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Potential use of topical film-forming sprays containing *Garcinia cowa* leaf extract nanoemulsion for pharmaceutical and cosmeceutical applications

Kanyawadee Bureekaew[a], Mayuree Kanlayavattanakul[b], Surat Laphookhieo[a,c], Yan Zhao[d], Suvimol Surassmo[e], Orawan Suwantong[a,c,*]

[a] School of Science, Mae Fah Luang University, Chiang Rai 57100 Thailand
[b] School of Cosmetic Science, Mae Fah Luang University, Chiang Rai 57100 Thailand
[c] Center of Chemical Innovation for Sustainability (CIS), Mae Fah Luang University, Chiang Rai 57100 Thailand
[d] Institute of Edible Fungi, Shanghai Academy of Agricultural Sciences, Shanghai 201403 China
[e] National Nanotechnology Center, National Science and Technology Development Agency, Pathum Thani 12120 Thailand

*Corresponding author, e-mail: orawan.suw@mfu.ac.th

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ABSTRACT: This study developed and characterized a topical film-forming spray incorporating *Garcinia cowa* leaf extract (GC) nanoemulsion for potential applications in pharmaceutical and cosmeceutical products. The formulations were prepared using Eudragit E100 (EuE100) as the polymeric matrix, dibutyl phthalate (DBP) as a plasticizer, GC nanoemulsion as the active ingredient, polyvinyl pyrrolidone (PVP) as a binder and enhancer, and ethanol as the solvent. Results indicated that viscosity increased with higher concentrations of EuE100 while the spray pattern diminished. All formulations (S1–S6) exhibited evaporation durations ranging from 3.27 to 7.10 min and demonstrated low contact angles between 19.05° and 30.04°. The developed film-forming sprays exhibited significant antibacterial and anti-inflammatory activities and no cytotoxicity. Skin irritation potential was assessed by a closed patch test, and no significant irritant effects were observed. These topical film-forming sprays containing GC nanoemulsion show potential for pharmaceutical and cosmeceutical applications.

KEYWORDS: *Garcinia cowa* leaf extract, nanoemulsion, topical film-forming spray, pharmaceutical and cosmeceutical products

INTRODUCTION

The skin is the largest organ in the human body and has three primary functions, including protection, regulation, and sensation. It acts as a barrier against temperature fluctuations and protects the body from the harmful effects of bacteria, radiation, and chemicals [1]. The three principal layers of the skin comprise the epidermis, dermis, and subcutis. The epidermis, as the outermost layer of the skin, safeguards against chemical exposure, bacterial invasion, and transepidermal water loss. This robust yet highly flexible layer contributes to maintaining a healthy skin appearance and youthfulness [2, 3]. When bacteria or pathogens breach this barrier, they trigger inflammation and tissue damage in the skin while impeding healing. To address these issues, pharmaceutical and cosmeceutical interventions are utilized to mitigate further injury and reduce bacterial contact with the skin surface. The epidermal layer predominantly consists of lipids and fatty acids; therefore, product formulations must possess lipophilic properties [4]. Besides, the particle size plays a critical role in determining the absorption efficacy through human skin. Numerous hair follicles have pore sizes ranging from 100 to 200 μm; thus, products should ideally have a particle diameter not exceeding 200 nm [5, 6].

There are many marketed pharmaceutical and cosmeceutical products, including hydrogels, facial masks, and topical film-forming sprays [7, 8]. Sprays are a popular choice for transdermal drug delivery and offer many advantages over conventional topical preparations. Sprays provide excellent coverage of the skin or wound, high drug penetration, reduced irritation, accelerated wound healing through moisture control, and protection of the skin or wound from bacteria or pathogens [8]. A topical film-forming spray is a liquid solution that forms a transdermal film after being applied to the skin. It comprises four main components: a polymer, a solvent, penetration enhancers, and a drug [9]. It is a favored route of drug administration with easy release, and the digestive tract or liver cannot destroy the drug. However, its application has limitations such as reduced drug delivery through the skin because the outermost layer of the epidermis, called the stratum corneum, prevents substance penetration [10].

