



Research article

Effect of naturally isolated hydroquinone in disturbing the cell membrane integrity of *Pseudomonas aeruginosa* MTCC 741 and *Staphylococcus aureus* MTCC 740Venkadapathi Jeyanthi^{a,b,*}, Palaniyandi Velusamy^{a,**}, Govindarajan Venkat Kumar^{a,c}, Kannan Kiruba^d^a Department of Biotechnology, School of Bioengineering, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu District, 603 203, India^b Department of Biotechnology, SRM Arts and Science College, Kattankulathur, Chengalpattu District, 603203, Tamil Nadu, India^c Department of Biotechnology, Ponnaiyah Ramajayam Institute of Science and Technology, Thanjavur 613403, Tamil Nadu, India^d Department of Biotechnology, University of Madras, Guindy Campus, Chennai 600 025, Tamil Nadu, India

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ABSTRACT

Nosocomial pathogens cause various health problems in human and many novel drugs are under investigation to combat the pathogens. The present study explains the naturally derived hydroquinone possible mode of action against *Pseudomonas aeruginosa* MTCC 741 and *Staphylococcus aureus* MTCC 740. Time kill studies, cell viability assays, membrane potential assays, and potassium release assays were carried out to study the mode of action. Time kill studies revealed the rapid death of bacterial pathogens exposed to 4X MIC (Minimum inhibitory concentration) of the hydroquinone. Cell viability assay results showed that nearly half of the cell destruction of test pathogens occurred within one hour. Transmission electron microscopic (TEM) observations revealed the disruption of the cell membrane, which caused severe ultrastructural changes in both test pathogens. Hydroquinone dissipated the membrane potential of test pathogens, as confirmed by the depolarization of membrane potential, increases in permeability and leakage of intracellular potassium ions. At the concentration of 2X MIC hydroquinone in 5 min, about 91.41% and 84.85% potassium ions were released from *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740, respectively. This is the first report on the mode of action of naturally derived hydroquinone against clinical pathogens.

1. Introduction

In recent years, a rapid increase in serious infections caused by clinical pathogens that are resistant to multiple antibiotics has been observed. Among various pathogens, infections caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus* are amongst the most serious threats to human health (Gerits et al., 2016). The opportunistic pathogen *Pseudomonas aeruginosa* is a Gram-negative, rod-shaped bacterium and responsible for 10–15% of nosocomial infections worldwide (Strateva et al., 2007). It is the common cause of hospital-acquired, health-care-associated, and ventilator-associated pneumonia. It is a major threat to public health because of its ubiquitous nature (Ullah et al., 2016). Furthermore, it represents a significant cause of morbidity in patients suffering from serious burns, cystic fibrosis, urinary tract infections,

sepsis, and receiving intensive care treatment (Youssef et al., 2011; Ouberai et al., 2011). The Gram-positive, cocci shaped *Staphylococcus aureus* is another important pathogen that is generally involved in nosocomial and community-acquired infections. The nosocomial-acquired infections include bacteremia, surgical wound infections, and implant-related infections. Infections caused by community-acquired *S. aureus* involve skin, soft tissue infections and pneumonia (Moellering, 2006; Gerits et al., 2016). Over the years, *S. aureus* has developed multiple strategies to suppress the human immune system and withstand antibiotic therapy. This led to the emergence and spread of methicillin-resistant *Staphylococcus aureus* (MRSA), which has caused a major problem to human society (Tong et al., 2015). *P. aeruginosa* and *S. aureus* are the most commonly found pathogens in the mucus of cystic fibrosis (CF) patients and both are associated with poor respiratory

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functions (Limoli and Hoffman, 2019; Orazi et al., 2019). Moreover, the emergence of antibiotic-resistant pathogens highlights the need for novel antimicrobial agents.

