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Maximising microbial control: Synergistic potential in polyhexamethylene biguanide-benzalkonium chloride combinations

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ABSTRACT

Aims: The exploration of new strategies for effective microbial control is one of the most significant studies in developing new formulations of antimicrobial agents. The increasing prevalence of microbial threats is a pressing threat to public health. Hence, this study aims to investigate the synergies between combinations of polyhexamethylene biguanide (PHMB) and benzalkonium chloride (BKC) compared to the individual PHMB or BKC as active agents for microbial control. A set of combinations of the active ingredient was tested against two Gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*, and two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*.
Methodology and results: The antibacterial activity of PHMB and BKC was investigated using the microdilution method to determine their minimum inhibitory concentrations (MIC). The results showed that PHMB was more effective against Gram-positive bacteria, with a significant effect on *B. cereus* with a MIC value of 31.25 ppm, while having a lesser impact on *E. coli* (125 ppm). The MIC value of BKC was 15.625 ppm for the Gram-positive bacteria and showed a more prominent effect on Gram-negative bacteria. The MIC values were used as the baseline for formulating PHMB and BKC mixtures. The formulated combinations were evaluated using disc diffusion (DD) and well diffusion (WD) methods. The results indicated that the combined active agents have an efficiency similar to the stand-alone effect. The cytotoxic effect of these compounds was also assessed, and they showed toxic traits towards Vero cells, indicating that these two cationic agents acted additively towards each other.
Conclusion, significance and impact of study: The combination of PHMB and BKC represented a potential strategy of mixed antimicrobial agents, which could be applied in applications such as healthcare and consumer products.

Keywords: Antimicrobial activity, antimicrobial agents, benzalkonium chloride, disinfectants, polyhexamethylene biguanide

INTRODUCTION

Effective control of microbial growth and transmission is crucial in public health and hygiene. Microorganisms, including pathogenic bacteria, viruses and fungi, pose a constant threat to human health by causing infectious diseases and contaminating various surfaces and environments (Zhao *et al*., 2019). High-touch elements, dry surfaces and multi-user objects, such as bed rails, doorknobs, light switches, keyboards, shopping carts and elevator buttons, are common targets for microorganisms that have relatively long lifespans, have high risks of pathogen infection and need to be continuously sterilised with potent biocidal chemicals, disinfectants and antiseptics (Møretrø and Langsrud, 2017; Kampf *et al*., 2020).

Disinfectant products are chemical combinations that sterilise and remove microbiological infectious agents, excluding bacterial spores from inanimate objects, including biotic and abiotic surfaces and water sources (Rutala *et al*., 2008). Public health organisations such as WHO encourage good personal hygiene, including frequent hand washing with soap and using disinfectants to deactivate virus infectivity and eliminate infection likelihood. Disinfectant mechanism and resistance have been extensively studied, especially concerning the chemical mechanism of action, as well as pathogen surface and molecular makeup as the factors, with an

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increasing focus on how disinfectants may contribute to antibiotic-resistant microbes (Bridier *et al*., 2011; Rutala and Weber, 2021). Resistance occurs when bacteria are not inhibited by an antimicrobial agent concentration, similar to antibiotic resistance. Using a suitable disinfectant and controlling bacterial resistance is crucial by combining different disinfectants (Maillard, 2005; Jiang *et al*., 2018). *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* are used in this study due to their pathogenic nature and their association with various human infections. The selected bacteria represent a diverse range of Gram types. *B. cereus* and *S. aureus* are Gram-positive, while *P. aeruginosa* and *E. coli* are Gram-negative, which allows us to study the effectiveness of antimicrobial agents against different cell wall structures.

Polyhexamethylene biguanide (PHMB) is a cationic polymer biocide from the polymeric guanidine family that is commonly used due to its broad range of antimicrobial activity (Dias *et al*., 2021). The mechanism of action of PHMB has long been thought to be dominated by membrane disruption; nevertheless, current research also showed that PHMB, in particular, has the ability to circumvent the cell membrane and condense bacterial chromosomes (Chindera *et al*., 2016; Sowlati-Hashjin *et al*., 2020). PHMB is well tolerated on human skin, eyes and wounds, and its market is rapidly growing, including liquids, gels and antimicrobial dressings (Kaehn, 2010). Benzalkonium chloride (BKC) is a quaternary ammonium compound (QAC) used in a wide range of goods, both clinically prescribed and over-the-counter (Pereira and Tagkopoulos, 2019). BKC has been proven to have high efficacy in antimicrobial activity, bacterial growth inhibition and virus inactivation (Bondurant *et al*., 2020; Chin *et al*., 2020; Ogilvie *et al*., 2021). The use of BKC as a disinfectant against SARS-CoV-2 has been proven by the US EPA and Health Canada (Bondurant *et al*., 2020; Ogilvie *et al*., 2021). Subsequently, the synergistic effect of combining PHMB and BKC as active ingredients for antimicrobial control is an intriguing avenue of investigation.

