

FORMULATION OF AN EMULGEL LOADING A SYNERGISTIC ANTIBACTERIAL COMBINATION OF α -MANGOSTIN ENRICHED EXTRACT AND LAWSONE METHYL ETHER

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Abstract

This study aimed to formulate a stable topical emulgel containing a synergized anti-methicillin-resistant *Staphylococcus aureus* (MRSA) combination of α -mangostin enriched extract (AME) and lawsone methyl ether (LME). AME (containing 84.9% w/w α -mangostin) was prepared using green technology, while pure LME was semi-synthesized from lawsone. Synergistic antibacterial effects against MRSA were evaluated using a broth microdilution assay coupled with the checkerboard method. Four AME and LME-loaded emulgels (F1–F4) containing 0.025% w/w AME and 0.2% w/w LME were formulated and evaluated for their physical, chemical, and biological stabilities under accelerated conditions for 6 months. The antibacterial assay followed by the checkerboard method revealed the best synergistic anti-MRSA effect between AME and LME at concentrations of 0.5 and 4 μ g/mL, respectively. Based on the stability evaluations, the F4 formula exhibited good physical, chemical, and biological stabilities for at least 6 months. These results indicate that the emulgel containing a synergistic cocktail of 0.025% w/w AME and 0.2% w/w LME possesses high potential for further *in vivo* studies on the treatment of wounds associated with MRSA infection.

Keywords: Emulgel; Lawsone methyl ether; Mangostin; MRSA; Synergistic

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Introduction

An antibiotic-resistant bacterium is a menace and a serious burden on the global health system. Antibiotic-resistance increases the chance of mortality, and it is expected that it will make modern medical care unsustainable (Peters *et al.*, 2019). In 2019, an epidemic of 4.95 millions deaths was caused by antibiotic-resistant bacterial infections. One of the most problematic pathogens related to multidrug resistance is MRSA, a Gram-positive bacterium. MRSA is a serious clinical and public health problem that caused more than 100,000 deaths of bacteremia (Murray *et al.*, 2022). Consequently, antimicrobial-resistant bacterial infections result in over three million days of hospitalization (Pumart *et al.*, 2012). MRSA is also a common antibiotic-resistant pathogen that causes skin and wound infections, i.e., boils, cellulitis, abscesses, pressure ulcers, and diabetic ulcers (Nery Silva Pirett *et al.*, 2012; Abrahamian *et al.*, 2019). In the health domain, MRSA strains are known as superbugs that have developed resistance to a wide range of commonly used antibiotics, including macrolides, tetracyclines, aminoglycosides, fluoroquinolones and β -lactams (Vestergaard *et al.*, 2019; Debasmita *et al.*, 2013). Although vancomycin, a drug of choice for MRSA infection, possesses a good therapeutic effect, it may cause common side effects, i.e., bladder pain, bloating or swelling of the face, arms, hands, lower legs, or feet, bloody or cloudy urine, decreased urine, difficult, burning, or painful urination, and ototoxicity (Van Bambeke, 2015).

Recently, there has been a surge in research aimed at identifying new molecules or modifying known agents to treat antibiotic-resistant bacteria. The new antibiotics are developed in a limited and delayed manner (Mundy *et al.*, 2016). As part of these investigations, some phytoalexins have been reported to possess antibacterial activity against MRSA, such as curcuminoids (Mun *et al.*, 2014), flavonoids (rutin, morin, and quercetin) (Amin *et al.*, 2015), α -mangostin (Sakagami *et al.*, 2005), and lawsone methyl ether (LME) (Yang *et al.*, 2001). However, the anti-MRSA potency of the afore-mentioned phytochemicals is not good enough for further drug development. Recently, it has been revealed that the combined use of two antibacterial compounds, α -mangostin enriched extract (AME) and LME, synergized their anti-MRSA potency. For examples, AME at 0.008 μ g/mL plus LME at 0.49 μ g/mL exhibited synergistic effects against several MRSA strains with the fractional inhibitory concentration indexes (FICI) of 0.008-0.009 (Meah *et al.*, 2020a).

Therefore, these two compounds might be a promising antibacterial cocktail for the formulation of a highly efficacious anti-MRSA preparation.