Quinones consist of an aromatic ring with two substitutions for ketones. The antimicrobial activity of quinones is derived from their ability to donate free radicals. Reactive oxygen species (ROS) are a group of free radicals having a significant antimicrobial effect against bacterial pathogens (Memar et al., 2018). The aromatic organic compound hydroquinone has been used for decades in the treatment of hyperpigmentation disorders (Parsaeimehr et al., 2017). It is also known as benzene-1, 4-diol and, most commonly used chemical agent for skin lightning. It is widely used for the production of antioxidants, agrochemicals, polymers, cosmetics, pharmaceuticals as well as in paper manufacturing and photographic developers (Ghadimi et al., 2017). Apart from this, it is also shown to have different biological activities such as antibacterial, insecticidal, cytotoxicity, phospholipase A2 (PLA2) inhibitor and anti-HIV properties (Qiu and Wang, 2008). Generally, hydroquinone is chemically synthesized using benzene as the starting material. In general, humans are exposed to hydroquinone by occupational, food and environmental sources and are associated with an increased risk of adverse health effects (DeCaprio, 1999). The benzene derivative compound named hydroquinone (benzene-1, 4-diol) has the molecular formula is $C_6H_6O_2$. It is soluble in water and has a molecular weight of 110.112 Da. In our previous study, we isolated a naturally derived hydroquinone from halotolerant *Bacillus methylotrophicus* MHC10 inhabiting a saltpan to counteract those drawbacks (Jeyanthi et al., 2016). It has been suggested that flow cytometry is a quick and reliable method to study the effect of antimicrobial agents on bacterial cells. It is a popular technique for the analysis of individual cells and yields information within minutes. To determine the cell viability, fluorescent nucleic acid binding dye, propidium iodide (PI) is widely used. PI can only penetrate cells with compromised bacterial membranes, binding to the nucleic acid of dead or damaged cells by intercalating between the bases (Novo et al., 2000). Meanwhile, the flow cytometric analysis of bacterial membrane potential was carried out for many years using membrane potential-sensitive fluorescent probe, DiSC3(5) (Zhang et al., 2000). Membrane potential (MP) is of crucial importance in bacterial physiology. It enables various processes such as ATP production, bacterial autolysis, transport of glucose, chemotaxis, and survival at low pH (Novo et al., 1999). For many antimicrobial agents, bactericidal activity through the disruption of membrane potential is the proposed mechanism of action (Silverman et al., 2003). Hence, the present study was undertaken to determine the possible mode of action of hydroquinone on the membrane of *S. aureus* and *P. aeruginosa* as test pathogens.

2. Materials and methods

2.1. Naturally derived hydroquinone and test pathogens

The production, purification, and characterization of antimicrobial compound hydroquinone from halotolerant *Bacillus methylotrophicus* MCC 2831 were previously reported by our research team (Jeyanthi et al., 2016). The MIC values of purified hydroquinone were found to be 7.81 $\mu\text{g}/\text{mL}$ and 15.625 $\mu\text{g}/\text{mL}$ against *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740 respectively. The same test pathogens were used for this study and the culture were maintained in Trypticase Soy Broth (TSB) medium (Hi-Media Laboratories, Mumbai, India) containing 20 % (v/v) glycerol at -70°C .

2.2. Time-kill kinetics assay

Time-kill kinetic studies of hydroquinone treated bacterial pathogens was performed according to the National Committee for Clinical Laboratory Standard (NCCLS) guidelines (NCCLS, 2006). The bacterial culture was diluted in Mueller Hinton Broth (MHB) medium to adjust the cell density to approximately 10^7 CFU/mL. The death rate was evaluated by

measuring the reduction in viable bacteria (log 10 CFU/mL) at 2 h time intervals for 24 h at 4X MIC of the compound. Samples were removed at defined intervals and the serially diluted samples were plated on (Mueller Hinton agar) MHA medium for the enumeration of colonies. The time-kill curves for bacterial pathogens were constructed by plotting numbers of viable cells versus time. The maximum killing was observed in the concentration of 4X MIC of the compound. All the experiments were performed in triplicates. The statistical analysis was performed using ORIGIN PRO software.

2.3. Determination of cell viability

The cell viability of *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740 treated with hydroquinone was determined using a BD FACS calibur flow cytometry system (BD Biosciences, USA) (Novo et al., 2000). The suspension (10^7 CFU/mL) of test bacterial pathogens was treated with 4X MIC of hydroquinone for 1 h and 2 h. The untreated bacteria were used as negative control. The bacterial cells were then harvested, washed twice with phosphate-buffered saline (PBS) and, resuspended in the same buffer. To the cell suspension, PI dye (final concentration 2 $\mu\text{g}/\text{mL}$) was added. It was then incubated for 15 min at room temperature to enable the uptake of dye. The dead bacteria are stained fluorescent red by PI. The PI fluorescence was excited at 485 nm and was measured at 620 nm using a BD FACSCalibur flow cytometry system (BD Biosciences, USA). For analyzing the data, BD CellQuest Pro software was used. Based on the side light scatter and PI, the R2 and R3 gates were used to identify live and damaged or dead cells, respectively. All the experiments were performed in triplicates.

