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Research paper

Bioadhesive dermal patch as promising approach for improved antibacterial activity of bioactive compound of Zingiber cassumunar Roxb in ex vivo *Staphylococcus aureus* skin infection model

Latifah Rahman [a,*], Reni Sriyani Lembang [a], Subehan Lallo [b], Sri Resky Handayani [a], Usmanengsi [a], Andi Dian Permana [a,**]

[a] Department of Pharmaceutics, Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia
[b] Department of Phytochemistry Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia

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ABSTRACT

Infected burn injury has become a significant challenge among skin health problems. Recently, the use of a natural product, as an antibacterial alternative, has increased, particularly in the management of skin infections. *Zingiber cassumunar* Roxb. has been reported to possess a high amount of curcumin, showing an excellent antibacterial activity against *Staphylococcus aureus*, a bacterium associated with skin infections. With respect to the selection of drug dosage form, the use of a bioadhesive patch could be beneficial as this system can easily adhere to the infected site in the skin. Here, we present the development of bioadhesive patches containing *Zingiber cassumunar* Roxb. extract with different types of bioadhesive polymers, namely HPMC, NaCMC, chitosan and Carbopol. The characterization results indicated that the patches were uniform in their drug content, weight and thickness, indicating the reproducibility of the method used. Importantly, the patches were found to have excellent folding endurance values, which were more than 300 times, with surface pH values close to skin pH. The *ex vivo* dermal delivery showed that among the four bioadhesive polymers studied, HPMC and NaCMC were 1.3–2 times better than chitosan and Carbopol in terms of delivery of curcumin. Essentially, more than 80% of bacterial burdens were killed following the application of HPMC and NaCMC patches in an *ex vivo Staphylococcus aureus* infection model in rat skin. However, considering that HPMC patches showed lowest bioadhesion property, patches prepared from NaCMC were considered as the optimized formulation. To conclude, this work shows a promising delivery approach for a natural product in the treatment of the skin infections.

1. Introduction

Skin is the principal body part that defends the body from the entry of microorganisms. However, burn wound injury affects the physiology of the skin by harming the local networks [11]. World Health Organization (WHO) has reported that every year, approximately more than 300,000 people suffer from fire burns. A fire burn could lead to impairment of main functions of skins owing to damage to tissue and fluid, as well as due to the loss of water [2,13]. In normal conditions, skin infection is mainly triggered by Gram-positive bacteria named as *Staphylococcus aureus* (*S. aureus*) [5,11]. Additionally, more than 80% of chronic wounds are expected to be associated with biofilm development of bacteria [22]. The main target in the treatment of skin infections caused by burning is, skin wound closure, to decrease the rate of mortality [3].

The common method of infection wound care is the administration of topical antimicrobial drugs. When patch dosage forms are compared with standard topical dosage forms, such as ointments, creams and lotions, they offer more comfort and convenience for the user and the user can also be assured that the active ingredients in the dosage form are not wiped out [4,6,16]. As bioadhesive formulations are able to adhere to moist environments in the body surface, the application of a bioadhesive patch system could be potentially beneficial for the clinical environment [18]; S. Pendekal and K. Tegginamat, 2012; [31], particularly in the condition of wounded and infected skin. Importantly, since the patch delivery system maintains a solidified dimension, no differences in its

* Corresponding author.
** Corresponding author.
E-mail addresses: latifahrahman@unhas.ac.id (L. Rahman), andi.dian.permana@farmasi.unhas.ac.id (A.D. Permana).

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thickness and, therefore, the dose uniformity after the application can be assured. It takes the form of a topical dosage system, containing a defined drug loading available for release within a defined surface area. Essentially, this kind of system would ideally be self-adhesive, stiffened with protecting compounds and capable of delivering a drug dose equivalent to that achieved by the other topical dosage forms [6,31].

Several antibacterial agents have been recommended as options for the treatment of *S. aureus* infections, namely aminoglycosides, macrolides, fluoroquinolones, chloramphenicol, tetracyclines, and β-lactam antibiotics [16,33]. However, numerous strains of *S. aureus* have been found to be resistant to those antibacterial agents [8,10]. Therefore, due to inadequate treatment options existing to treat *S. aureus* infections and the associated increase in multidrug resistance, development of a novel efficient antibacterial agent against *S. aureus* is highly needed.

