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Original article

Combined effect of brazilin-rich extract and lawsone methyl ether against infection-causing bacteria

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ABSTRACT

Bacterial contamination and infection widely affect the food, pharmaceutical and biomedical industries. Additionally, these bacteria developed resistance to synthetic antibiotics causing public health danger, globally. Natural plant extracts (NPE) are suitable alternatives to synthetic antibiotics to tackle antimicrobial resistance problems. Furthermore, a blend or combination of different NPEs exerts a wide spectrum of antimicrobial activity. Therefore, the combined effect of brazilin-rich extract (BRE) and lawsome methyl ether (LME) against infection-causing common bacteria were evaluated. BRE had a lower minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against most of the Gram-negative bacteria (*Salmonella typhi, Salmonella typhimurium* and *Pseudomonas aeruginosa*) while LME was active against most of the Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus,* and *Staphylococcus epidermidis*). The combination of BRE and LME at 2:1 and 1:1 concentration significantly reduced the MIC value of each compound as compared to either BRE or LME concentration alone ($P < 0.05$). Further time-kill kinetics revealed a 3.0–3.5 log reduction in Gram-positive bacteria and a 2.5–3.0 log reduction in Gram-negative bacteria during 120 min of incubation, respectively. Therefore, a combination of BRE and LME was recommended as natural antibacterial to synthetic antibiotics for food and pharmaceutical applications.

1. Introduction

Bioactive compounds from herbal plants hold significant therapeutic implications, and their isolation and characterization to yield novel drugs have been explored for centuries (Jamaddar et al., 2023; Nirmal et al., 2015; Rajput et al., 2022). According to the World Health Organization, almost 80 % of people around the globe, especially in developing countries, use medicinal plants for managing various disorders (Shomudro et al., 2023), while researchers increasingly explore natural drugs from plant extracts. Moreover, resistant bacterial infections are increasingly prevalent and pose a significant global public health concern due to their challenging treatment and association with high rates of illness and death. In the United States, there has been a consistent rise in extended-spectrum rates of bacterial infection since 2000 (Kaye & Pogue, 2015). Antibiotic resistance imposes a considerable clinical and economic burden, leading to elevated mortality rates, higher expenses for antibiotics and hospital care, and prolonged hospital stays, particularly in intensive care units (Salam et al., 2023). In the 21st century, the progress in molecular biotechnology and medicine has effectively controlled and even eradicated certain human pathogens in developed nations; however, the evolution of pathogens has given rise to the emergence of novel infectious diseases. The accelerated global mobility of humans, driven by socioeconomic activities, technological advancements, and transportation, poses a formidable challenge in curtailing the dissemination of pathogens. This challenge is underscored by instances such as 2009′s influenza pandemic, 2014′s Ebola outbreak, and recent COVID-19 pandemic (El-Saadony et al., 2021; Hiscott et al., 2020). Amidst this challenge, the significance of natural agents derived

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from plant sources gains prominence as next-generation antimicrobial alternatives (El-Saadony et al., 2021). These natural products offer a promising avenue for therapeutic exploration, harnessing the potential of plant-derived compounds in combating evolving pathogens and contributing to ongoing efforts in global health security.