2.4. Electron microscopic analysis

The electron microscopic analysis of hydroquinone treated bacterial pathogens was examined according to the method of Hartmann et al. (2010). TEM was used to examine the ultrastructural changes in test pathogens. The bacterial suspension (10^7 CFU/mL) was treated with 4X MIC of the compound for 30 min, 1 h and 2 h against *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740, individually. A drop of treated bacterial suspension was placed onto the TEM grid. It was then fixed with 2 % glutaraldehyde for 1 h and washed thrice with distilled water. Then, it was post fixed with 0.2% osmium tetroxide for 1 h followed by the dehydration of samples with ethanol series (30, 50, 70, 85 and 100 %) and air-dried. Finally, ultrastructural changes in the bacterial cells were examined by using FEI-Philips TECHNAI 10 TEM (FEI Company, USA) under standard operating conditions.

2.5. Membrane potential assay

The membrane potential of *S. aureus* MTCC 740 and *P. aeruginosa* MTCC 741 was tested using the fluorescence probe DiSC₃(5) by the method of Huang and Yousef (2014). Bacterial cells in the mid-logarithmic phase were centrifuged, washed in 5mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.8) and resuspended in the same buffer to an optical density at 600nm of 0.05. The fluorescent probe DiSC₃(5) was added to the cell suspension to a final concentration of 0.5 μM . The mixture was incubated for 15 min at room temperature to allow the uptake of the DiSC₃(5) probe. After incubation, potassium chloride was added to the cell suspension at a final concentration of 100mM. To the 900 μL of cell suspension, 100 μL of 4X MIC Hydroquinone was added and incubated for 30 min and 1h separately. For the negative control, the live (untreated) bacteria were used. The proton ionophore, CCCP (10 μM) treated with the bacterial pathogen for 1h was used as the positive control. A change in fluorescence due to membrane depolarization was recorded using a BD FACSCalibur flow cytometry system at an excitation wavelength of 622 nm and an emission wavelength of 670 nm. All the experiments were performed in triplicates.

2.6. Potassium release assay

Potassium ions released from *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740 cells were determined followed by the method of Huang and Yousef (2014) using a K^+ -sensitive probe, PBFI which is impermeable to bacterial cells. Bacterial cells in the mid-logarithmic phase were centrifuged, washed in 5 mM HEPES buffer, and resuspended in the same buffer to an optical density at 600 nm of 0.05. To 900 μ L of the cell suspension, 10 μ L of the 1mM PBFI probe was added. This was followed by the addition of 100 μ L of 1X and 2X MIC of hydroquinone. A change in fluorescence corresponding to potassium concentration for every 1 min over 5 min was recorded using the BD FACSCalibur flow cytometer at an excitation wavelength of 346 nm and an emission wavelength of 505 nm. All the experiments were performed in triplicates. The statistical analysis was performed using ORIGIN PRO software.

3. Results and discussion

3.1. Time-kill kinetics studies

The killing kinetics were analyzed to evaluate the activity of hydroquinone against test pathogens. It can determine the bactericidal or bacteriostatic activity of an agent over time. Both test pathogens, *P. aeruginosa* MTCC 741, and *S. aureus* MTCC 740 showed an effective response to 4X MIC of hydroquinone treatment. In *P. aeruginosa* MTCC 741, there was a 3-log reduction in the number of CFU/mL in 2 h and further 3-log reduction was observed in 4 h (Figure 1A). In *S. aureus* MTCC 740, 1-log reduction was observed in 2 h, and a further 3-log reduction was noticed in 4 h (Figure 1B). The error bars in graph represented the standard error obtained from triplicate samples and P values included. Similar results were obtained with all three replicates. Many researchers have investigated the effect of quinone derivative compounds against clinical pathogens. Ooi et al. (2013) evaluated the time-kill kinetics of tert-butylhydroquinone (TBHQ) and tert-butylbenzoquinone (TBBQ) against *Staphylococcus aureus* SH1000. For the first 120 min of analysis, TBHQ had no effect on bacterial viability. The conversion of TBHQ to TBBQ initiated the bactericidal effect and showed a 5 log reduction in cell number within 3 h. Kim et al. (2010) reported the bactericidal effect of 2, 6-dimethoxy-1, 4-benzoquinone (DMBQ) by a time-kill curve experiment. The bactericidal effect of DMBQ was observed against *S. typhimurium* and *S. aureus* after 12 h of incubation. It indicated that doubling the MIC of DMBQ showed a significant reduction in the growth rate of bacterial pathogens.