A topical antimicrobial preparation is commonly used in the treatment of infectious wounds. The most common topical antibacterial preparations involve ointments, creams, gels, and solutions. However, the stability of active compounds and patient compliance usually play an important role in the dosage design. Emulgels are a semisolid dosage form in combination with an emulsion (o/w or w/o) and gel that comprise oil droplets entrapped in hydrogel that have various advantages for hydrophobic drugs, i.e., controlled release, smooth, improved loading capacity, easily removable, greaseless, production feasibility, low cost of production as well as better stability than other topical formulations (Sohail *et al.*, 2018; Sushma *et al.*, 2021). Recently, various emulgels have been developed via loading with phytochemicals for treating bacterial and fungal skin infections (Afzal *et al.*, 2023; Singh *et al.*, 2023). Up to date, there is no report on the topical antibacterial preparation containing a synergistic cocktail between AME and LME. Therefore, the present study aimed to formulate a stable topical emulgel containing the synergistic cocktail AME and LME.

Materials and Methods

Plant materials and chemicals

The fresh *G. mangostana* fruits were purchased from a market in Hat-Yai city, Songkhla (Thailand). Standard α -mangostin and lawsone methyl ether, lawsone, citric acid, Diaion[®] HP-20, dimethyl sulfoxide (DMSO), and resazurin were purchased from Sigma-Aldrich (Darmstadt, Germany). Butylene glycol (BP grade), Carbopol 934, Tween 60 and Span 60 were purchased from the PC Drug center (Bangkok, Thailand). Light liquid paraffin (BP grade) was purchased from S Tong Chemicals (Nonthaburi, Thailand). Polyethylene glycol 400 (BP grade) was purchased from Vidhyasom (Bangkok, Thailand). Butylated hydroxytoluene (BHT) was purchased from Fluka (Buchs, Switzerland). HPLC grade solvent, acetonitrile, and analytical grade solvents, ethanol, isopropanol, and acetone were purchased from RCI Labscan (Bangkok, Thailand). Water was purified using a Mili-Q system purchased from Millipore, Bedford (MA, USA). Brain heart infusion (BHI) was obtained from Becton, Dickinson and Company

(NJ, USA). *Staphylococcus aureus* (ATCC 25923) and MRSA (DMST 20654) were obtained from the Department of Medical Sciences, Ministry of Public Health (Bangkok, Thailand).

Preparations of AME and LME

AME was prepared using a green microwave extraction and fractionation method previously described (Meah *et al.*, 2020b). The obtained yellow powder of AME was subsequently subjected to determining the α -mangostin content using an HPLC method. The AME used in this study contained 84.9% w/w α -mangostin. LME was prepared using a semisynthetic method through the methylation of lawsone under acidic conditions (Meah *et al.*, 2020a).

Quantitative HPLC method for AME and LME

The HPLC method of AME and LME was performed using the HPLC method previously described (Bundeesomchok *et al.*, 2016), with some modifications. Briefly, the method was performed on an LC-20 Shimadzu HPLC system (Tokyo, Japan) equipped with an LC-20AD pump, an SIL-20A autosampler, and an SPD-M20A photodiode-array detector. The HPLC column was a 150×4.6 mm, 5 μ m ACE 5 C18-PFP (Advanced Chromatography Technologies, Aberdeen, Scotland). The column was eluted with a mobile phase consisting of acetonitrile and 0.2% formic acid at a ratio of 70:30 (% v/v) at a flow rate of 1 mL/min, and the quantitative determinations of α -mangostin and LME were carried out at a wavelength of 240 nm.

The stock solutions of standard α -mangostin and LME (0.2 mg/mL) were prepared in methanol and diluted to provide five concentrations (200, 100, 50, 25 and 12.5 μ g/mL) to establish their calibration curves.

Determination of antibacterial and synergistic effect

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the tested compounds against MRSA were determined using a broth microdilution method as previously described (Meah *et al.*, 2020b). The living bacterial cell (pink color) was determined using a resazurin test (Coban, 2012). The BHI broth was used as a negative control, while ampicillin was used as a positive control. The MIC and MBC were determined at the lowest concentration of compounds that produced suppression of visible growth. The experiments were performed in triplicate.

A synergistic antibacterial interaction among AME and LME against MRSA was evaluated using

the checkerboard method (Meah *et al.*, 2020a). Briefly, AME was diluted by two-fold dilutions along the X-axis side of the well-plates, while two-fold dilutions of LME were performed along the Y-axis side. Each well was subsequently added with the bacterial suspension (1×10^6 CFU/mL), the plates were incubated at 37°C for 24 h, and then 0.02% resazurin (20 μ L) was added and incubated at 37°C for 1 h. The experiments were performed in triplicate.