Caesalpinia sappan L., a medicinal plant of the Fabaceae family, is geographically distributed in Asia, including Burma, India, Vietnam, China, and Sri Lanka (Jung et al., 2015). It is utilized as a component in traditional foods in South Asia and is also used in traditional Ayurvedic, Chinese, and Thai medicine (Settharaksa et al., 2019). It was used as an emmenagogue and strong astringent in Thai traditional medicine. It is also used as a tonic and diuretic and finds application in the management of skin conditions, acute diarrhea, dysentery, and pulmonary hemorrhaging (Settharaksa et al., 2019). *C. sappan* extract has demonstrated diverse pharmacological effects, including anti-acne, anti-hypercholesterolemic, anti-hyperglycemic, hepatoprotective, anti-inflammatory, etc. (Jamaddar et al., 2023; Rajput et al., 2022). The petroleum-ether and ethanol extracts comprise tannins, steroids, phenols, saponins, and flavonoids, while, the water extract is abundant in phenols and tannins. Numerous compounds, such as brazilein, brazilin, brazilide A, butein, sappanone B, and protosappanins, have been identified in *C. sappan* (Nirmal et al., 2015). Further, Nirmal and Panichayupakaranant (2015) developed a standardized BRE and indicated various its potential bioactivities. On the other hand, *Impatiens balsamina* L. commonly known as garden balsam has been utilized as traditional herbal remedy for various diseases (Qian et al., 2023). Phytochemical analysis revealed the presence of various compounds, including phenolics, alkaloids, terpenoids, flavonoids, coumarins, naphthalene derivatives, and polysacchrides, with the notable inclusion of 2-methoxy-1,4-naphthoquinone (Qian et al., 2023). The plant exhibits diverse pharmacological effects, including antibacterial, antiparasitic, antioxidant, hepatoprotective, anticancer, etc (Qian et al., 2023). Further, LME was synthesized by methylation of 1,4-naphthoquinone (Lawsone) through an acidic process (Sakunphueak & Panichayupakaranant, 2012). Studies have shown that LME in combination with artocarpin exhibits synergistic effects against MRSA (Panichayupakaranant et al., 2019). Although there are some reports on the combination of LME with other bioactive compounds including α-mangostin-rich extract (Meah et al., 2020) and artocarpin-rich extract (Bazmi & Panichayupakaranant, 2022). There is no knowledge of the combined effect of BRE and LME against infection-causing bacteria. Therefore, this research investigated the combined antibacterial effect of BRE and LME against a few infectious bacteria including five Gram-positive strains and four Gram-negative strains. Additionally, time-kill analyses of the combined concentrations were evaluated against each bacterium. The outcome of this study will shed light on the efficacy of BRE and LME and further research avenues on the topic.

2. Material and methods

2.1. Chemicals

Brain-heart infusion (BHI) was procured from Becton Dickinson (Franklin Lakes, NJ, USA). Sodium benzoate and ampicillin were purchased from Sigma (Sigma-Aldrich, UK). All other chemicals used for experiments were analytical grade unless mentioned anywhere.

2.2. Preparation of BRE and LME

BRE was prepared according to the method mentioned by Nirmal and Panichayupakaranant (2015). Briefly, *C. sappan* dried powder (500 g) was extracted with 95 % ethanol (3 L × 3 times) under reflux conditions for 1 h. The extract pooled over and evaporated to dryness to obtain crude sappan extract (CSE). Further, 25 g of CSE was dissolved in 35 % ethanol and subjected to Diaion® HP-20 column (Shimadzu, Tokyo, Japan). The column was eluted with 35 % ethanol to obtain brazilin rich extract (39 % w/w). The extract evaporated to dryness to achieve BRE powder.

LME was prepared according to the methylation method of lawsone in an acidic condition, as mentioned by Panichayupakaranant and Reanmongkol (2002). Lawsone was dissolved in methanol (1 g/ 50 mL) and then acidified with concentrated hydrochloric acid (0.8 mL). The reaction mixture was refluxed for 4 hrs and then cooled at room temperature. The cooled suspension was vacuum filtrated to obtain a crude precipitate. Further, this precipitate retreated with a mixture of ethyl acetate and methanol (1: 1) to obtain a yellow crystal of LME.

2.3. Microorganisms

Pseudomoas aeruginosa (DMST 15442), *Salmonella typhimurium* (DMST 562), *Staphylococcus aureus* (ATCC 25923), *Escherochia coli* (ATCC 25922) and *Staphylococcus epidermidis* (ATCC 14990) were obtained from the Faculty of Medicine, while *Bacillus subtilis* was obtained from the Faculty of Science, Prince of Songkhla University, Thailand. *Cutibacterium acnes* (DMST 14916), *Streptococcus pyogenes* (DMST 17020), and *Salmonella typhi* (ATCC 19430) were procured from the Department of Medical Science Center, Thailand. All bacterial cultures were received as a slant with screw-capped tubes. Upon receiving, all bacterial cultures were regrown in BHI broth for 24 h at 37 °C incubation for further experiments.