Antimicrobial products in medical, industrial and household settings often contain multiple disinfectants, with the synergistic effect being a key mechanism. The synergistic combination of antimicrobials broadens the spectrum of antimicrobial action and prevents the emergence of resistant strains (Basavegowda and Baek, 2022). Combining antimicrobial agents reduces toxicity and provides long-lasting residual effects. Therefore, this study aims to explore the potential benefits of this approach. This study aims to explore the potential benefits of combining these two agents and assess whether their synergistic action can provide a more effective solution for microbial control in various applications, such as healthcare settings, food processing and environmental disinfection.

MATERIALS AND METHODS

Bacterial cultures

Two Gram-positive bacteria (*B. cereus* and *S. aureus*) and two Gram-negative bacteria (*E. coli* and *P. aeruginosa*) that have been found in the human environment and commonly selected for studies related to antimicrobial susceptibility were used in this study (Rozman *et al*., 2017; Upreti *et al*., 2018). All bacteria were obtained from the environmentally sourced bacterial library of the Marine Biotechnology Laboratory, Universiti Malaysia Terengganu. The bacteria were maintained weekly on nutrient agar at 30 °C for 12 h followed by storage at 4 °C. For the preparation of inoculum, the bacterial colonies were inoculated in sterile distilled water and vigorously mixed to match the 0.5 McFarland standard. The final inoculum concentration or colony-forming unit (CFU) for every test bacterium was set at 1×10^8 CFU/mL.

Resazurin-aided microdilution assay

The minimum inhibitory concentrations (MIC) of PHMB and BKC against selected bacteria of *B. cereus*, *S. aureus*, *E. coli* and *P. aeruginosa* were determined by using the resazurin-aided microdilution method (Elshikh *et al*., 2016). Overnight-grown cultures of *B. cereus*, *S. aureus*, *E. coli* and *P. aeruginosa* were inoculated and diluted to match the 0.5 McFarland standard, along with the growth medium and the wells of 96-well plates. A concentration of 2000 ppm PHMB and BKC was prepared in Mueller-Hinton (MH) broth and added to the first wells of the first row of the plate wells. Then, a double serial dilution of PHMB-BKC broth mix was done and the diluted bacterial suspensions were added in a ratio of 1:1. The whole process did not exceed 15 min before the incubation period. The plate was incubated at 30 °C for 24 h. After the incubation, 0.015% resazurin was added to each well. Colour changes of blue-coloured resazurin into pink or colourless were observed. The wells with no colour changes before the first wells with colour changes were recorded as the MIC level of each bacterium.

Preparation of polyhexamethylene biguanide-benzalkonium chloride (PHMB-BKC) formulations

The MIC values obtained from the resazurin-aided microdilution assay were used to establish the baseline for the formulation of PHMB and BKC mixtures (Table 1). PHMB and BKC were diluted in phosphate-buffered saline (PBS) following appropriate ratios between them.

Disc diffusion (DD) assay

The disc diffusion (DD) method was referred to study the inhibition zone for bacterial growth (Bauer *et al*., 1966; Bonev *et al*., 2008; Matushcek *et al*., 2014). For this experiment, discs containing antibacterial formulations (Table 1) were prepared by soaking sterile paper discs in

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a PHMB-BKC mixture for 1 h and air-dried for 24 h in a biological safety cabinet at room temperature. The test bacteria were cultured overnight using MH broth at 37 °C. Then, the cultured bacteria were streaked on MH agar using a sterile cotton swab. The antimicrobial discs were placed onto the streaked agar and incubated for 24 h at 37 °C. Conventional antibiotics, including penicillin, gentamicin, kanamycin and streptomycin, were used as the positive control in this experiment. Meanwhile, a disc without any agent was used as the negative control. After the incubation period, inhibition zones were measured and recorded for data analysis.