3.2. Determination of cell viability and biocompatibility

Many researchers have used the flow cytometry technique to determine the effect of antibacterial compounds on pathogens using

fluorescent probes (Ruger et al., 2012). Novo et al. (2000) studied the cell viability of *S. aureus* and *Micrococcus luteus* treated with standard antibiotics such as amoxicillin, tetracycline, and erythromycin. Here the fluorescent probe PI, bound to the nucleic acid of dead and damaged cells with compromised bacterial membranes after 4 h of exposure to antibiotics. Similarly, in our previous study (Jeyanthi and Velusamy 2016), the cell viability of MRSA treated with 4X MIC of the phenolic compound for different time intervals was investigated. The nucleic acid probe, PI bound to DNA by intercalating between the bases and emitted red fluorescence. In our current study, the cell viability test was performed using flow cytometry to determine the antibacterial effect of 4X MIC of hydroquinone (Figure 2). Flow cytometry observation showed that the bacterial cell membrane was disrupted as the nucleic acid dye PI binds with bacterial nucleic acid. Therefore, the PI dye exhibited red fluorescence in the dead cells and the live cells emitted green fluorescence. It was observed that, within 1 h, nearly half the cell population was destroyed as 46.12 % and 50.52 % in *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740 respectively. Hydroquinone at 2–4% concentration is widely used for hyperpigmentation. No toxicity has been observed in human volunteers with the topical application (Talakoub et al., 2009; Stephens et al., 2018). However, its prolonged exposure at very high doses leads to skin disorders. Hence, the US Food and Drug Administration approved this compound as the triple-combination cream (TCC) containing 4% hydroquinone, 0.05% tretinoin and 0.01% fluocinolone acetonide for the topical treatment (Shin and Park, 2014). On the other hand, *S. aureus* and *P. aeruginosa* were the most common bacteria isolated from the skin lesions of patients with dermatitis. Hence, hydroquinone could act as a potent agent for treatment of skin disorders as well as an antibacterial agent. Shimada et al. (2012) studied the *in vivo* hydroquinone toxicity in mice by inhalation. Their findings showed that high dose exposure causes hepatic and renal adenoma. Likewise, *in vitro* hydroquinone toxicity studies on cultured human cell lines have been shown to cause mononuclear cell leukemia [Regev et al., 2012]. Similarly, tert-butylhydroquinone (tBHQ), a hydroquinone derivative is widely used as a food preservative. The acceptable daily intake of tBHQ is 0.7 mg/kg body weight as approved by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Some *in vitro* studies indicated that exposure to high levels of tBHQ was carcinogenic in animals (Gharavi et al., 2007). Hence, hydroquinone at higher concentration is a potential toxic agent to mammalian and human cells.

3.3. Ultrastructural changes in test pathogens

The bacterial cell membrane is responsible for essential cellular functions. The membrane integrity is important for all functions and its disruption causes metabolic dysfunction, which leads to cell death (Lunde et al., 2009). In TEM analysis (Figure 3), the ultrastructural