Particle size affects the delivery of an active substance to various sites in the body. Thus, nanoencapsulation has the potential to protect the substance against enzymatic breakdown, temperature, or moisture, while improving controlled release and increasing bioavailability [11, 12]. Many researchers have investigated drug delivery using nano-sized particles that

Table 1 Compositions of topical film-forming sprays containing GC nanoemulsion.

Formulation	EuE100 (%w/v)	EuE100:PVP weight ratio	DBP (%w/v)	GC2 (%v/v)	GC3 (%v/v)
B1	8	7:3	2	–	–
B2	10	7:3	2	–	–
B3	12	7:3	2	–	–
S1	8	7:3	2	16	–
S2	10	7:3	2	16	–
S3	12	7:3	2	16	–
S4	8	7:3	2	–	16
S5	10	7:3	2	–	16
S6	12	7:3	2	–	16

can penetrate the stratum corneum [13, 14] using nanoencapsulation techniques for antibiotics, lipophilic drugs, hydrophilic drugs, vitamins, and polyphenolic compounds [15–21]. Nanoencapsulation of the active substances plays an important role in developing topical film-forming sprays.

This study developed topical film-forming sprays containing *Garcinia cowa* leaf extract (GC) nanoemulsion for pharmaceutical and/or cosmeceutical applications. These developed film-forming sprays were characterized for their physicochemical properties (pH, viscosity, evaporation time, weight per spray, contact angle, and spray pattern), as well as their encapsulation efficiency, release characteristics, antibacterial activity, anti-inflammatory activity, cytotoxicity, stability, and skin irritation.

MATERIALS AND METHODS

Materials

Garcinia cowa leaf extract (GC) was obtained from Laphookhieo's laboratory. Span 80, Tween 20, Tween 80, glycerol, Griess reagent, polyvinylpyrrolidone (PVP), dibutyl phthalate (DBP), and lipopolysaccharide (LPS) (from *Escherichia coli* O55:B5) were purchased from Sigma-Aldrich (USA). Ethanol and chloroform were purchased from RCI Labscan (Thailand). Eudragit® E100 (EuE100) was a gift from Jebsen & Jessen Ingredients (T) Ltd. (Thailand). The Finn chamber was purchased from Epitest Ltd. (Finland).

Preparation of topical film-forming sprays containing GC nanoemulsion

The GC nanoemulsions used in this study were prepared according to the method described by Bureekaew et al [22]. The selected GC2 and GC3 nanoemulsions exhibited nano-sized droplets (66–68 nm), low PDI values (< 0.2), negative zeta potentials (approximately −10 to −12 mV), encapsulation efficiencies of 42–45%, and good physical stability during storage at 30 °C for 3 months [22]. First, Eudragit E100 (EuE100) was dissolved in ethanol to prepare various solution concentrations (8, 10, and 12% (w/v)). Next, 16% (v/v) of GC nanoemulsion (based on the volume of the EuE100 solution) was added to EuE100 solution. The mixture was stirred until a homogenous solution was obtained. After that, PVP was added to the mixture at a weight ratio of 7:3 (EuE100:PVP) and stirred continuously. Then, 2% (w/v) of DBP was added and stirred until a homogenous solution was obtained. The compositions of topical film-forming sprays containing GC nanoemulsion are shown in Table 1. These film-forming sprays were kept in a closed container.

Physicochemical properties of topical film-forming sprays containing GC nanoemulsion

Stability testing

Stability testing was conducted to observe the physical properties of topical film-forming sprays containing GC nanoemulsion under three conditions (4 °C, room temperature, and 45 °C). The solutions were stored for 0, 1, 2, and 3 months.

pH study

The pH of the formulations was measured with a pH meter calibrated with standard solutions at pH 4, 7, and 10.

Viscosity study

The viscosity of the formulations was measured using a Brookfield viscometer (AMETEK Brookfield, USA) with a spindle rotational speed of 200 rpm at room temperature.

Contact angle

The topical film-forming spray formulations were characterized by contact angle and surface tension (SL200KS, KINO Scientific Instrument Inc., USA). The sessile drop mode was used for contact angle examination at 25 °C. The sample was dropped on a glass slide, and the droplet was captured and analyzed by the software.