Well diffusion (WD) assay

Antibacterial activities of PHMB-BKC solutions were also evaluated using a well diffusion (WD) method on MH agar (Jahangirian *et al*., 2013). Overnight cultured bacteria in MH broth at 30 °C were prepared. Under the aseptic condition, MH agars for this experiment were prepared by punching 6 mm holes using a glass capillary for placement of the active ingredient. Then, the cultured bacteria were streaked in MH agar and 50 µL of test samples were added into the punched hole. The plates then were incubated for 24 h at 37 °C. After the incubation period, the inhibition zones were measured and recorded for data analysis.

3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) assay

The solutions (1000 ppm PHMB, 1000 ppm BKC and a mixture of 500 ppm PHMB and 500 ppm BKC) and their ten-fold dilutions were tested for cytotoxic effect using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay (ISO, 2009; Generali *et al*., 2020). Vero cells were seeded in a 96-well plate and incubated at 37 °C overnight, supplemented with 5% CO_2. PHMB and BKC solutions were diluted in Dulbecco's Modified Eagle Medium (DMEM) to achieve the desired concentrations. Once the cells reached 80% confluency, the culture medium was removed and the cells were treated with 100 µL of diluted PHMB and BKC in triplicate wells. Cells treated with formulations were incubated at different time points; 1 h at 37 °C. After each time point, the solutions were removed and the treated cells were washed twice with 100 µL fresh DMEM for 2 min. Fresh DMEM 100 µL was added, followed by the addition of 10 µL MTT solution into each well. The cells were then incubated for 4 h at 37 °C in the dark. After 4 h, the MTT solution was removed and 100 µL DMSO were added to each well and mixed gently. Then, the presence of viable cells was visualised by the development of purple colour due to the formation of formazan crystals. The absorbance was measured using a microplate reader at 570 nm. The data were recorded and calculated as a percentage of cell cytotoxicity using the formula below:

% viability = [(Average negative control – Average of treatment)/Average negative control] × 100

Table 1: Total 14 formulations for different ratios of PHMB and BKC on antibacterial tests.

Formulations	Concentrations (ppm)	
	PHMB	BKC
A	Highest MIC [a]	Highest MIC [b]
B	Highest MIC [a]	0
C	0	Highest MIC [b]
D	0	1000
E	100	900
F	200	800
G	300	700
H	400	600
I	500	500
J	600	400
K	700	300
L	800	200
M	900	100
N	1000	0

[a]Based on the highest MIC value of PHMB (Table 2); [b]Based on the highest MIC value of BKC (Table 2).

Table 2: Determination of the MIC for PHMB and BKC by resazurin-aided microdilution method against selected pathogenic microbes.

Bacterial species	MIC (ppm)	
	PHMB	BKC
B. cereus	31.25	15.6250
S. aureus	62.50	15.6250
P. aeruginosa	62.50	0.9766
E. coli	125.00	1.9531

Data analysis

The statistical analysis of the results was carried out using GraphPad Prism Version 10.0.2 for Windows (GraphPad Software, USA). One-way analysis of variance (ANOVA) was referred to and used for the study in order to validate the effectiveness of antimicrobial actions between formulations. The results are presented as the mean of triplicate ± standard deviations (SD). The *p* values where $p<0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Minimum inhibitory concentrations (MIC)

The studied chemical agents, PHMB and BKC, are widely used in commercial solutions by health centres, hospital environments and industrial settings, for both topical and surface applications. Determination of MIC for PHMB and BKC was carried out using *B. cereus*, *S. aureus*, *P. aeruginosa* and *E. coli*. Colour changes of blue-coloured resazurin dye into pink or colourless were observed. The concentrations of the column with no colour changes were taken as the MIC values (Table 2).

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Benzalkonium chloride, which is a type of QAC, does not have sporicidal action, but its activity hinders the development of microorganisms and the germination of spores (Jones and Joshi, 2021). The MIC values of 15.625 ppm were determined for *B. cereus* and *S. aureus*, corresponding to eight times the MIC values for Gram-negative bacteria of *P. aeruginosa* and E. *coli* (MIC between 0.9-2.0 ppm). The most sensitive microorganism among the selected pathogenic bacteria towards BKC was *P. aeruginosa* (0.9766 ppm).