2.4. MIC and MBC protocol

The MIC and MBC of all samples alone or in combination were determined towards 5 Gram-positive and 4 Gram-negative infectious causing bacteria. Freshly grown bacterial colonies were lopped into the sterile 0.85 % NaCl to prepare the cell suspension. The obtained cell suspension was adjusted to 1×10^8 CFU/ml by comparing it with a 0.5 McFarland solution. The stock bacterial suspension (10^8 CFU/ml) was diluted to 1×10^6 CFU/ml before using for microdilution assay. In brief, the bacterial suspension was added to each well containing the tested compound. The initial concentration of BRE (2 mg/ml), LME (2 mg/ml), and different combinations of BRE and LME (BRE: LME; 2:1 and 1:1) (2 mg/ml) were used for the microdilution assay. Ampicillin (1 mg/mL) and sodium benzoate (2 mg/mL) were used as the standard drugs. All plates were placed at 37 °C for 24 h of incubation. Anaerobic conditions were used for *C. acne* incubation. The MIC value of the compound is that concentration where no visible growth of microorganisms was observed, whereas MBC denotes the lowest concentration required to kill the microorganisms.

2.5. Time kill kinetics assay

The time-dependent inhibition of infection causing bacteria using BRE, LME, and their selected was examined over 2 hr at room temperature. The bacteria grown overnight in BHI broth were centrifuged at 4000 rpm for 10 min and bacterial cells were obtained. The cells were cleaned with sterile saline, and suspended in fresh nutrients to achieve 10^6 CFU/mL (Nirmal et al., 2023). Thereafter, 4.5 mL of bacterial suspension (1×10^6 CFU/ml) was added with 0.5 mL of the MIC value of each tested compound. The control sample consisted of only bacterial culture. An aliquot of 50 μl was drawn every 5, 15, 30, 60, 90, and 120 min and diluted properly then spread on BHI agar plates. The plates were incubated at 37 °C for 24 h and viable colonies (limit 100 CFU/ml) were counted.

2.6. Statistical analyses

All experiments have been performed in triplicate until and unless mentioned elsewhere. The results were indicated as the mean of triplicate. The Significant difference between the samples was analyzed using EXCEL stat software through one-way ANOVA and Turkey's test. The

value with $p < 0.05$ indicates the significant difference.

3. Results

3.1. MIC and MBC

Table 1 represents the MIC and MBC values of BRE, LME and their combinations against various Gram-positive infectious bacteria including *S. aureus, S. epidermidis, B. subtilis, S. pyogenes* and *C. acnes.* In general, ampicillin as a standard drug showed the lowest MIC and MBC (0.12 μg/ml) against all tested organisms. While sodium benzoate a food preservative showed higher MIC and MBC (500 or > 500 μg/ml**)** among all the samples tested. For *S. aureus, S. epidermidis*, and *B. subtilis*, LME consistently shows lower MIC and MBC values compared to BRE. In the case of *S. pyogenes*, BRE demonstrates a lower MIC (15.6 μg/ml) compared to LME (31.3 μg/ml), but the same MBC (31.3 μg/ml) was noted for both samples. Interestingly, for *C. acnes*, LME displays substantially higher MIC and MBC values (500 and 1000 μg/ml, respectively) compared to BRE (31.3 and 62.5 μg/ml, respectively). The 1:1 combination was found to be more effective at inactivating *S. epidermidis.* and *B. subtilis* compared to the 2:1 combination. Conversely, the 2:1 combination exhibited pronounced effects for *S. pyogenes* and *C. acnes*. Whereas no significant difference was found between the MIC and MBC concentrations of both the 2:1 and 1:1 combination for *S. aureus.*

The antibacterial activities of BRE, LME and their combinations against Gram-negative infectious bacteria is presented in Table 2. A standard drug ampicillin showed the lowest MIC and MBC against *E. coli* and *P. aeruginosa* (31.3 and 0.12 μg/ml, respectively), while a higher concentration was needed for *Salmonella* strains (250 μg/ml). However, sodium benzoate required > 1000 μg/ml to inhibit any Gram-negative bacteria tested. LME was reported with MIC and MBC of 150/250 μg/ml against *E. coli,* respectively compared to BRE (250/500 μg/ml). Whereas BRE demonstrates higher efficacy against *S. typhi, S. typhimurium,* and *P. aeruginosa* compared to LME. There was no significant effect of either combination against studied bacteria except *S. typhi* where BRE: LME with 2:1 was more effective than the 1:1 combination.