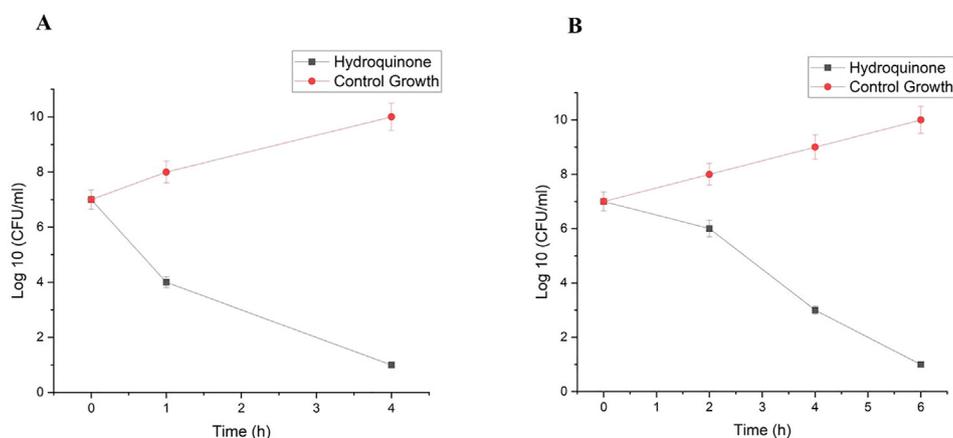


Figure 1. Time kill curves with (A) *Pseudomonas aeruginosa* MTCC 741 and (B) *Staphylococcus aureus* MTCC 740. Symbols: ● growth control; ■ hydroquinone 4X MIC. The results are given as the mean \pm SD (n = 3) and *p-values of ≤ 0.05 are considered to be significant.

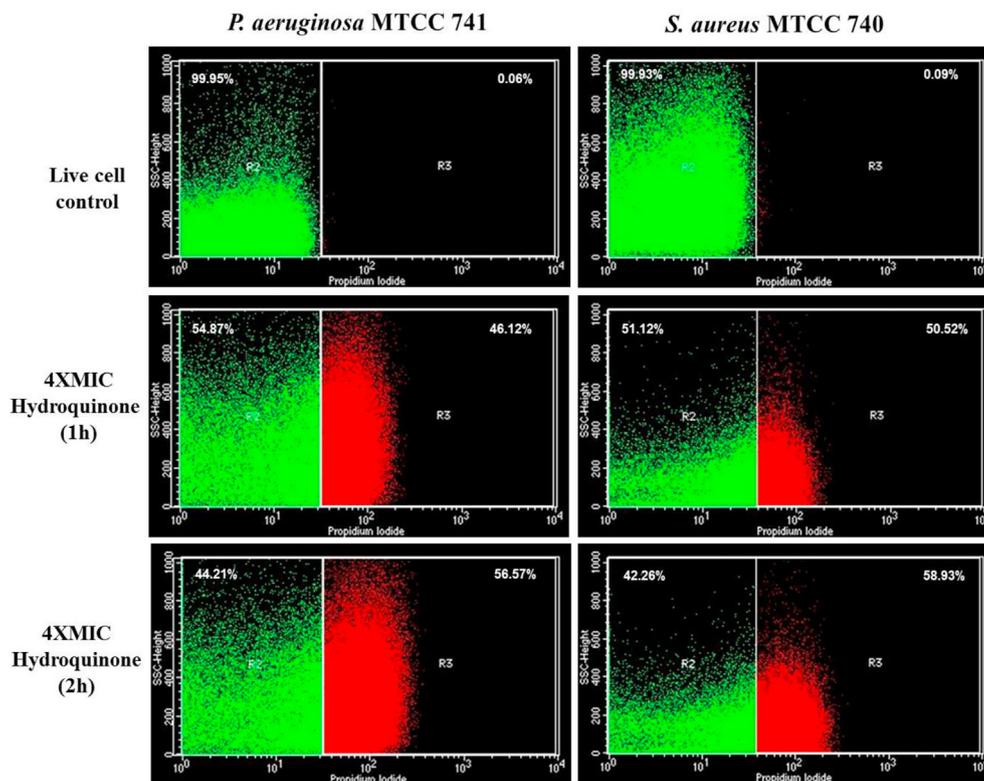


Figure 2. Flow cytometric analysis represents the dot plot profiles of bacterial cells treated with 4X MIC of hydroquinone. The percentages of live (green fluorescence) and dead cells (red fluorescence) are mentioned.