The interaction among AME and LME was determined by calculating the fractional inhibitory concentration (FIC) and the fractional inhibitory concentration index (FICI) using equations (1) and (2), respectively (Milne and Gould, 2012).

$$\text{FIC} = \text{MIC}_b (\text{MIC of compounds in combination}) / \text{MIC}_a (\text{MIC of a single compound}) \quad (1)$$

$$\text{FICI} = \text{FIC of AME} + \text{FIC of LME} \quad (2)$$

Based on a calculated FICI value, the results were interpreted as a synergistic effect when the FICI value was less than 0.5.

Preparation of emulgels containing AME and LME

Emulgels were prepared by combining o/w emulsion and gel bases (Sohail *et al.*, 2018).

Briefly, the oil phase of the emulsion was prepared by dissolving Span 60 in light liquid paraffin (LLP), while the aqueous phase was prepared by dissolving Tween 60 in purified water, and then heated in a water bath at 70-75°C. The oil phase was then transferred into the aqueous phase and stirred. Whereas, the gel base was prepared using Carbopol 934 dispersed in purified water with constant stirring. Subsequently, four emulgels were

Table 1. Formulas of emulgels containing AME and LME

Formulations	Concentrations (%w/w)			
	F1	F2	F3	F4
AME	0.025	0.025	0.025	0.025
LME	0.2	0.2	0.2	0.2
BG	10	10	10	10
Citric acid	0.1	0.1	0.1	0.1
BHT	0.1	0.1	0.1	0.1
LLP	16	12	8	4
Tween 60	2.68	2.01	1.34	0.67
Span 60	1.32	0.99	0.66	0.33
Purified water	20	15	10	5
Carbopol 934	1	1	1	1
Paraben concentrate*	1	1	1	1
Purified water q.s. to	100	100	100	100
TEA q.s. to	pH 6-6.5			

*Paraben concentrate consisted of methyl paraben (10% w/v) and propyl paraben (2% w/v) in propylene glycol.

formulated based on the ratios of emulsion to gel, as shown in Table 1. AME, LME, BHT and citric acid were dissolved in butylene glycol (BG), added to the emulgel bases, and then stirred until the homogeneous emulgels were produced. After that, paraben concentrate was then added and stirred, and the pH was adjusted by triethanolamine (TEA) to 6-6.5 (Table 1).

Antibacterial evaluation of emulgel

The antibacterial effects of emulgel against *S. aureus* and MRSA were determined by a broth microdilution assay. The emulgel (1 g) was mixed with BHI broth (1 mL) and then 2-fold serial dilutions were performed on a 96-well microplate to contain 50 μ L of the mixture in each well. Bacterial suspension (50 μ L) was subsequently added to each well and incubated at 37°C for 24 h. The resazurin test was used to determine bacterial death (Coban, 2012). The emulgel without AME and LME was used as a control, while Betadine® and 1% silver sulfadiazine cream were used as positive controls. The highest dilution showing no visible growth was determined as MIC. The experiment was performed in triplicate.

Physical stability evaluation of the emulgel

Emulgel was kept in a tight container, protected from light, and performed a freeze-thaw stability test at -20°C for 48 h. It was and then placed at 25°C for 48 h, for 3 cycles. Before and after freeze-thaw cycles, general appearances, including texture, homogeneity, and phase separation were observed, and some physical properties were determined as follows:

Color testing: The color test of the emulgel was determined using a color matching spectrophotometer (CM-700d, Konica Minolta). Color valuation was expressed as follows: On the Munsell color systems, hue is the basic color that is a category of abbreviation of the color spectrum (Y = Yellow, GY = Greenish yellow, R = Red), value is lightness (a scale is 0 to 10 or black to white), and chroma is color saturation (0 to 20 or grey to color). CIE color systems: L* for perceptual lightness, a* are red and green, and b* are blue and yellow, the four unique colors of human vision.

Viscosity measurement: The viscosity of emulgel was measured using a Brookfield viscometer (Stoughton, MA 02072, USA) with a No. 4 spindle, rotated at 0.6 rpm, and at room temperature. The experiments were performed in triplicate.

pH measurement: Emulgel (1 g) was dissolved in 10 mL of distilled water. The pH value of the sample was measured using a pH meter (Mettler Toledo, USA). The experiments were performed in triplicate.