3.2. Time kill kinetic

Fig. 1 represents the time kill kinetics of BRE, LME and selected BRE: LME combination against Gram-positive bacteria. Cumulatively, a 3.0—3.5 log reduction of the bacterium was observed during 120 min of incubation.

A time kill kinetics of BRE, LME and selected BRE: LME against Gram-negative bacteria during 120 min is shown in Fig. 2. Overall, 2.5–3.0 logs of reduction were noted in all tested organisms during 120 min of incubation with the tested sample. None of the samples tested was able to completely kill the tested organism during 120 min of incubation, indicating that more exposure time was needed for complete eradication.

4. Discussion

4.1. MIC and MBC

The low MIC and MBC values suggest strong antibacterial activity. When examined individually, both BRE and LME exhibited notable MIC and MBC values, indicating their effectiveness in inhibiting and killing the specified Gram-positive bacterial strains. When considering individual bacterial strains, both BRE and LME display varying degrees of effectiveness. LME showed lowered MIC and MBC against the majority of the bacteria indicating its superior antibacterial activity against these strains ($P < 0.05$). BRE and LME had similar efficacy in inhibiting *S. pyogenes*. While, BRE was more effective than LME against *C. acnes* ($P < 0.05$), indicating potential differences in their mechanisms of action or bacterial susceptibility. The efficacy of BRE against *C. acnes* was well established, and the current results were concurrent with Nirmal and Panichayupakaranant (2014) who reported the antibacterial activities of Brazilin, BRE, and *C. sappan* Linn heartwood extract (CSE) against acne-associated bacteria, namely *S. aureus*, *P. acnes,* and *S. epidermidis*. Moreover, another study indicated that methanolic and 50 % ethanolic extracts of C. *sappan* wood showed the highest anti-acne potency among 28 plant extracts (Batubara et al., 2009). Extract components from *C. sappan* were isolated using column chromatography and preparative HPLC, resulting in the isolation of protosappanin A, brazilin, and sappanone B from methanolic extracts, with brazilin exhibiting superior antimicrobial properties (MIC = MBC = 0.50 mg/ml). The combined effect of BRE: LME in the ratios of 2:1 and 1:1, varies across the tested Gram-positive bacteria. The possible explanation for the combined effect of BRE and LME could be attributed to the differential interactions between BRE and LME at varying ratios. The 1:1 combination might lead to enhanced antimicrobial activity for *S. epidermidis* and *B. subtilis* due to a more balanced ratio of the two compounds, facilitating synergistic interactions and thereby augmenting their combined efficacy.

When tested in Gram-negative bacteria such as *P. aeruginosa* and *S. typhi,* LME shows limited efficacy with MIC and MBC values exceeding 1000 μg/ml. While BRE showed lower MIC and MBC against the majority of the Gram-negative bacteria tested. BRE was reported to possess MIC/MBC of 250/250 μg/ml and 250/250 μg/ml against *S. typhimurium* and *P. aeruginosa,* respectively (Nirmal and Panichayupakaranant (2015). The combination of BRE and LME at different ratios reveals intriguing insights into their combined antibacterial effects. In general, Gram-positive bacteria exhibited greater susceptibility to BRE and LME compared to Gram-negative bacteria. The antibacterial effects of BRE were found to be associated with the brazilin content within the extracts (Nirmal & Panichayupakaranant, 2015).

The susceptibility of pathogens to antimicrobial compounds depended on the origin and strain of the bacteria. Also, cell membrane structure in bacteria has a significant influence on their resistance levels to antibiotics and disinfectants (Russell, 2003). Gram-positive bacteria possess thicker peptidoglycan layers in their membranes, which absorb antibiotics effectively, rendering them more susceptible to antimicrobial

Table 1
MIC and MBC of BRE and LME alone and in combination against Gram-positive infectious bacteria.