Curcumin or diferuloylmethane is the main phytoconstituent of Zingiberaceae family, which is also well-known as turmeric. With respect to its antibacterial activity, curcumin is reported to inhibit the growth of both Gram-positive and Gram-negative bacteria [34,36,38]. Importantly, one of the Gram-positive strains that is sensitive to curcumin-mediated inhibition is *S. aureus* [35]. Curcumin exhibited minimal inhibitory concentrations (MICs) ranging from 125 to 250 μg/mL against 10 strains of *S. aureus* (including 2 ATCC MSSA and MRSA standard strains, 4 MRSA clinical isolates, and 4 MRSA from culture collection) [19]. Also, Wang et al. reported that the MIC of curcumin against MSSA was 256 μg/mL [37]. *Zingiber cassumunar* Roxb. is one of the plants from Zingiberaceae family that has been reported to possess a high amount of curcumin. Moreover, this plant has been found to show antibacterial activity against several bacteria, including *S. aureus* [9,12,32].

In this study, we show, for the first time, the formulation and optimization of bioadhesive dermal patch for the topical delivery of curcumin from *Zingiber cassumunar* Roxb extract using various type of polymers. The formulated patches were further characterized for their physical characteristics, moisture absorption capability and mechanical properties. The *ex vivo* dermal delivery study in rat's skin was also carried out. Finally, the antimicrobial efficacy studies against *S. aureus* were carried out in *ex vivo* skin infection model.

2. Material and methods

2.1. Materials

Polyvinylpyrrolidone (PVP K-90) and Carbopol (carbomer 934) were obtained from BF Goodrich chemical, USA. Sodium carboxymethylcellulose (NaCMC), Potassium chloride, anhydrous calcium chloride, magnesium chloride, sodium nitrite, potassium sulphate, hydroxypropylmethylcellulose (HPMC), chitosan and curcumin were purchased from Sigma–Aldrich Pte Ltd, Singapore. All other reagents used were analytical grade.

2.2. Collection and extraction of Zingiber cassumunar Roxb

Zingiber cassumunar Roxb was collected from South Sulawesi, Indonesia. The samples were dried at 50 °C for 48 days. The dried samples were then ground into a fine powder that passed through a sieve of number 20. The fine powders were extracted using maceration technique with 70% ethanol as a solvent. The extraction was carried out for three days in a room with no direct sunlight. The ethanol was evaporated by subjecting the extract obtained from 70% ethanol in a rotary evaporator (Büchi Rotavapor R-114, Büchi, Switzerland), obtaining *Zingiber cassumunar* Roxb Extract.

2.3. Determination of curcumin content

In this study, curcumin was selected as the analyte of interest. The analysis of curcumin was carried out using reverse-phase high-performance liquid chromatography (HPLC) (Shimadzu Prominence, Shimadzu, Kyoto, Japan) system with LC-20AT quaternary gradient pump and Shimadzu LC solution software (ver. 1.21 SP1). The separation was carried out using an Xselect CSH™ C18 column (Waters, 3.0 × 150 mm with particle sizes of 3.5 μm) with the flow rate of 0.5 mL/min. A mixture of 2% v/v acetic acid in water and acetonitrile (50:50 v/v) was used as the mobile phase and UV detector was set at 425 nm. The analytical method was validated according to the International Committee of Harmonization (ICH) 2005 [24].