Spray pattern study

The spray pattern was determined by actuating the colored formulations onto a piece of white paper positioned perpendicularly to the spray nozzle. First, all formulations were colored using methylene blue. Then, each formulation was sprayed onto the white paper at a distance of 5.0 cm. The resulting spray pattern was evaluated by measuring the diameter of the blue spot at four different orientations.

Weight per spray study

The weight per spray is a quantitative test used to determine the weight of the formulations per actuation. First, each formulation was filled into a spray container. The containers were weighed before and

after spraying. The weight per spray was calculated using Eq. (1).

$$\text{Weight per spray} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Number of actions}} \quad (1)$$

Evaporation time

The evaporation time is defined as the time required for the formulations to form a film. Each formulation was sprayed onto a polystyrene plate, with the spray area set to 3.5 cm in diameter and the solution volume to 130 µl. The evaporation time was recorded at room temperature.

Encapsulation efficiency

The encapsulation efficiency was measured to determine GC loaded into the topical film-forming sprays. First, each sample was added to a centrifuge tube containing chloroform at a ratio of 0.2:2 ml (sample:chloroform). The mixtures were then sonicated in an ultrasonic bath at a power level of 9 at room temperature for 30 min. Each solution was then placed in a refrigerator to accelerate the stratification. Finally, the GC content in the samples was analyzed using a UV-Vis spectrophotometer (Perkin-Elmer, USA) at a wavelength of 413 nm. Since a specific marker compound was not isolated from the extract, the GC itself was used as the calibration reference. Standard solutions of GC with concentrations ranging from 0.03 to 0.5 mg/ml were prepared and analyzed at 413 nm. The calibration curve exhibited excellent linearity, with a regression equation of $y = 1.8795x + 0.0036$ ($R^2 = 0.9997$). The encapsulation efficiency (%EE) was calculated using Eq. (2).

$$\text{EE (\%)} = \frac{\text{Weight of loaded GC}}{\text{Weight of initial GC}} \quad (2)$$

Drug release study of topical film-forming sprays GC nanoemulsion

The *in vitro* release of GC from topical film-forming sprays containing GC nanoemulsion was determined by the Franz diffusion cell method. First, 130 µl of each topical film-forming spray formulation was dropped on a glass slide (3 cm in diameter). Then, each sample was placed on a polyethersulfone membrane in a modified Franz diffusion cell system containing a receptor medium composed of 94% (v/v) PBS (pH 5.5) and 1% (v/v) Tween 80 at 32.5 °C. The receptor solution was withdrawn at specified time points (0.5, 1, 2, 3, 4, 6, 8, 10, 24, and 48 h). At each time point, 1 ml of the receptor solution was removed and replaced with an equal volume of fresh buffer solution. Then, the released amounts of GC were measured using a UV-Vis spectrophotometer at 413 nm and calculated from the GC calibration curve.

Antibacterial activity of topical film-forming sprays containing GC nanoemulsion

Staphylococcus aureus TISTR 746, *Staphylococcus epidermidis* DMST 15505, *E. coli* TISTR 527, and *Pseudomonas aeruginosa* TISTR 1287 were used to evaluate the antibacterial activity of topical film-forming spray formulations. One loopful of each bacterium was inoculated into a test tube and shaken in an incubator shaker at 37 °C for 24 h. Bacterial cultures containing approximately 10^5 CFU/ml were prepared. To estimate the antibacterial activity, 130 µl of each topical film-forming spray formulation was dropped onto a 6-well plate and then dried in a fume hood. The resulting film sample was immersed in 1 ml of medium [PBS (pH 5.5), 1% (v/v) Tween 80, and 5% (v/v) ethanol] for 4 h to obtain the extraction medium. Subsequently, 50 µl of the bacterial suspension was added to the wells containing 50 µl of the extraction medium. The plates were incubated at 37 °C for 24 h, and and the optical density (OD) was measured at 625 nm using a microplate reader (BioTek Instruments, USA) to observe bacterial growth.