The QACs are considered low-level disinfectants in health facilities, and they are often used in concentrations of 2000 mg/L (2000 ppm), with no formaldehyde in the formulation (Mazzola *et al*., 2009). BKC, a positively charged quaternary nitrogen, not only interacts with anionic lipids, but also allows hydrophobic tails to enter bilayers, disrupts lipid structure and bridges permeability barriers. This results in low-molecular-weight material leakage, proton motive force loss and oxidative phosphorylation decoupling (Noel *et al*., 2021). The studied antiseptics are commonly used due to their non-aggressive action and residual activity on surfaces, acting as a replacement for harmful phenolic compounds.

The MIC values of PHMB were higher compared to BKC, which clearly indicated that the antibacterial activity of PHMB was significantly lower than that of BKC. Although it is lower than BKC, PHMB's bacterial capabilities have been established against a variety of species, with first-order kinetics and within 1 h at doses less than 10 µg/mL (Chindera *et al*., 2016). The MIC values of 125 ppm were determined for *E. coli*, corresponding to two times the MIC values for *S. aureus* and *P. aeruginosa* (MIC = 62.50 ppm). The most sensitive bacterium among the selected bacterial strains towards PHMB was *B. cereus* (31.25 ppm).

It Is well known that PHMB is a broad-spectrum antimicrobial biocide with a high therapeutic index that kills bacteria, fungi, parasites and some viruses and is frequently used in clinics, households and industries. As a biguanide group, PHMB binds with and sequesters anionic lipids, resulting in homogenous lipid domains. This alters the bilayer structure, resulting in membrane permeability and intracellular leakage. Evidence also showed that PHMB can penetrate the bacterial membrane, compress bacterial deoxyribonucleic acid (DNA) and inhibit DNA replication (Noel *et al*., 2021).

Antibacterial potential of PHMB-BKC formulations

Using the result from the MIC experiment, the concentration that was picked to be included in the formulations (Table 1 and 2) was the highest MIC, which is 125 ppm and 15.625 ppm, for PHMB and BKC respectively. The PHMB and BKC solutions were measured and mixed with PBS as their carrier solvent. The combination of PHMB and BKC has been arranged according to respective ratios in order to study its efficiency as a combined antimicrobial solution. The antibacterial activity of PHMB-BKC was tested using DD and WD methods against *B. cereus*, *S. aureus*, *P. aeruginosa* and *E. coli* (Table 3). The control antimicrobial discs, penicillin, streptomycin, kanamycin, and gentamicin with diameters of 6 mm with a concentration of 30 µg/disc were used for this experiment (Table 4).

Among the four bacteria from DD results, two of them were Gram-positive bacteria (*B. cereus* and *S. aureus*) that have shown positive results towards the PHMB-BKC combinations with *B. cereus* having an inhibitory zone as high as 23.33 ± 0.58 mm when treated with the combination of 900 ppm BKC and 100 ppm PHMB (Table 3), which is similar or greater when treated with positive controls. It was consistent with previous studies describing the porous nature of the outer peptide-glycan layer of Gram-positive bacteria (Huang *et al*., 2008). The bacteria strains that showed minimal inhibition (*P. aeruginosa* and *E. coli*) both belong to the Gram-negative bacteria category. It is due to the lipopolysaccharide (LPS) composition and cation concentration of these Gram-negative bacteria's outer membrane contribute to the strength of the LPS-LPS linkages, forming a barrier with comparatively smaller porins for disinfectants to pass through (Wilson and Ho, 2023).

Meanwhile, out of four strains tested using WD assay (*B. cereus*, *S. aureus*, *P. aeruginosa* and *E. coli*) screened for antibacterial potential test activity towards PHMB-BKC mixtures, *S. aureus* provided more consistent and prominent susceptibility even on the lowest concentration combination at the range of 12 mm to 20 mm inhibitory zones. The Gram-negative bacteria (*P. aeruginosa* and *E. coli*) exhibited the least susceptibility towards the formulations as compared to the Gram-positive bacteria (*B. cereus* and *S. aureus*). Statistical analysis using one-way ANOVA was carried out to compare the effectiveness of all concentrations of PHMB and BKC towards its ability to inhibit the selected bacteria through DD and WD methods. The one-way ANOVA from both methods revealed that there was not a statistically significant difference in bacteria inhibition between at least two groups (p = 0.2667 and 0.1215 for DD and WD, respectively).