Chemical stability evaluation of the emulgel

Emulgel was kept in a tight container, protected from light, and stored in a stability chamber (Mettler, Germany) at 30°C, with 65% relative humidity (RH) as well as at 40°C, with 75% HR for 6 months (ICH, 2003). An aliquot of the sample was taken at 0, 3, and 6 months. The contents of AME and LME were determined using the HPLC method. The experiments were performed in triplicate. The percentage labelled amount (%LA) was calculated by equation 3.

$$\%LA = (\text{Analytical concentration} / \text{Concentration indicated on the label}) \times 100 \quad (3)$$

Biological stability evaluation of the emulgel

The biological stability of the emulgel was determined based on its antibacterial effects using a broth microdilution. Antibacterial evaluations were performed before and after accelerated conditions at 3 and 6 months.

Statistical analysis

Results were expressed as the mean $\pm$ S.D. The significant differences in data analysis between the groups were analyzed using an ANOVA followed by Tukey's test. *p*-Values of less than 0.05 were considered statistically significant.

Results and Discussion

Preparation of standardized AME and LME

AME was prepared by a green extraction and purification method (Meah *et al.*, 2020b). Based on this method, 14.9 g of AME was obtained from 1.5 kg of dried powders of *G. mangostana* pericarps. The percentage yield was almost 1%. AME was subjected to determination of α -mangostin content using an HPLC method and found to contain $84.9 \pm 1.90\%$ w/w of α -mangostin. LME was prepared from lawsone via a semi-synthesis method (Meah *et al.*, 2020a). In this study, a methylation of lawsone (10 g) in acidic conditions produced 8.2 g of LME, which was calculated at 77% yield. These methods produce a high quality of AME and LME and are practically applied in the herbal industries due to their simplicity, environmental friendliness, and cost-effectiveness. The obtained AME and LME were subjected to further studies.

Synergistic anti-MRSA effects of AME and LME

Based on the broth microdilution assay (Table 2), AME exhibited good anti-MRSA activity with the MIC and MBC values of 4 and 8 $\mu\text{g/mL}$, respectively, whereas LME and ampicillin exhibited weak antibacterial activity with the MIC of 32 and 64 $\mu\text{g/mL}$ and MBC of 128 and 256 $\mu\text{g/mL}$, respectively. Although a synergistic anti-MRSA effect among AME, containing 95.0% w/w of α -mangostin, and LME has been previously described (Meah *et al.*, 2020a), the present study re-determined the synergistic effect because the α -mangostin content of AME used (84.9% w/w) was different from the previous study.

Table 2. MIC and MBC of AME, LME and ampicillin against MRSA (n=3)

Compounds	MIC ($\mu\text{g/mL}$)	MBC ($\mu\text{g/mL}$)
AME	4	8
LME	32	128
Amp	64	256

MIC: minimal inhibitory concentration; MBC: minimal bactericidal concentration

Table 3. Synergistic effect of AME and LME against MRSA (n=3)

Compounds	MICa ($\mu\text{g/mL}$)	MICb ($\mu\text{g/mL}$)	FIC	FICI	Interaction
AME	4	0.5	0.125	0.25	Synergistic
LME	32	4	0.125		

MICa: MIC of the sample alone; MICb: MIC of the sample combination

Based on the checkerboard assay, the interaction between AME (0.5 $\mu\text{g/mL}$) and LME (4 $\mu\text{g/mL}$) against MRSA was synergistic with a FICI value of 0.25 (Table 3). These results confirm the previous reports on synergistic anti-MRSA effects among AME and LME (Meah *et al.*, 2020a). α -Mangostin has been reportedly to inhibit MRSA via altering membrane permeability by its isoprenyl groups and resulting in loss of intracellular fluid (Koh *et al.*, 2013). The mechanism of a synergistic effect of LME with AME against MRSA is relate to the various target sites of actions. LME has been shown to have antibacterial properties through changing microorganism mitochondrial respiration (Vanichayachart *et al.*, 1992) and the synergistic effect of AME and LME has been studies

bacteriolysis activity that effect of a cell membrane rupture of microbial which resulted in the leakage of intracellular fluid of MRSA (Meah *et al.*, 2020a).