In this study, bactericidal activity (bacterial killing) was defined as the ability of the extraction medium from the topical film-forming sprays containing GC nanoemulsion to kill 99.9% of the bacteria. A drop from each well was streaked onto a nutrient agar plate and incubated at 37 °C for 24 h. Finally, if no bacterial growth was observed, the extraction medium was considered to possess bactericidal activity.

Anti-inflammatory activity of topical film-forming sprays containing GC nanoemulsion

RAW 264.7 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. First, each topical film-forming spray formulation was dropped onto a 6-well plate and then dried in a fume hood. The film sample was immersed in a serum-free medium (SFM) for 4, 8, and 24 h to produce the extraction medium. After the specified incubation time, the extraction medium was collected. Concurrently, RAW 264.7 cells were seeded into a 96-well plate at 1×10^5 cells/well and incubated in a humidified incubator with 5% CO_2 at 37 °C for 24 h to allow cell attachment. The cells were then treated with the extraction medium for 1 h. After that, the inflammatory response was induced with LPS at 37 °C for 24 h. Then, the Griess reagent was added to monitor nitric oxide (NO) production from the cells. The plate was then incubated at room temperature for 10 min. Finally, the anti-inflammatory activity of the topical film-forming spray formulations was evaluated by measuring the absorbance at 540 nm using a microplate reader (BioTek Instruments, USA). The NO level is expressed based on the nitrite calibration curve, and the relative NO inhibition (%) was

calculated using Eq. (3).

$$\text{NO inhibition (\%)} = \frac{(A_{control} - A_{blank}) - (A_{sample} - A_{background})}{A_{control} - A_{blank}} \times 100\% \quad (3)$$

Where $A_{control}$ is the absorbance of the control (cells, media, and LPS, but no sample), A_{sample} is the absorbance of the sample (cells, media, LPS, and sample), $A_{background}$ is the absorbance of the background (sample and media), and A_{blank} is the absorbance of cells and media.

Indirect cytotoxicity test of topical film-forming sprays containing GC nanoemulsion

NCTC clone 929 cells were cultured in DMEM containing 10% FBS at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. First, 130 µl of each topical film-forming spray formulation was dropped onto a 6-well plate and dried in a fume hood. The resulting film sample was immersed in 1 ml of SFM for 4 and 24 h to produce the extraction medium. After a specified incubation time, the extraction medium was collected. Concurrently, the cells were seeded into a 96-well plate at a density of 8,000 cells/well and incubated in a humidified incubator with 5% CO_2 at 37 °C for 24 h to allow cell attachment. The cells were then starved with SFM for 24 h. After that, the medium was replaced with the extraction medium, and the cells were incubated for an additional 4 h. After the incubation period, cell viability was determined using the MTT assay, with cells cultured in fresh SFM serving as the control.

Clinical evaluation

Volunteer recruitment

This study protocol was approved by the Mae Fah Luang University Ethics Committee (Approval No. COA: 097/2022) on June 16, 2022, before the evaluation of human volunteers commenced. All investigation methods concerning human volunteers filed in the study protocol followed the guidelines of the Declaration of Helsinki and Tokyo for humans. Healthy Thai volunteers aged between 20 and 60 years old with no skin diseases were registered in this study. All volunteers were informed about the study and signed a consent form which was approved by the ethical committee of Mae Fah Luang University before enrollment.

Skin irritation test

The skin irritation investigation of the topical film-forming sprays containing GC nanoemulsion was performed using a single-application, closed patch test on 30 healthy Thai volunteers (22 female and 8 male) for 24 h. Finn chambers (8 mm) on Scanpor tape were used. A 20-µl sample, including distilled water (negative control), the blank topical film-forming spray (B1), and the topical film-forming spray containing GC nanoemulsion (S1), was added to the Finn chambers. Then, the test materials were placed on the upper outer arms and closed for 24 h. The sites were graded at 24 h after patch removal using a 6-point (0–4) erythema grading scale to evaluate irritation severity as follows:

0: No irritation, erythema;

0.5: Weak positive reaction, with little erythema and difficult to observe;

1: Weak positive reaction, with little erythema across most of the treatment site;

2: Moderate positive reaction, with obvious erythema;

3: Strong positive reaction, with spreading erythema and edema in the test area;

4: Strong positive reaction, with spreading erythema and edema extending beyond the test area.