Based on the statistical analysis, it was shown that the formulations or mixtures perform similarly with each concentration in terms of effectiveness on bacteria in any ratio. The observed variations in bacterial inhibition were not deemed significant enough to conclude that one formulation was consistently more effective than another. This suggested that varying the concentration levels of PHMB and BKC did not lead to discernible differences in their bacterial efficacy. A study by Noel *et al*. (2021) also concluded that the combination of PHMB and BKC acted additively in exhibiting their antibacterial actions.

PHMB is a cationic polymer with broad-spectrum antibacterial properties. It disrupts bacterial membranes, leading to leakage of cellular contents and subsequent cell death (Deka *et al.*, 2015). PHMB has been used in wound care, disinfectants, and contact lens solutions due to its efficacy against Gram-positive and Gram-negative bacteria, as well as fungi. Meanwhile, BKC is another cationic surfactant with antimicrobial properties. It disrupts cell membranes and interferes with cellular processes,

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Table 3: Inhibition of selected bacterial strains by a total of 14 formulations of PHMB-BKC mixtures through DD and WD methods.

Concentrations (ppm)		Bacterial inhibition zones (mm)							
PHMB	BKC	DD method				WD method			
		B. cereus	*S. aureus*	*E. coli*	*P. aeruginosa*	*B. cereus*	*S. aureus*	*E. coli*	*P. aeruginosa*
125 [a]	15.6 [b]	8.00	9.00	7.00	6.00	7.33 ± 0.58	10.00	8.33 ± 0.58	7.00
125 [a]	0	7.33 ± 0.58	9.00	7.00	6.00	-	7.00	7.33 ± 0.58	6.00
0	15.6 [b]	9.00	7.00	-	8.00	8.33 ± 0.58	9.67 ± 0.58	-	-
0	1000	23.00	21.67 ± 0.58	10.00	8.00	23.00	20.33 ± 0.58	14.33 ± 0.58	12.00
100	900	23.33 ± 0.58	21.00 ± 1.00	9.00	7.00	21.00	20.00	12.33 ± 0.58	9.33 ± 0.58
200	800	22.00	22.00	9.67 ± 0.58	8.00	22.00	20.33 ± 0.58	14.67 ± 0.58	9.33 ± 0.58
300	700	21.33 ± 0.58	20.33 ± 0.58	9.33 ± 0.58	8.00	24.00	20.00	11.00	10.00
400	600	23.00	20.67 ± 0.58	10.00	8.00	22.00	19.33 ± 0.58	13.67 ± 0.58	9.33 ± 0.58
500	500	21.00	20.00	10.00	8.00	21.00	17.67 ± 0.58	13.00	8.00
600	400	20.00	18.67 ± 1.15	10.00	8.00	19.33 ± 0.58	17.33 ± 0.58	11.00	8.00 ± 1.00
700	300	20.00	18.67 ± 0.58	10.00	8.00	20.00	18.33 ± 0.58	11.33 ± 0.58	9.00
800	200	16.67 ± 1.15	16.33 ± 0.58	10.00	8.00	20.00	16.00	11.33 ± 1.15	8.00
900	100	15.00	14.67 ± 0.58	11.00	8.00	17.33 ± 0.58	14.33 ± 1.33	11.33 ± 0.58	7.67 ± 0.58
1000	0	9.67 ± 0.58	12.33 ± 0.58	10.00	7.33 ± 0.58	10.33 ± 0.58	9.33 ± 0.58	11.33 ± 0.58	12.00

[a]Based on highest MIC of PHMB (Table 2); [b]Based on highest MIC of BKC (Table 2).

Table 4: Inhibition of different bacteria by antibiotics.

Bacteria	Inhibition zones (mm)			
	Penicillin	Streptomycin	Kanamycin	Gentamicin
B. cereus	10	20	24	23
S. aureus	13	10	16	17
P. aeruginosa	0	10	0	16
E. coli	0	10	13	13

ultimately causing bacterial cell death (Butucel *et al.*, 2022). BKC has found applications in various industries, including healthcare, personal care products, and household disinfectants. Figure 1 summarises the mechanism of action (MOA) of PHMB and BKC individually and illustrates the MOA of these two compounds together as a mixture.