2.4. Preparation of bioadhesive patches containing Zingiber cassumunar Roxb Extract

Bioadhesive patches were prepared using PVP K-90 as a polymer to form films. Additionally, propylene glycol was used as a penetration enhancer and plasticizer in the patches. Different bioadhesive polymers were also used, namely NaCMC, HPMC, chitosan and Carbopol. The compositions of the formulations are shown in Table 1. Initially, PVP K-90 was dissolved in distilled water. Afterwards, propylene glycol was added into the aqueous gel. For formulations containing NaCMC and HPMC, the bioadhesive polymers were directly mixed with the aqueous gel formulations. For formulations containing chitosan, the bioadhesive polymer was firstly dissolved in 1.5% v/v acetic acid solution and mixed with the aqueous gel formulations. For formulations containing Carbopol, the bioadhesive polymer was dispersed in water, neutralized with triethanolamine (1.5 times the concentration of Carbopol, pH observed was 7.01 ± 0.03) and mixed with the aqueous gel formulations. Following this, *Zingiber cassumunar* Roxb extract was added into each formulation. The mixtures were then stirred using a magnetic stirrer at 500 rpm for 30 min at room temperature. The formed polymeric hydrogels were kept in a dark place in sealed vials at 4 °C until further investigation. The final mixtures were finally poured into glass petri dishes and were dried in room temperature for 48 h.

2.5. Characterization of bioadhesive patches

2.5.1. Uniformity of drug content evaluation

The uniformity of content of curcumin in the patches was carried out by dispersing each patch in 100 ml of distilled water. Specifically, for formulations containing chitosan, the patches were dispersed in 100 ml of 1.5% v/v of acetic acid solution. The dispersions were further diluted with methanol and sonicated for 1 h to dissolve the curcumin. The mixtures were then centrifuged at 14,000 rpm for 15 min. The amount of curcumin in the supernatant was determined by HPLC [6].

2.5.2. Uniformity of weight and thickness evaluation

The weight uniformity of patches was carried out by weighing six patches randomly and the average weight were calculated. For the thickness uniformity, a calibrated digital calipers was used for the purpose of determining the uniformity of thickness. The calipers was placed at five different points on a patch and the values of thickness were recorded. The average thickness of the patch was calculated. This procedure was carried out for six patches of each variety of curcumin patches prepared [6]; S. Pendekal and K. Tegginamat, 2012).

2.5.3. Folding endurance test

The folding endurance evaluation of patch formulations was conducted by folding the bioadhesive patches at the same position until the films broke. The folding endurance was denoted as the number of times of folding taken to rupture the patches [29].

2.5.4. Surface pH measurement

The pH of the surface was evaluated as per a method described previously [15], with a slight modification. Briefly, the patches were soaked in 10 mL of phosphate buffer for 2 h and the surface pH was determined using a digital pH meter.

Table 1
Compositions of bioadhesive patch formulations containing *Zingiber cassumunar* Roxb extract (%w/w).

Composition	Extract	PVP K-90	Propylene glycol	NaCMC	HPMC	Chitosan	Carbopol
F1	1	10	0.5	2	–	–	–
F2	1	10	0.5	–	2	–	–
F3	1	10	0.5	–	–	2	
F4	1	10	0.5	–	–	–	0.3

2.5.5. Moisture absorption ability of the patches

The ability of the prepared patch to absorb moisture from different environments was evaluated as previously described (S. Pendekal and K. Tegginamat, 2012), with a minor modification. Initially, patches were placed in a desiccator containing anhydrous calcium chloride for 24 h prior to use. Afterwards, three desiccators with three different relative humidity (RH) values were prepared using saturated solutions of magnesium chloride, sodium nitrite and potassium sulphate to represent 33% RH, 65% RH and 97% RH, respectively. The patches were placed into each desiccator and the weight of the patches was determined every 48 days for 14 days. Finally, the percentage of moisture absorption was determined using the following calculation:

$$\text{The percentage of moisture absorption} = \frac{mass\ of\ patch\ in\ dessicator - initial\ mass}{initial\ mass} \text{x } 100\%$$

2.5.6. In vitro bioadhesive evaluation

The bioadhesive property of the patches was evaluated using a modified physical balance, as previously described [7], with a slight modification. Initially, a piece of rat skin was secured to the mouth of a glass vial containing PBS pH 5.5. Afterwards, the glass vial was strongly attached in the center of a glass beaker containing PBS of pH 5.5 at 37 °C. The patches being evaluated were fixed to the lower part of rubber stopper with glue. The patch were then attached to the skin and the mass (g) required to separate the patch from the skin surface was denoted as the bioadhesive strength (shear stress) of the patch. The force of adhesion and the bond strength was calculated using the following equations:

$$\text{Force of adhesion (N)} = \frac{Bioadhesion\ strength\ (g)}{1000} \text{ x } 9.81$$

$$\text{Bond strength (N/m2)} = \frac{Force\ of\ adhesion\ (N)}{surface\ area\ (m^2)}$$