The Mean Irritation Index (M.I.I.) was calculated following Eq. (4).

$$\text{M.I.I.} = \frac{\Sigma \text{ erythema grade at 24 h}}{\text{number of volunteers}} \quad (4)$$

The irritation degree was classified based on M.I.I. values: non-irritation (M.I.I. < 0.2), slight irritation ($0.2 \leqslant$ M.I.I. $\leqslant 0.5$), moderate irritation ($0.5 \leqslant$ M.I.I. $\leqslant 1$), and severe irritation (M.I.I. $\geqslant 1$).

Statistical analysis

All the experimental data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using either one-way ANOVA followed by Tukey's post hoc test or an independent samples *t*-test, using IBM SPSS Statistics 24 (USA). Statistical significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Physicochemical properties of topical film-forming sprays containing GC nanoemulsion

The topical film-forming sprays were formulated using EuE100, PVP, and DBP. EuE100 was utilized as the film-forming polymer matrix for controlled drug release, while DBP was added as a plasticizer to improve film flexibility and softness. PVP was incorporated to enhance the release rate [13, 19]. To determine the optimal composition, nine formulations were prepared and evaluated for their physicochemical properties, as shown in Table 2. Results indicated that before the addition of GC nanoemulsion, increasing the concentration of EuE100 (B1, B2, and B3) increased viscosity but reduced the spray pattern area, attributed to higher cohesive forces of EuE100. A narrower spray pattern resulted in a smaller coverage area and extended the evaporation time.

The topical film-forming sprays containing GC nanoemulsion (S1–S6) gave evaporation times ranging from 3.27 to 7.10 min. The pH of the formulations

Table 2 Physicochemical properties of the topical film-forming sprays.

Formulation	pH	Viscosity (cP)	Contact angle (°)	Evaporation time (min)	Weight/spray (g)	Spray pattern (cm)	Stability
B1	8.77 ± 0.01	4.01 ± 0.15	28.33 ± 1.39	3.16 ± 0.07	N/A	3.76 ± 0.08	N/A
B2	8.82 ± 0.02	5.48 ± 0.32	31.54 ± 2.91	3.66 ± 0.31	N/A	3.22 ± 0.08	N/A
B3	8.92 ± 0.00	12.89 ± 0.18	37.10 ± 0.41	4.03 ± 0.05	N/A	3.05 ± 0.02	N/A
S1	8.14 ± 0.07	3.33 ± 0.15	20.97 ± 1.38	3.27 ± 0.05	0.08 ± 0.01	4.01 ± 0.01	Unchanged
S2	8.53 ± 0.02	5.25 ± 0.20	28.50 ± 1.44	4.31 ± 0.12	0.09 ± 0.02	3.23 ± 0.09	Unchanged
S3	8.58 ± 0.01	12.57 ± 0.68	30.04 ± 1.03	4.87 ± 0.37	0.12 ± 0.03	3.06 ± 0.04	Precipitated
S4	8.49 ± 0.09	4.02 ± 0.04	19.05 ± 0.80	4.41 ± 0.10	0.12 ± 0.00	3.87 ± 0.03	Unchanged
S5	8.48 ± 0.06	5.38 ± 0.18	26.26 ± 0.57	6.26 ± 0.06	0.11 ± 0.00	3.32 ± 0.01	Unchanged
S6	8.49 ± 0.00	11.08 ± 0.37	27.68 ± 0.95	7.10 ± 0.02	0.10 ± 0.04	3.07 ± 0.04	Precipitated