changes in bacteria were observed after treatment with 4X MIC of hydroquinone. The bacterial membrane damage of both *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740 was observed at 30 min and the membranes were deformed severely in 1 h. After 2 h, *P. aeruginosa* MTCC 741 cells appeared to be broken and in the case of *S. aureus* MTCC 740, the cell contents were expelled into the extracellular space. Hartmann et al. (2010) described the TEM analysis of bacterial cell damage in *E. coli* DSM 1103 and *S. aureus* DSM 1104 caused by antimicrobial peptides gramicidin S and peptidyl-glycylleucine-carboxyamide (PGLa). It revealed several distinct signs of damage to the cell envelope such as blisters,

protruding bubbles on the cell surface of *E. coli*, and non-membrane-enclosed bodies were observed in *S. aureus*. Ultrastructural changes in *P. aeruginosa* and *S. aureus* treated with Burow's solution were studied by Hyo et al. (2012). Burow's solution is an aqueous solution of aluminium triacetate. It has antibacterial properties and is used as a topical treatment for skin infections. In both bacteria, they found that the cytoplasmic membrane was detached from the cell and the cytoplasmic contents were released out of the cell. The Gram-positive *S. aureus* is primarily composed of peptidoglycan. The hydroquinone treatment caused the accumulation of ROS and affects the peptidoglycan.

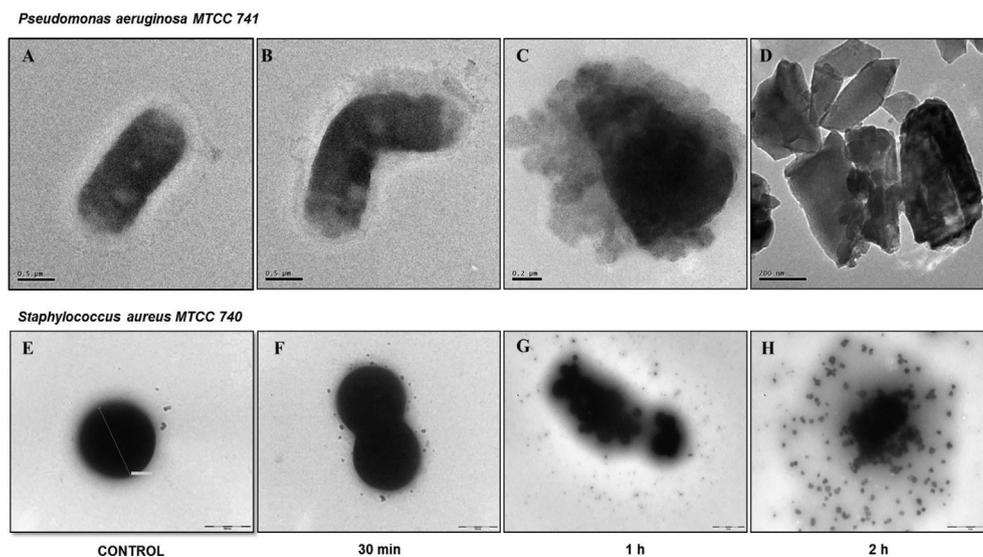


Figure 3. TEM images of *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740 treated with 4X MIC hydroquinone. Untreated control cells (A, E). Cells treated for 30 min (B, F), 1h (C, G), 2h (D, H). The width of the images corresponds to a distance of 0.5 µm (A, B, E, F) and 0.2 µm (C, D, G, H).

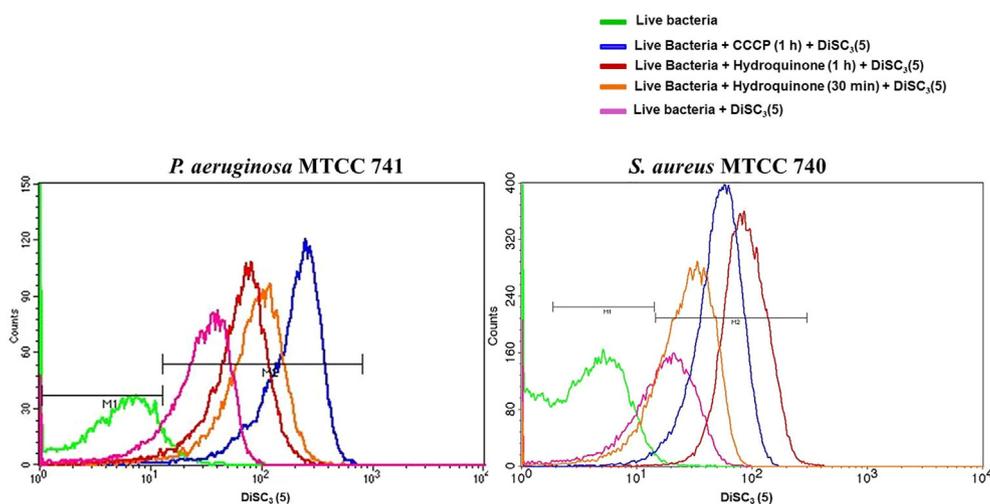


Figure 4. Changes in bacterial membrane potential of *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740 in the presence of 4X MIC hydroquinone.

