. aureus and B. substilis) apparently were more resistant to quinine-derived compounds (table 1).
Table 1. Zone of Inhibition at different concentration of quinine-derived compounds for different pathogenic bacteria using disk-diffusion method.

| Compound                  | Concentration of extract (%) | Zone of inhibition (mm)\(^a\) | Gram-negative bacteria | Gram-positive bacteria |
|---------------------------|------------------------------|--------------------------------|------------------------|------------------------|
|                           |                              |                                | E. coli               | P. aeruginosa          | S. aureus | B. subtilis |
| Quinine                   | 0.5                          | -                              | -                      | -                      | -         | -          |
|                           | 1.0                          | 7.5±0.70                       | -                      | -                      | -         | -          |
|                           | 1.5                          | 11.5±0.58                      | 10±0.21               | -                      | -         | -          |
|                           | 5.0                          | 12.5±0.56                      | 15±0.34               | 8.5±0.60               | 12.5±0.19 | -          |
|                           | 10.0                         | 15.5±0.42                      | 20±0.30               | 10.5±0.33              | 15±0.18   | -          |
| Ester quinine propionate  | 0.5                          | -                              | -                      | -                      | -         | -          |
|                           | 1.0                          | -                              | -                      | -                      | -         | -          |
|                           | 1.5                          | 7±0.36                         | 13±0.2                | -                      | -         | -          |
|                           | 5.0                          | 8.5±0.23                       | 18±0.16               | 8±0.32                 | 10±0.22   | -          |
|                           | 10.0                         | 10±0.42                        | 23.5±0.14             | 9±0.24                 | 14.5±0.19 | -          |
| Ester quinine hexanoate   | 0.5                          | -                              | -                      | -                      | -         | -          |
|                           | 1.0                          | -                              | 7±0.19                | -                      | -         | -          |
|                           | 1.5                          | 8±0.20                         | 7.5±0.26              | -                      | -         | -          |
|                           | 5.0                          | 8.5±0.23                       | 8±0.11                | -                      | 7±0.30    | -          |
|                           | 10.0                         | 9.25±0.36                      | 10±0.23               | -                      | 10±0.21   | -          |
| Ester quinine benzoate    | 0.5                          | -                              | -                      | -                      | -         | -          |
|                           | 1.0                          | -                              | -                      | -                      | -         | -          |
|                           | 1.5                          | -                              | -                      | -                      | -         | -          |
|                           | 5.0                          | 9±0.09                         | -                      | -                      | -         | -          |
|                           | 10.0                         | 11±0.17                        | -                      | -                      | -         | -          |
| Ester quinine 2-chloro benzoate | 0.5 | -                              | -                      | -                      | -         | -          |
|                           | 1.0                          | -                              | -                      | -                      | -         | -          |
|                           | 1.5                          | -                              | -                      | -                      | -         | -          |
|                           | 5.0                          | -                              | -                      | -                      | -         | -          |
|                           | 10.0                         | -                              | -                      | -                      | -         | -          |
| Ester quinine heptanoate  | 0.5                          | -                              | -                      | -                      | -         | -          |
|                           | 1.0                          | -                              | -                      | -                      | -         | -          |
|                           | 1.5                          | 5±0.16                         | 8±0.25                | -                      | -         | -          |
|                           | 5.0                          | 7±0.13                         | 8±0.35                | -                      | -         | -          |
|                           | 10.0                         | 8.5±0.25                       | 11±0.32               | -                      | -         | -          |
| Streptomycin              | 0.125                        | 12±0.17                        | 12±0.20               | 12±0.10                | 8±0.26    | -          |

\(^a\)value of minus disc diameters.  
*Data are represented as mean of inhibition ±SEM, P<0.005 versus positive control value using one-way ANOVA and Duncan post-hoc test. SEM: standard error of mean.
Figure 2. Growth inhibition of some tested pathogenic bacterial strains by different concentration of ester quinine propionate using disk diffusion method.