(S1–S6) decreased with the addition of acidic GC nanoemulsion. Although the formulations exhibited relatively alkaline pH values (approximately 8.1–8.9) compared to the physiological skin surface pH, no significant skin irritation was observed during the patch test. This acceptable skin compatibility could be attributed to the rapid solvent evaporation and subsequent film formation upon application, which minimizes prolonged contact of the liquid formulation with the skin [7]. Furthermore, the natural buffering capacity of human skin can efficiently counteract slightly alkaline exposure, thereby preventing the induction of erythema or irritation during short-term contact. However, further long-term studies are necessary to confirm skin compatibility during extended use [23]. The contact angle is defined as the angle of interaction between a liquid and a solid surface [24]. The contact angle values of the developed formulations ranged from 19.05° to 30.04°, indicating good wettability and spreadability. These relatively low contact angle values suggest that the formulations could spread effectively on the skin surface, thereby facilitating uniform film formation and improving surface coverage after spray application. However, the formulations S3 and S6 exhibited longer evaporation times and narrower spray patterns. Furthermore, after the stability study under various storage conditions (4 °C, room temperature, and 45 °C) for 3 months, formulations S3 and S6 developed visible white precipitates at the bottom of the containers, particularly at elevated temperatures, indicating compromised physical stability. In contrast, formulations S1, S2, S4, and S5 remained visually homogeneous, with no observable precipitation or phase separation throughout the entire storage period. Thus, formulations S1, S2, S4, and S5 were selected for further evaluation to study their biological activities and release profiles.

Encapsulation efficiency of topical film-forming spray containing GC nanoemulsion

The %EE represents the percentage of GC successfully entrapped into the topical film-forming nanoemulsion spray. The %EE of the samples was quantified via UV-Vis spectrophotometry at 413 nm using a calibration curve of GC ($y = 1.8795x + 0.0036$, $R^2 = 0.9997$). The %EE values of S1, S2, S4, and S5 were 71.81 ± 3.56, 65.66 ± 1.71, 60.01 ± 0.03, and 62.25 ± 1.27, respectively. The %EE values of S1 and S2 were higher than those of S4 and S5, possibly due to the larger particle size of GC2, as reported by Bureekaew et al [22], loaded into S1 and S2 that increased the ability to entrap GC [25].

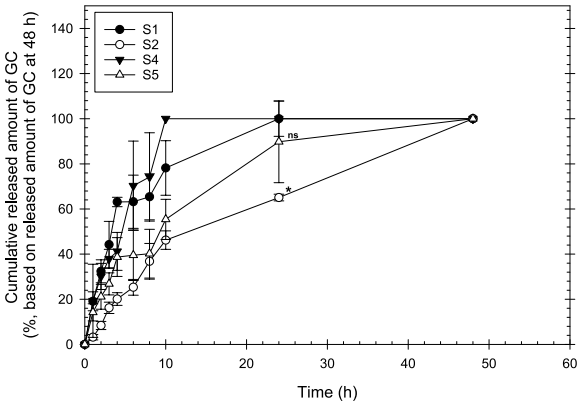

Fig. 1 Cumulative release profiles of GC from topical film-forming sprays containing GC nanoemulsion ($n = 3$). The asterisk (*) indicates a statistically significant difference between S1 and S2 ($p < 0.05$), whereas 'ns' denotes no significant difference between S4 and S5 ($p > 0.05$) at 24 h.

Drug release study of topical film-forming sprays containing GC nanoemulsion

The drug release study was carried out in PBS containing Tween 80 (1% (v/v)) at 32.5 °C for 48 h. The release profiles of the topical film-forming sprays containing GC nanoemulsion are shown in Fig. 1. All samples exhibited a gradual and continual release of GC over time. Specifically, the GC released from formulations S4 and S1 reached a plateau at 10 h and 24 h, respectively. At 24 h, the cumulative released amount of GC from S2 and S5 was lower than that from S1 and S4. Statistical analysis confirmed a significant difference between S1 and S2 ($p < 0.05$), indicating that higher concentrations of EuE100 formed a denser polymeric matrix that hindered GC release. On the other hand, no significant difference was observed

between S4 and S5 ($p > 0.05$). This indicates that although S5 contained a higher polymer concentration than S4, its hindering effect on drug release was less pronounced at 24 h, as both formulations had already achieved relatively high cumulative release levels (100% and 90%, respectively). All formulations eventually reached 100% cumulative release at 48 h.