Lipopolysaccharide is an essential component found on the outer membrane of Gram-negative *P. aeruginosa*. Many pieces of evidence showed that antimicrobial compounds could inhibit the lipid synthesis and blocked the movement of solutes between the periplasm and external cell surface (Eband et al., 2016). Porins are proteins found in the outer membrane of Gram-negative bacteria. They form water-filled open channels across the membrane that allows the transportation of ions, sugars and amino acids. Previous studies reported that antimicrobial compounds could pass through porins and create pores in the cell membrane resulting in cellular damage (Pages and James, 2008). In the present study, TEM studies revealed that the hydroquinone treatment damaged the outer membrane of *P. aeruginosa* and *S. aureus* by ROS accumulation, through the creation of pores and resulting in bacterial death.

3.4. Effect of hydroquinone on cell membrane potential

Bacteria maintain a proton gradient known as the proton motive force (PMF), generated across the cell membrane. The PMF is necessary for ATP synthesis and to transport nutrients into the cells (Farha et al., 2013). The structure of the bacterial cell membrane can be disrupted by hydroquinone that causing membrane components and ions to be released into the bacterial broth. Many researchers also proved that aromatic compounds could interfere with the bacterial cell wall to make

modifications in the integrity of the cytoplasmic membrane. This leads to the dissipation of proton-motive force (PMF) and prohibits the activity of membrane enzymes (Pumbwe et al., 2007). Herein, the aromatic derivative compound hydroquinone could have targeted the cytoplasmic membrane components on both Gram-negative as well as Gram-positive bacteria. The PMF consists of the sum of the electric potential ($\Delta\psi$) and the transmembrane proton gradient (ΔpH). This gradient was assessed with a membrane potential-sensitive fluorescent probe, DiSC₃(5). This dye can penetrate the bacterial outer membrane, and is taken up by normal bacterial cells, depending on the magnitude of the cytoplasmic membrane's electrical potential gradient. It is then accumulated in the cytoplasmic membrane and causes self-quenching (Winkel et al., 2016). Herein, the effect of hydroquinone was studied on the membrane potential of *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740 (Figure 4). DiSC₃(5) probe accumulates in the healthier polarized cell membrane and the fluorescence decreases due to the partition of dye on the surface of polarized cells. Hence control cells (no drug treatment) produced a low signal. Hydroquinone at the concentration of 4X MIC significantly permeabilized the cell membrane of both bacterial species in 30 min. It led to the loss of membrane potential and the DiSC₃(5) was released into the medium leading to an increase in fluorescence. Within 1h, the membrane potential was further reduced and fluorescence intensity was increased. The positive control, carbonyl cyanide 3-chlorophenylhydrazone (CCCP), which is a membrane inhibitor, effectively permeabilized the

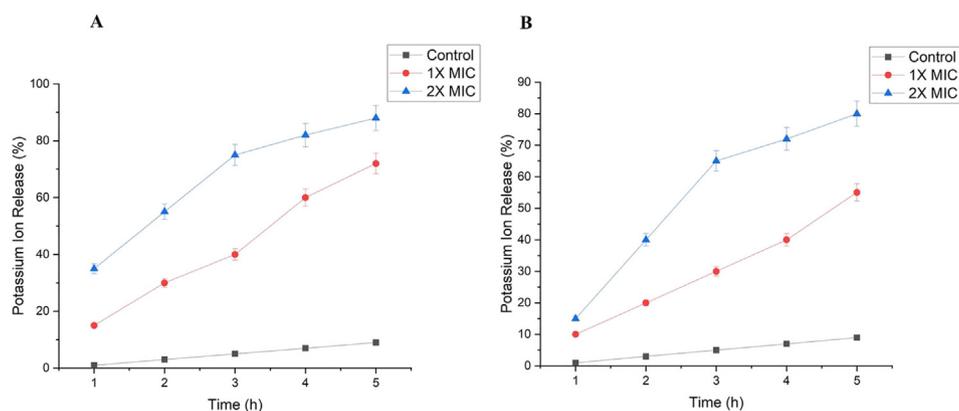


Figure 5. Release of potassium ions from *P. aeruginosa* MTCC 741 (A) and *S. aureus* MTCC 740 (B) induced by 1X and 2X MIC of hydroquinone. The results are given as the mean \pm SD ($n = 3$) and * p -values of ≤ 0.05 are considered to be significant.