Out of all derivatives, ester quinine propionate was found to be the most active compound among other quinine derivatives since it significantly inhibited growth inhibition activity to all bacterial strains tested and produced zones greater than 8 mm. Ester quinine propionate showed the highest activity against \textit{P. aeruginosa} with a range inhibition zone from 13 to 23.5 mm. The same derivative was quite active against other tested bacterial strains as well with a range of inhibition zone from 7 to 13 mm (table 1). On the other hand, long alkyl chain substitute quinine derivatives (ester quinine hexanoate and ester quinine heptanoate) showed moderate activity only against gram-negative bacteria strains. Those derivatives showed no activity for gram-positive bacteria. Likewise, the shorter alkyl chain quinine derivative exhibited better antimicrobial activity against the microorganisms tested, compared to the long chain ones.

Quinine have been reported in numerous reports to exhibit antimicrobial activity against \textit{E. coli} and \textit{Streptococcus pneumoniae} [11,12,15]. Quinine has been reported to inhibit the proteolipid subunit of the F0F1 H+ - ATPase of \textit{Streptococcus pneumoniae} [17]. Combination of quinine and other phytochemicals, such as quercetin and reserpine, showed significant reduction of biofilm formation on \textit{S. aureus} [18]. Straube et al. 1993 also revealed that quinine sulfate showed antibacterial activity by inhibiting the adhesion and invasion of \textit{E. coli} in host cells during the urinary tract infections [19]. Study by Wolf et al in 2005 also indicated that quinine sulfate protects the host cell by interfering the invasion of \textit{Salmonella typhimurium} and \textit{Shigella flexeri}, even it did not show any inhibitory effect on their bacterial growth [20]. A quinine-derived compound, quinolones, also had been reported to be have bactericidal effect by preventing biofilm formation and interfering the maintenance of chromosomal topology by targeting enzyme topoisomerase II and IV [21,22]. Meanwhile, ester quinine 2-chloro benzoate exhibited no inhibition effect against any tested microorganisms.

| Table 2. Minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) of quinine derivatives for different pathogenic bacterial strains. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Quinine         | Ester quinine propionate | Ester quinine hexanoate | Ester quinine heptanoate | Ester quinine 2-chloro benzoate |
| \textit{E. coli} | MIC %           | 0.25-0.5         | 0.5-1.25         | 2.5-5           | 2.5-5           |
|                 | MLC %           | 0.5-0.125        | 2.5-5            | >5              | >5              |
| \textit{P. aeruginosa} | MIC % | 0.5-1.25         | 0.5-1.25         | 2.5-5           | 2.5-5           |
|                 | MLC %           | 2.5-5            | 2.5-5            | 2.5-5           | 2.5-5           |
| \textit{S. aureus} | MIC % | 2.5-5            | 2.5-5            | -               | -               |
|                 | MLC %           | 2.5-1.25         | -                | -               | -               |
| \textit{B. subtilis} | MIC % | 2.5-5            | 1.25-2.5         | 2.5-5           | -               |
|                 | MLC %           | 2.5-5            | >5               | -               | -               |
Table 2 demonstrated that out of all quinine derivatives, ester quinine propionate effectively inhibited the growth of all the bacterial pathogens tested though their sensitivity quite varied in each pathogens. Ester quinine propionate remarked strong inhibition to the growth of *E. coli*, *P. aeruginosa*, and *B. substilis* with the MIC of 0.5-1.25% and MLC of 2.5-5%. However, *S. aureus* were mildly sensitive to ester quinine propionate. In addition, other quinine-derived compounds did not present any antimicrobial activity against previously-mentioned bacterial strain (figure 4). Apparently, *S. aureus* has demonstrated an ability to respond to new active compound and develop the resistant mechanism, cause it more resistant to compound tested [23]. In the present study, few of quinine derivatives were found to have antibacterial properties though the MIC was considered high. Therefore, it may be potential for additional compound to increase the synergistic activity of available drugs.