AME and LME-loaded emulgel formulations

In this study, the concentrations of AME and LME in the emulgels were set to be 500 times their synergistic MICs to ensure their topical antibacterial effect. Therefore, the concentrations of AME and LME in the formulations were fixed at 0.025% and 0.2%, respectively. Carbopol 934 was used for the formulation of emulgel bases, because it has been reported that Carbopol 934 possessed better drug release profiles than Carbopol 940 (Nikumbh *et al.*, 2015). Four emulgel formulations, namely F1, F2, F3, and F4, were formulated as shown in Table 1. Based on the freeze-thaw cycle test, F4-emulgel was the most suitable formula since it exhibited better characteristics, i.e., smooth, homogeneous texture, and non-sticky as well as being more stable than the others (Figure 1). The viscosity values of emulgel formulations are presented in Table 4. Viscosity increased with an increase in the emulsion concentration at F1 > F2 > F3 > F4, respectively. There were no significant differences in the pH of all formulation. Thus, F4-emulgel was then subjected to evaluation for antibacterial effects.

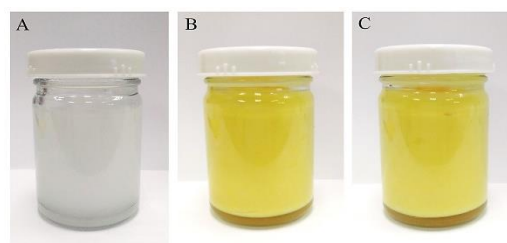


Figure 1. F4 emulgel base (A), F4 emulgel before (B), and after (C) freeze-thaw cycle test.

Antibacterial effects of F4 emulgel against MRSA and *S. aureus* were carried out compared with two standard drugs, namely Betadine[®] solution, 1% silver sulfadiazine cream, and the emulgel base. The results are shown in Table 5. F4-emulgel exhibited antibacterial effects against MRSA and

Table 4. Characteristics of emulgels after the freeze-thaw cycles

Formulations	Appearances	pH value	Viscosity ($\times 10^4$ cps)
F1	Yellow, homogenous, smooth and slightly sticky	6.18 $\pm$ 0.04	58.05 $\pm$ 0.75
F2	Yellow, homogenous, smooth and slightly sticky	6.17 $\pm$ 0.05	56.18 $\pm$ 0.47
F3	Yellow, homogenous, smooth and slightly sticky	6.18 $\pm$ 0.04	54.01 $\pm$ 0.20
F4	Yellow, homogenous and smooth	6.15 $\pm$ 0.02	52.66 $\pm$ 0.57

S. aureus at dilutions of 1:512 and 1:1024, respectively, while the emulgel base was inactive against both bacteria. In addition, Betadine® and 1% silver sulfadiazine cream exhibited antibacterial activity against MRSA and *S. aureus* at dilutions lower than F4 emulgel. The results confirm that the concentrations of AME (0.025%) and LME (0.2%) in the emulgel are enough to exhibit good antibacterial effects against MRSA and *S. aureus*, and these antibacterial effects were better than those of Betadine® and 1% silver sulfadiazine cream.

Physical stability of F4 emulgel

The visual appearance and physical properties of F4 emulgel before and after the freeze-thaw cycle test were determined as shown in Table 6. The visual appearance and physical properties of the emulgel were not changed upon subjection to the freeze-thaw cycles.

A color determination by the Munsell color system revealed that based on three specific colors, F4 emulgel had a greenish yellow (GY) color, with the L^* a^* b^* values not changing after the freeze-thaw cycles. F4 emulgel had a mild acidic pH of 6.15, which was in the accepted ranges of topical formulations (pH 5-8) (Saraf *et al.*, 2010), and the pH was stable after the freeze-thaw cycles. In addition, the viscosity of the emulgel was 52.66×10^4 cps and did not significantly change (52.33×10^4 cps) after the freeze-thaw cycles. These results indicated that the emulgel exhibited good physical stability (Sohail *et al.*, 2018; Khullar *et al.*, 2012).

Chemical stability of F4 emulgel

The chemical stabilities of F4 emulgel were evaluated using a stability chamber at $30 \pm 2^\circ\text{C}$ and 65% RH, and $40 \pm 2^\circ\text{C}$ and 75% RH (accelerated conditions) over a period of 6 months. The concentrations of AME and LME were periodically determined at 0, 3, and 6 months using an HPLC method, and it was revealed that the concentrations of AME and LME in the emulgel were not significantly decreased under $30 \pm 2^\circ\text{C}$ and 65% RH over the period of 6 months (Figure 2). In contrast, under the accelerated conditions, the concentration of LME was significantly decreased by approximately 30%, while the concentration of AME was not significantly decreased after 6 months (Figure 2). LME may undergo hydrolysis because the emulgel base contains water. LME can be hydrolyzed with the aid of water at high temperatures to be lawsone. AME was entrapped in the oil phase of the emulsion, which may increase its chemical stability. Our results agreed with the previous report on the stability of AME at $4 \pm 2^\circ\text{C}$ and $30 \pm 2^\circ\text{C}$ but were not stable under the accelerated conditions (45°C and 75% RH) for a 4-month-period (Meah *et al.*, 2020b).