Substances	*S. aureus* (μg/ml)		*S. epidermidis* (μg/ml)		*B. subtilis* (μg/ml)		*S. pyogenes* (μg/ml)		*C. acnes* (μg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
BRE	62.5	125	62.5	62.5	62.5	125	15.6	31.3	31.3	62.5
LME	15.6	31.3	15.6	31.3	15.6	31.3	31.3	31.3	500	1000
BRE: LME (2:1)	31.3	62.5	31.3	125	31.3	62.5	15.6	31.3	31.3	62.5
BRE: LME (1:1)	31.3	62.5	15.6	31.3	15.6	31.3	31.3	31.3	62.5	125
Sodium benzoate	>500	ND	125	250	>500	ND	500	500	>500	ND
Ampicillin	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12

The MIC and MBC value of BRE: LME (2:1) indicates the presence of 2 part of BRE and 1 parts of LME (e.g. MIC value of 31.3 contained 20.86 parts of BRE and 10.43 parts of LME). Similarly, MIC and MBC value of BRE: LME (1:1) indicates the presence of 1 part of BRE and 1 parts of LME (e.g. MIC value of 31.3 contained 15.65 parts of BRE and 15.65 parts of LME).

Table 2
MIC and MBC of BRE and LME alone and in combination against Gram-negative infectious bacteria.

Substances	*E. coli* (μg/ml)		*S. typhi* (μg/ml)		*S. typhimurium* (μg/ml)		*P. aeruginosa* (μg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
BRE	250	500	250	500	125	250	250	500
LME	125	250	500	1000	250	500	>1000	ND
BRE: LME (2:1)	250	250	250	500	250	250	250	500
BRE: LME (2:2)	125	250	500	1000	125	250	500	500
Sodium benzoate	>500	ND	>1000	ND	>1000	ND	>1000	ND
Ampicillin	31.3	31.3	125	250	250	250	125	250

The MIC and MBC value of BRE: LME (2:1) indicates the presence of 2 part of BRE and 1 parts of LME (e.g. MIC value of 31.3 contained 20.86 parts of BRE and 10.43 parts of LME). Similarly, MIC and MBC value of BRE: LME (1:1) indicates the presence of 1 part of BRE and 1 parts of LME (e.g. MIC value of 31.3 contained 15.65 parts of BRE and 15.65 parts of LME).