According to studies by Noel *et al*. (2021), every combination of cationic membrane-active antibacterial agents interacted additively. This shows that disinfectants with comparable processes and cellular targets benefit from being combined, but not synergistically. They hypothesised that this is because similar-acting drugs have a limited but consistent ability to enhance each other's actions when administered together. At the sub-lethal quantities evaluated, the cationic membrane-active chemicals both disturb membrane stability and promote intracellular leakage; consequently, their presence benefits each other mechanistically. With widely overlapping

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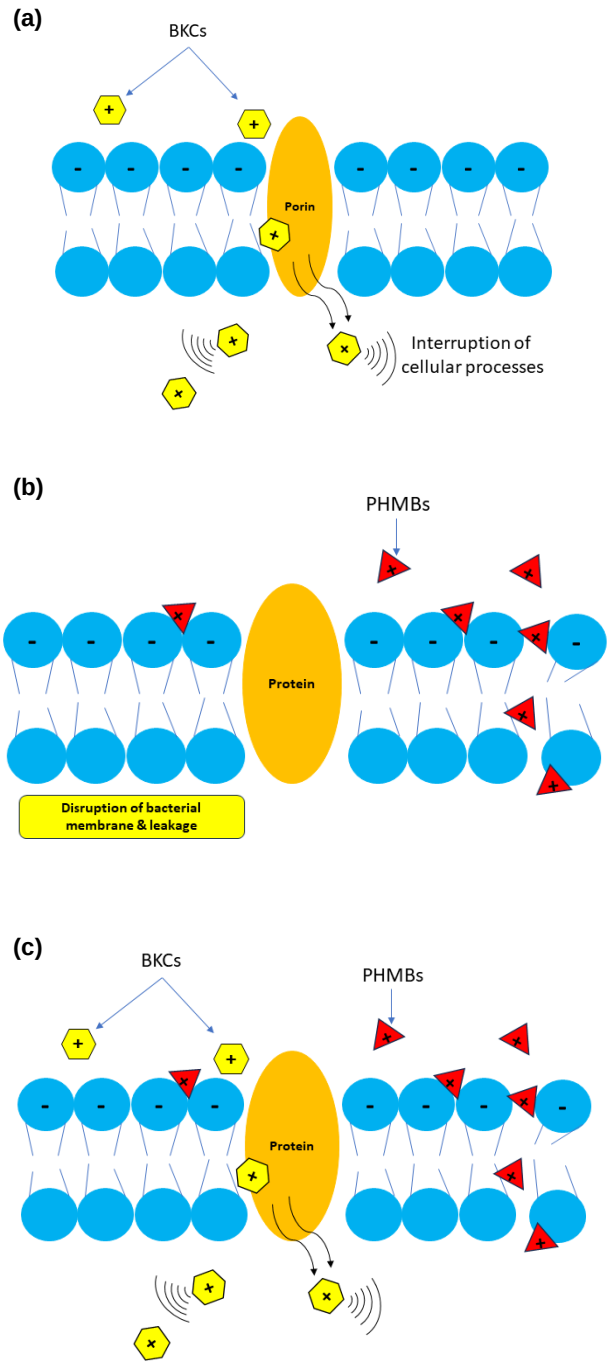

Figure 1: Mechanism of action of (a) PHMB, (b) BKC and (c) mixture of PHMB and BKC.

mechanisms, the total activity can never be more than the sum of its parts; hence, the interaction is confined to additivity instead of synergism.

Cytotoxic effect of PHMB, BKC and their combination

Polyhexamethylene biguanide (PHMB) and benzalkonium chloride (BKC) are commonly used antimicrobial agents known for their effectiveness against various microorganisms. However, as with any chemical compound, the cytotoxicity of these agents must be carefully evaluated to ensure their safe use. This experiment delves into the cytotoxicity properties of a mixture of PHMB and BKC on Vero cells, derived from the African green monkey kidney, shedding light on the potential impact of this combination on cell viability**.**

Absorbance (ABS) readings were recorded after 1 h treatment by 1000 ppm PHMB, 1000 ppm BKC and 500 ppm + 500 ppm BKC (Figure 2). A series of ten-fold dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}) were also done to test their toxicity in lower concentrations. The Vero cells exhibited lower toxicity patterns to the ten-fold PHMB and BKC formulations when exposed to lower concentrations. From the results recorded on the graphs, it was shown that the ABS readings for concentrations starting from 10 ppm and lower have low cytotoxicity towards the Vero cells. Based on the cytotoxicity test, the IC50 value for PHMB and BKC on Vero cells was obtained (1.4185 and 1.4308, respectively). The shallow slope in the dose-response curve for BKC and PHMB may suggest a more gradual cellular response to increasing concentrations, potentially leading to a wider safety margin for lower doses.