A decrease in α -mangostin concentration by 25%, approximately, has been found in α -mangostin-loaded film-forming gel after being kept at room temperature for 6 months (Tanngoen *et al.*, 2018). In addition, it has been reported that α -mangostin-loaded nanoemulsion was stable at room temperature ($27 \pm 2^\circ\text{C}$) after 90 days and

Table 5. Antibacterial activity evaluation of the F4 emulgel (n=3)

Samples	MIC in terms of dilutions of samples	
	<i>S. aureus</i>	MRSA
F4 emulgel	1:1024	1:512
Emulgel base	n.a.	n.a.
Betadine®	1:64	1:64
1% Silver sulfadiazine cream	1:256	1:128

n.a. = not active at the dilution of 1:8

Table 6. Physical stability evaluation of the F4 emulgel

Physical properties	Before the freeze-thaw cycle	After the freeze-thaw cycle
Appearances	Yellow semi-solid	Yellow semi-solid
Color test		
- Munsell color		
Hue/value/chroma	0.8GY/5.1/3.2	0.7GY/5.1/3.3
- CIE Lab		
L^*	52.70	52.70
a^*	-6.75	-6.76
b^*	22.65	23.16
pH value	6.15 ± 0.02	6.15 ± 0.01
Viscosity ($\times 10^4$ cps)	52.66 ± 0.57	52.33 ± 0.57

L^* : lightness; a^* : redness; b^* : yellowness.

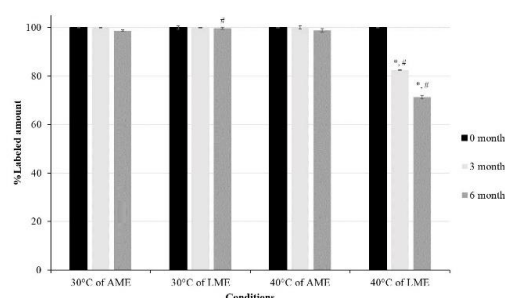


Figure 2. Percentage labeled amount of AME and LME in emulgel, *Significant difference ($p < 0.05$) when compared to the initial date (0 month). #Significant difference ($p < 0.05$) when compared between AME and LME under the same conditions

Table 7. Biological stability of F4 emulgel (n=3)

Accelerate conditions	MIC in terms of dilution of samples	
	<i>S. aureus</i>	MRSA
30 ± 2°C, 65% RH		
0 month	1:1024	1:512
3 months	1:1024	1:512
6 months	1:1024	1:512
40 ± 2°C, 75% RH		
0 month	1:1024	1:512
3 months	1:512	1:128
6 months	1:512	1:128

under the heating-cooling cycles (Asasutjarit *et al.*, 2019), while α -mangostin in the propolis cream was stable when kept at 30 ± 2°C and 65% RH, and 40 ± 2°C and 75% RH for 45 days (Chuapaditphun *et al.*, 2019). These imply that the hydrophobic drugs, AME and LME, might be more stable when they were loaded in oil droplets of the emulsion structure of the emulgel base.

Biological stability of F4 emulgel

The biological stability of F4 emulgel was evaluated on the basis of the antibacterial activity of the emulgel before and after storage at 30 ± 2°C and 65% RH, and under accelerated conditions (40 ± 2°C and 75% RH) for 6 months. The results are shown in Table 7. The emulgel exhibited good biological stability at 30 ± 2°C and 65% RH over a period of 6 months. However, the antibacterial effects of the emulgel were slightly reduced (one dilution) after being kept under the accelerated conditions for 6 months. The results agree with the chemical stability evaluation of the emulgel.

Conclusions

F4 emulgel containing 0.025% AME and 0.2% LME was successfully formulated using 1%

Carbopol 934 as gelling agent. The emulgel exhibited good antibacterial effects against MRSA and *S. aureus* as well as good stability when it was kept in an air tight container protected from light and stored at a temperature not exceeding 30 ± 2°C to prolong its shelf-life. This emulgel might be used as an alternative topical treatment for skin and wound infections. However, further studies of *in vivo* wound healing and toxicity are needed prior to its clinical applications.

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