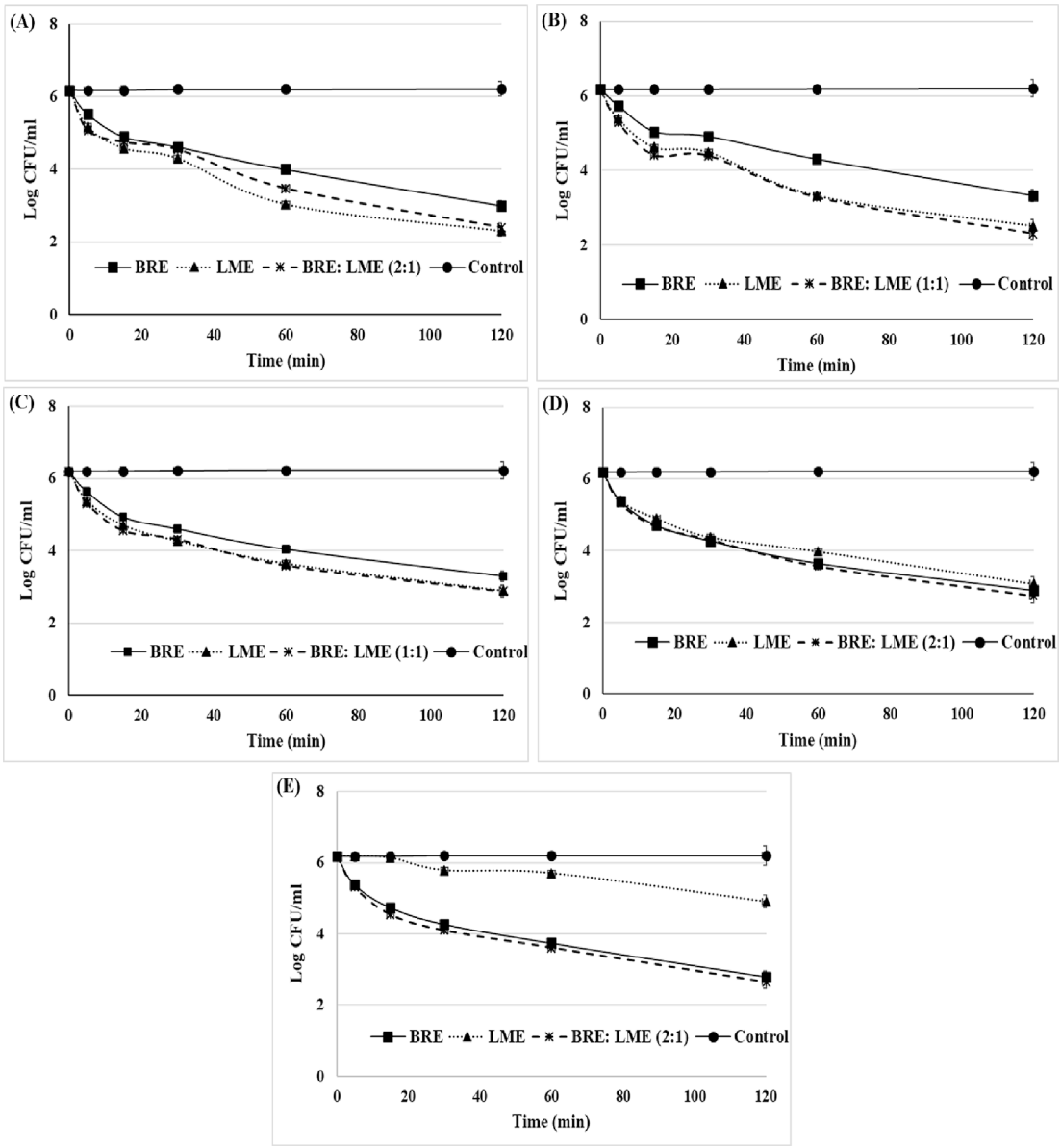

Fig. 1. Time kill analyses of BRE, LME and selected BRE: LME against Gram-positive bacteria (A) *S. aureus*, (B) *S. epidermidis,* (C) *B. subtilis,* (D) *S. pyogenes, and* (E) *C. acnes.*

agents. Conversely, Gram-negative bacteria are less vulnerable to certain physical attacks as they have a reduced capacity to absorb foreign substances in their surroundings (Breijyeh et al., 2020). The antibacterial properties of plants are associated with their capacity to produce multiple secondary metabolites, which possess complex structures with antimicrobial properties (Hemeg et al., 2020). The effectiveness of plant extracts in combating bacteria is influenced by the presence of active compounds and synergistic combinations with other