membrane in 1h and produced more signal. Similarly, Zhang et al. (2000) indicated that the antibiotics such as Polymyxin B, Polymyxin E1, and Gramicidin S effectively permeabilized the cytoplasmic membrane of *P. aeruginosa*. The membrane was depolarized with the release of DiSC₃ (5) and a consequent increase in fluorescence was observed. Huang and Yousef (2014) also demonstrated that DiSC₃ (5) probe accumulated in the healthy polarized bacterial cell membrane and becomes self-quenched. The lipopeptide antibiotic paenibacterin at a concentration of 32 µg/mL significantly permeabilized the cell membrane of *S. aureus*. This led to the membrane depolarization by releasing the DiSC₃ (5) probe from the cell membrane, resulting in increased fluorescence.

3.5. Release of potassium ions (K⁺) by hydroquinone

Potassium is the most abundant intracellular cation present in bacteria and an essential part of the cellular process. The internal ionic environment of bacterial cells is generally rich in potassium ions. The leakage of this ion has been used to monitor the disruption of bacterial membrane caused by antibacterial agents (Orlov et al., 2002; Ochrombel et al., 2011). In the current study, membrane depolarization caused by hydroquinone led us to investigate whether membrane-associated functions affected or not. Treatment with hydroquinone triggered concentration-dependent leakage of the K⁺ from *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740. The leakage of potassium ions from *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740 bacterial cells were determined after treatment with 1X and 2X MIC of hydroquinone at every 1 min over 5 min. Hydroquinone treatment caused a concentration-dependent K⁺ leakage from bacterial cells. At low concentration (i.e., 1X MIC), the compound did not lead to significant potassium ion leakage from cells (Figure 5A). But at the concentration of 2X MIC in 5 min, about 91.41% and 84.85% potassium ions were released from *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740, respectively (Figure 5B). The error bars in graph represented the standard error obtained from triplicate samples and P values included. Similar results were obtained with all three replicates. Bonelli et al. (2006) performed the potassium release assay in *M. flavus*. They observed the most pronounced pore formation and rapid leakage of intracellular potassium ions induced by gallidermin and nisin at a concentration of 500 nM. In the present study, the action of the hydroquinone on test pathogens appeared to have similar effects. Higgins et al. (2005) also studied the potassium sensitive fluorescent probe PBFI to detect the leakage of K⁺ ions from MRSA exposed to a lipoglycopeptide antibiotic, telavancin. They found that the telavancin triggered the release of K⁺ ions from MRSA in a concentration-dependent manner, which confirmed the dissipation of cell membrane potential.

4. Conclusion

From the present study, it was concluded that the hydroquinone is showing anti-bacterial efficacy against *P. aeruginosa* MTCC 741 and *S. aureus* MTCC 740. Hydroquinone caused bacterial cell death mainly by compromising cell membrane integrity. Time kill studies, cell viability assays, membrane potential assays, and potassium release studies revealed that the hydroquinone (different concentration at different time intervals) damage the cell membrane of the bacteria through oxidative stress caused by ROS and intracellular K⁺ ions was released from the bacteria cell membrane. Moreover, the cell membrane damage was visually confirmed through TEM analysis. TEM analysis revealed the destabilization of bacterial membrane and leakage of the cell contents. However, further molecular mechanism studies should be performed to better understand the effect of hydroquinone on bacterial outer membrane. Our study showed that naturally derived hydroquinone has strong antibacterial activity and it could be considered as a potent agent against bacterial pathogens. Further, studies of hydroquinone against other nosocomial pathogens are underway.

Declarations

Author contribution statement

Venkadapathi Jeyanthi: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Palaniyandi Velusamy: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Govindarajan Venkat Kumar, Kannan Kiruba: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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