Antibacterial activity of topical film-forming sprays containing GC nanoemulsion

Four bacterial candidates (Gram-negative: *E. coli* TISTR 527 and *P. aeruginosa* TISTR 1287, and Gram-positive: *S. epidermidis* DMST 15505 and *S. aureus* TISTR 746) were used to evaluate the antibacterial activity of the topical film-forming spray formulations. The susceptibility testing results, evaluated qualitatively based on bacterial growth inhibition and bactericidal activity, are reported in Table 3. Although the blank formulations (B1 and B2) exhibited inherent antibacterial activity—primarily due to the well-documented intrinsic antimicrobial properties of the cationic polymer Eudragit E100 [26]—the incorporation of the GC nanoemulsion significantly enhanced the overall antibacterial performance. Notably, while the blank formulation B1 failed to exhibit bactericidal (killing) activity against *S. aureus*, all GC nanoemulsion-loaded formulations (S1, S2, S4, and S5) demonstrated complete bactericidal efficacy against all tested strains, including *S. aureus*. These findings clearly indicate that the incorporation of the GC nanoemulsion provides a critical synergistic effect, improving the bactericidal efficacy of the topical film-forming sprays beyond the baseline activity provided by the polymeric matrix alone.

Anti-inflammatory activity of topical film-forming sprays containing GC nanoemulsion

The NO assay was used to evaluate the anti-inflammatory activity of the formulations. As shown in Fig. 2, formulations S1, S2, S4, and S5 exhibited good NO inhibition, confirming that the topical film-forming sprays containing GC nanoemulsion possess anti-inflammatory activity. Notably, the RAW 264.7 cells treated with the extraction medium from the topical film-forming sprays containing GC nanoemulsion showed higher NO inhibition compared to those treated with the extraction medium from the blank (topical film-forming spray without GC nanoemulsion). This enhanced efficacy can be attributed to the presence of xanthone compounds in the GC, which are well-documented to exhibit strong anti-inflammatory properties [27].

Indirect cytotoxicity of topical film-forming sprays containing GC nanoemulsion

The MTT assay was used to evaluate the indirect cytotoxicity of the spray formulations on NCTC clone 929 cells. The relative cell viability compared with the untreated control is illustrated in Fig. 3. Cell viability was determined after 4 and 24 h of incubation with different extraction media from the spray formulations. At 4 h, the viability of cells cultured with the extraction medium from the topical film-forming sprays containing GC nanoemulsion ranged between

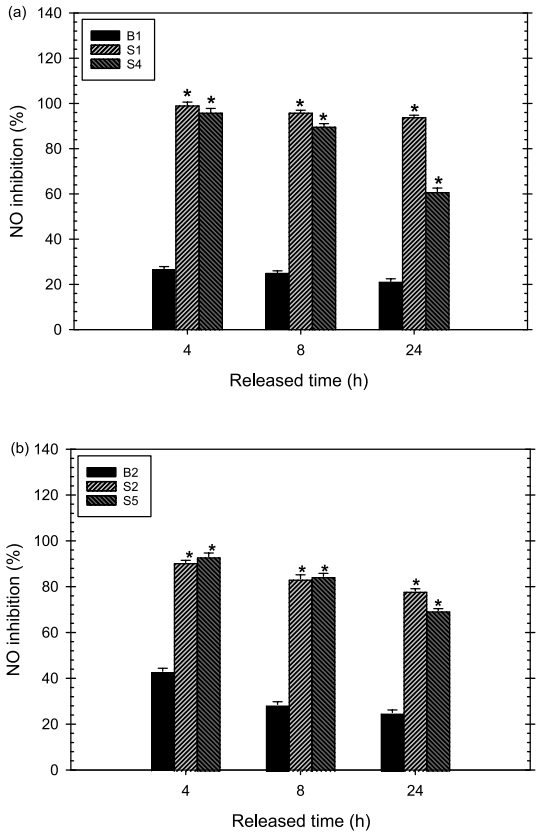

Fig. 2 Anti-inflammatory activity of (a) B1, S1, and S2 (* $p < 0.05$ compared with B1) and (b) B2, S4, and S5 (* $p < 0.05$ compared with B2).