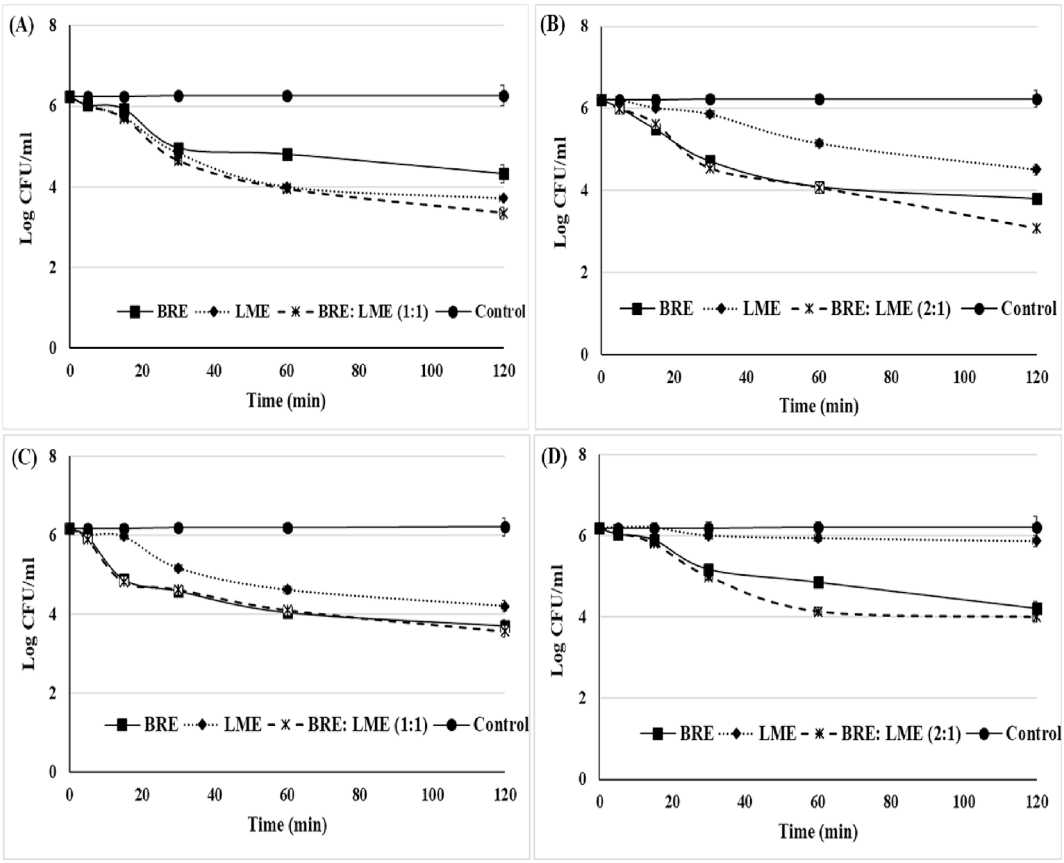

Fig. 2. Time kill analyses of BRE, LME and selected BRE: LME against Gram-negative bacteria (A) *E. Coli*, (B) *S. typhi*, (C) *S. typhimurium*, and (D) *P. aeruginosa.*

molecules (Gorlenko et al., 2020). Brazilin, as a flavonoid, exhibits antibacterial properties by interacting with intracellular proteins or bacterial cell walls leading to cell leakage (Xu & Lee, 2004). Research findings demonstrate that extracts from *C. sappan* possess antimicrobial capabilities, effectively inhibiting various foodborne pathogens such as *S. aureus, E. coli, S. enteritidis,* and *V. parahaemolyticus* (Pattananandecha et al., 2022). Particularly, *S. aureus*, a Gram-positive bacterium, appears to be most susceptible to *C. sappan* extracts. However, the susceptibility of Gram-negative bacteria may be attributed to differences in the outer membrane properties of the bacterial strains. LME have been reported for antimicrobial potential towards methicillin-resistant *S. aureus, Candida albicans, Trycophyton rubrum* (Panichayupakaranant et al., 2019; Sritrairat et al., 2011). These findings underscore the potential of BRE: LME combinations as promising therapeutic agents against bacterial infections, warranting further investigation into their mechanistic interactions and clinical applications.

4.2. Time kills kinetic

The MIC of BRE, LME and their combination were used for time kill kinetic determination against each bacterium for 120 min of incubation. Overall, different organism responds differently to each active compound during the incubation. However, the combined effect observed when LME was added with BRE in a 2:1 ratio (BRE: LME) offers a promising avenue for enhancing antibacterial activity. The varying efficacy observed among the compounds underscores the importance of understanding their mechanisms of action. In Gram-positive bacteria like *S. aureus, B. subtilis*, and *S. epidermidis*, the time kill curve suggests that BRE exhibits a relatively lower potential for bacterial eradication compared to LME. This discrepancy may stem from differences in their mechanisms of action, with LME's ability to disrupt the bacterial cell membrane and interfere with mitochondrial respiration likely conferring greater antibacterial potency. Meah et al. (2020) studied the combined effect of LME and α-mangostin-rich extract towards methicillin-resistant *Staphylococcus aureus* and reported that LME targets the bacterial membrane, potentially acting on different sites, thereby amplifying its antibacterial activity against MRSA. Additionally, the membrane disruption caused by LME can facilitate BRE's antimicrobial activity, resulting in morphological changes that lead to bacterial cell death. The notable exception of *C. acnes* highlights the importance of considering bacterial species-specific responses to antimicrobial agents. Despite LME's overall efficacy against Gram-positive bacteria, its effectiveness against *C. acnes* may be less pronounced, indicating the need for tailored treatment approaches. However, the combined effect observed when LME was added with BRE in a 2:1 ratio (BRE: LME) offers a promising avenue for enhancing antibacterial activity. This synergism likely arises from the complementary mechanisms of action of LME and the bioactive compounds present in BRE. By combining, they may exert a more potent and comprehensive antibacterial effect, surpassing the individual efficacy of either compound alone.