According to ISO 10993-5, cell viability beyond 80% was classified as non-cytotoxic; those between 80% and 60% were weak; those between 60% and 40% were moderate; and those below 40% were high cytotoxic (López-García *et al.*, 2014). From the graph in Figure 2, starting from a concentration of 10^{-2} for a PHMB-BKC mixture of 5 ppm each, they exhibit weak cytotoxicity. Meanwhile, the stand-alone PHMB and BKC only exhibit weak and low cytotoxicity at 10^{-3} dilutions at a concentration of 5 ppm. This suggested that the combination of PHMB and BKC can reduce their cytotoxicity compared to the individual concentrations.

Both PHMB and BKC have been individually assessed for their cytotoxicity on Vero cells, PHMB's cytotoxicity is often concentration-dependent, with higher concentrations causing cell membrane disruption, mitochondrial dysfunction, and, ultimately, cell death (Christen *et al.*, 2017). Similarly, BKC's cytotoxic effects are attributed to its membrane-destabilising properties, which can lead to cellular damage (Deutschle *et al.*, 2006). Comparing the cytotoxicity of PHMB and BKC, it becomes evident that these disinfectants have relatively similar toxicities toward Vero cells. The minor difference in values could be attributed to factors such as differences in the mechanism of action, cellular uptake, and metabolic processing of the two compounds. Moreover, it also showed that the combined PHMB and BKC exhibited slightly lower toxicity compared to individual application.

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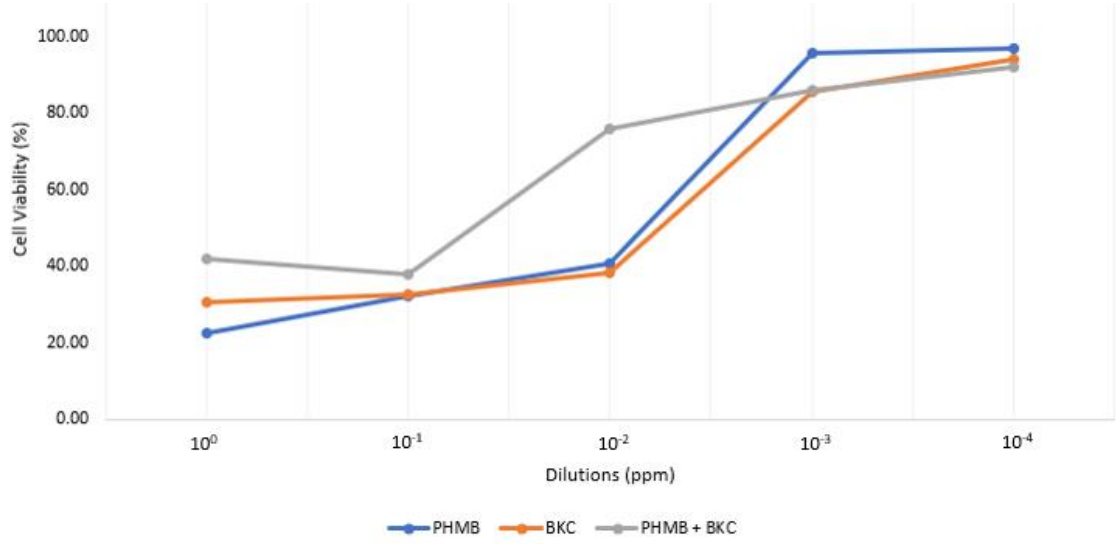

Figure 2: Cytotoxicity activity of PHMB, BKC and their combinations towards Vero cells after 1 h treatment.

CONCLUSION

Results of the current study indicated that the combination of PHMB and BKC had antibacterial effect against the tested Gram-positive and Gram-negative bacteria but only acted additively, not synergistically, as their efficiency is similar to the individual effect. Moreover, their combinations also exhibited slightly lower toxicity compared to individual concentrations. Further studies on their ability in real-life situations, such as on facilities and surfaces, could heighten the understanding about these potential combinations towards applications in healthcare and consumer products.

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