4. Conclusion
In summary, this current preliminary screening study suggested that quinine-derived compounds reduced the growth of human pathogenic microorganisms tested thus the compounds potentially work as antimicrobial agents for both variety of gram-negative and gram-positive bacterial strains. Although some quinine derivatives may serve as a beneficial antibacterial agent, the mechanisms remain unclear. Further well designed and detailed studies are required to be conducted to evaluate the mechanism of quinine derivatives and their toxicity *in vitro*, notably ester quinine propionate, and the possibility use of that derivative compound as a combination to increase the synergistic activity with conventional available drugs. Additionally, the structure-activity relationship (SAR) analysis is necessary to investigate the relationship between chemical structure and its biological properties.

Acknowledgments
This study was financially supported by the grants from The Ministry of Research, Technology and Higher Education of the Republic of Indonesia through INSINAS (2019). The authors also are thankful for the support from Research Center for Chemistry – Indonesian Institute of Sciences for the facilitation of the process to conduct this study.

References
[1] Escaich S 2010 Expert. Opin. Ther. Pat. 20 1401-18
[2] O’Neill J 2014 Review on Antimicrobial Resistance: Tackling a crisis for the health and wealth of nation (London: Review on Antimicrobial Resistance)
[3] Stojković D, Petrović J, Soković M, Glamočlija J, Kukić-Marković J and Petrović S 2013 J. Sci. Food. Agr. 93(13) 3205-8
[4] Othman L, Sleiman A and Abdel-Massih RM 2019 Front Microbiol. 10 911
[5] Lai P and Roy J 2004 Curr Med Chem. 11(11) 1452-60
[6] Santos FA and Rao VSN 2011 J. Pharm. Pharmacol. 50(2) 225-9
[7] Krishnavenil M and Suresh K 2015 Int. J. Curr. Res. Aca. Rev. 3(3) 169-78
[8] Ning X, Shi X and Yang G 2016 Int. J. Mol. Sci. 17(5) 504
[9] Wolf R, Baroni A, Greco R, Donnarumma G, Ruocco E and Tufano MA 2002 Ann. Clin. Microbiol. Antimicrob. 1 5
[10] Kharal SA, Hussain Q, Ali F and Fakhruddin 2009 J. Pak. Med. Assoc. 59(4) 208-11
[11] Završnik D, Muratović S, Špirtović S, Softić D and Medić-Šarić M 2008 Bosn. J. Basic. Med. Sci. 8(3) 277-81
[12] Farhadi F, Khameneh B, Iranshahi M, Iranshahy M 2018 Phytother. Res. 33(1) 13-40
[13] Gatti R, Gioia MG, Cavrini V 2004 Anal. Chim. Acta. 512 85–91
[14] Bauer AW, Kirby WMM, Serris JC and Turck M 1966 American. J. Clin. Pathol. 45 493-6
[15] Brown RE, Stancato FA and Wolfe AD 1979 Life Sciences 25 1857-64
[16] Kunin CM and Ellis WY 2000 Antimicrob. Agent. Ch. 44(4) 848-52
[17] Muñoz R, Garcia E and De la Campa AG 1996 J. Bacteriol. 178(8) 2455-8
[18] Abreu AC, Saavedra MJ, Simões LC and Simões M 2016 Biofouling. 32(9) 1103-14
[19] Straube E, Schmidt G, Marre R and Hacker J 1993 *Zbl. Bakt.* **278** 218-28
[20] Wolf R, Grimaldi E, Donnarumma G, Greco R, Auricchio L, De Filippis A and Tufano MA 2005 *J. Travel. Med.* **12** 343-6
[21] Skogman ME, Kujala J, Busygin I, Leino R, Vuorela PM and Fallarero A 2012 *Nat. Prod. Commun.* **7**(9) 1173-6
[22] Kohanski MA, Dwyer DJ and Collins JJ 2010 *Nat. Rev. Microbiol.* **8** 423-35
[23] Kane TL, Carothers KE and Lee SW 2018 *Curr. Drug. Target.* **19**(2) 111-27