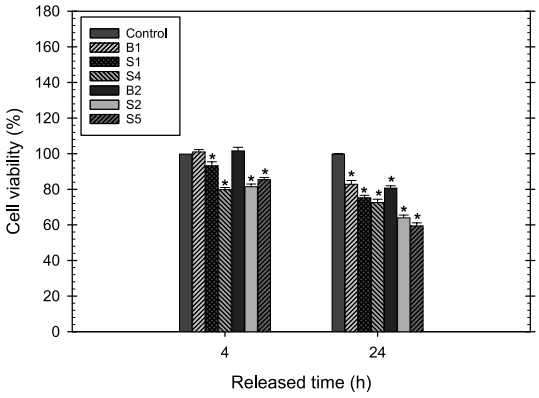

Fig. 3 Indirect cytotoxicity of extraction medium released from B1, S1, S2, B2, S4, and S5 at 4 and 24 h cultured with NCTC clone 929 cells (* $p < 0.05$ compared with the control).

Table 3 Bacterial inhibition and killing of the extraction medium from topical film-forming sprays containing GC nanoemulsion ($n = 3$).

Microorganism	Bacterial inhibition						Bacterial killing					
	B1	B2	S1	S2	S4	S5	B1	B2	S1	S2	S4	S5
E. coli TISTR 527	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
P. aeruginosa TISTR 1287	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
S. epidermidis DMST 15505	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
S. aureus TISTR 746	✓	✓	✓	✓	✓	✓	–	✓	✓	✓	✓	✓

✓= inhibited or killed bacteria; – = not inhibited or killed bacteria.

Table 4 M.I.I. and classification of 30 healthy volunteers.

Formulation	M.I.I.	Classification
B1	0.02	Non-irritation
S1	0.02	Non-irritation
DI water (negative control)	0.00	Non-irritation

∼80% and ∼93%. However, after 24 h of exposure, cell viability ranged from ∼59% to ∼75%. According to ISO 10993-5, a cell viability greater than 70% is generally considered non-cytotoxic. Although some formulations showed cell viability values approaching or below this threshold after 24 h of exposure, formulation S1 consistently maintained the highest cell viability among all tested groups, demonstrating a superior biocompatibility profile. Therefore, S1 can be considered the most promising formulation with respect to cytocompatibility and safety under the tested conditions.

Skin irritation of topical film-forming sprays containing GC nanoemulsion

The skin irritation potential of the topical film-forming spray (B1), the topical film-forming spray containing GC nanoemulsion (S1), and distilled water (negative control) was evaluated in 30 healthy Thai volunteers using a single-application, closed patch test. The erythema scores were recorded and used to calculate the M.I.I., as shown in Table 4. No significant skin irritation was observed for B1 (M.I.I. = 0.02), S1 (M.I.I. = 0.02), or distilled water (M.I.I. = 0). These findings suggest that the topical film-forming sprays containing GC nanoemulsion exhibited acceptable skin compatibility under the tested conditions, indicating their potential for pharmaceutical and cosmeceutical applications. However, further long-term safety evaluations are required to confirm their suitability for practical use.

CONCLUSION

The topical film-forming sprays incorporating GC nanoemulsion were successfully developed for potential pharmaceutical and cosmeceutical applications. Various concentrations of EuE100 (8, 10, and 12% (w/v)) were integrated with GC nanoemulsions (GC2 and GC3). Physicochemical analyses revealed that increasing the concentration of EuE100 resulted in increased viscosity but a narrower spray pattern. The evaporation times for the topical film-forming sprays containing GC nanoemulsion ranged from 3.27 to 7.10 min, with contact angles measured between 19.05° and 30.04°. Formulations S1, S2, S4, and S5 demonstrated good stability characteristics. Notably, the topical film-forming sprays exhibited antibacterial activity against both Gram-negative and Gram-positive bacteria. Furthermore, the formulations displayed similar release profiles, characterized by sustained drug release profiles over extended periods. Specifically, formulation S1 achieved the highest percentage of NO inhibition and maintained superior viability of NCTC clone 929 cells at both 4 h and 24 h. Crucially, S1 demonstrated acceptable skin compatibility without causing any skin irritation in human subjects. Therefore, these findings highlight formulation S1 as the most promising candidate for future development as a pharmaceutical or cosmeceutical product; however, further comprehensive studies are warranted to fully establish its long-term efficacy and safety.

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