BRE demonstrated greater efficacy in inactivating Gram-negative bacteria, except for *E. coli*. Interestingly, when BRE was combined with LME, a significant enhancement in efficacy against *E. coli* and *S. typhi* was noted. This suggests a synergistic effect between BRE and LME, particularly against certain Gram-negative bacterial species. The biofilm inhibition of *S. mutans* (Puttipan et al., 2018) and *S. aureus* (Peng et al., 2018) was reported. The researchers demonstrated that brazilin was able to effectively damage the biofilm by impeding the quorum sensing (QS) system in *S. aureus* and *S. mutans strains*, reducing extracellular polymeric matrix production, and displaying substantial inhibition on QS organization. Similarly, García-Heredia et al. (2016) confirmed that brazilin was capable of inhibiting growth and altering biofilm formation as well as motility mediated by the swarming mechanism. They also observed that this compound reduced the virulence

potential of both types of *E. coli* through modulation of expression levels. Jamaddar et al. (2023) suggest that the observed antibacterial effects of BRE across different bacterial strains indicate its ability to disrupt bacterial cell membranes. This disruption likely compromises membrane integrity, leading to intracellular content leakage and ultimately bacterial inactivation. Xu and Lee (2004) investigated the antibacterial potential of brazilin against 14 bacterial species and reported that brazilin exhibits a spectrum of antimicrobial activity against clinically significant bacterial species. Furthermore, the antibacterial mechanism of brazilin against MRSA was elucidated through time-kill studies, revealing its bactericidal action. The variations in effectiveness observed among different combinations of BRE and LME against various bacterial strains could be attributed to several factors, including differences in the susceptibility of bacterial species to specific bioactive compounds present in the extracts. Additionally, variations in the interaction between Brazilin and LME at different ratios may influence the bioavailability and potency of the active compounds against different bacterial targets. Also, the presence of synergistic or antagonistic interactions between Brazilin and LME compounds could also impact their overall antibacterial activity. Moreover, variations in the composition and concentration of secondary metabolites within the extracts may contribute to the observed differences in effectiveness against different bacterial strains. Further research is warranted to elucidate the underlying mechanisms driving these variations and optimize the combination ratios for enhanced antibacterial efficacy.

5. Conclusion

The study explores the antibacterial potential of BRE and LME alone or in combinations, against Gram-positive and Gram-negative infection-causing bacteria. Both BRE and LME exhibit significant efficacy against various Gram-positive strains, with LME generally demonstrating lower MIC and MBC values than BRE, particularly against *S. aureus, S. epidermidis,* and *B. subtilis*. While, BRE was more effective against Gram-negative bacteria, except *E. coli*. Moreover, the combined effect of BRE and LME varied with different ratios depending on the different bacteria and their composition. The observed variations in effectiveness among different combinations of BRE and LME suggest differential interactions and bioavailability of active compounds, influenced by bacterial species susceptibility and compound ratios. The time kill kinetics assay results not only validate the proposed mechanisms of action but also underscore the potential of LME and BRE, both individually and in combination, as effective antibacterial agents. Further elucidation of the underlying mechanisms governing the antibacterial activity of BRE and LME, both individually and in combination, is imperative for optimizing their therapeutic potential. Overall, the results underscore the potential of BRE and LME as antimicrobial agents against Gram-positive and Gram-negative infectious bacteria, with the combination approach introducing a dynamic element that warrants further exploration in the development of novel therapeutic strategies against infectious diseases. Moreover, BRE or LME or their combination could be used as a food preservative and antibacterial in pharmaceutical applications.

CRediT authorship contribution statement

Nilesh Nirmal: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Pankaj Koirala:** Writing – original draft, Investigation, Formal analysis, Data curation. **Anandu Chandra Khanashyam:** Writing – original draft, Visualization, Methodology, Data curation. **Pharkphoom Panichayupakaranant:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition. **Abdi Wira Septama:** Writing – review & editing, Visualization, Resources, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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