2.6. Ex vivo dermal delivery studies

Ex vivo dermal delivery studies of bioadhesive patches were performed using a 25 mL Franz diffusion cell, as previously described [21,25,26]. In this study, PBS (pH 7.4) containing 1% w/v Tween 80 was used as a medium prior. The skin was obtained from the abdominal skin of Male Sprague-Dawley rats. The experiments were approved and by the health ethical Committee of the Faculty of Medicine, Hasanuddin University and were performed in compliance with the ethical guidelines for experimentation on animals. The animals were sacrificed and then the skins were taken. Prior to the experiment, the skin of rat was trimmed to remove the hairs and the skins were equilibrated in the medium prior to each experiment. The receptor section of Franz diffusion cell was filled with the medium and the skin was sandwiched between the receptor and donor section. The experiment was carried out at 37 °C and the cells were stirred at 100 rpm. Patches with sizes of 1 × 1 cm^2 were attached to the skin in the donor compartment. At various interval time points, the patches were removed, and the skin was cleaned three times with distilled water to eliminate any excess patch formulation. Afterwards, the skin was cut into small pieces and 10 mL of methanol was added into the skin. The mixture was sonicated in a bath sonicator for 6 h to extract the curcumin retained the skin. The mixtures were then centrifuged at 14,000 rpm for 15 min. The amount of curcumin in the supernatant was determined by HPLC method described in Section 2.3.

2.7. Antibacterial activity in an ex vivo infection model in rat skin

2.7.1. Culture of Staphylococcus aureus

The bacterial strain used was *Staphylococcus aureus* (ATCC® 25923) (Thermo Fisher Scientific, Waltham, MA). Before the antibacterial study, *S. aureus* was cultured in tryptic soy broth (TSB) at 37 °C overnight. The pellets were obtained by centrifugation at 3000 rpm for 30 min. The obtained pellet was resuspended in fresh TSB. To achieve an equivalent to 1.5 × 10^8 CFU/mL, optical density at 550 nm of the bacterial suspensions was set.

2.7.2. Preparation of bacterial infection model on rat skin

To prepare *ex vivo* infection model on rat skin, initially, the skins were disinfected by immersing in 10% povidone iodine for 1 h. Briefly, burn wound was created using red-hot brass knob with a diameter of 5 mm [22]. The burn skins were aseptically put on TSA plates using a metal tweezer in Class II Microbiological safety cabinet. Following this, an aliquot of 50 μL of *S. aureus* suspensions 2 × 10^5 CFU/mL were dropped on to the wound of the skin. The plates were cultivated at 37 °C. To allow the formation of *ex vivo* infection, every day, the skins were aseptically transferred to new TSA plates for five days.

2.7.3. Antibacterial activity in ex vivo bacterial infection model on rat skin

Ex vivo antibacterial activity of bioadhesive patches of *Zingiber cassumunar* Roxb extract in infection model on rat skin was performed using the technique reported previously [17,22,23], with a minor modification. Initially, the *ex vivo* skin infection model was sandwiched in the Franz diffusion cells. The patch formulations were applied on to the infected skin. After 12 h, 24 h and 48 h, the patches were removed, and the skin samples were placed into 2 mL Eppendorf tube. Sterile water (1.5 mL) was added to the skin and the mixtures were vortexed for 15 min. Subsequently, 20 μL of homogenized samples were inoculated into TSA plates. The plates were then incubated at 37 °C for 24 h. To evaluate the antibacterial activity, the numbers of viable CFU of *S. aureus* were counted and compared to the initial number of viable CFU at the beginning of the experiment [20]. A piece of infected skin, to which there was no application of a test patch, was taken as a control.

2.8. Statistical analysis

All results were reported as means ± standard deviation (SD) of the mean. Microsoft® Excel® 2016 (Microsoft Corporation, Redmond, USA) was used to calculate SD of all data. GraphPad Prism® version 6 (GraphPad Software, San Diego, California, USA) was used to statistically analyze the data obtained. In all cases, $p < 0.05$ was denoted as a significant difference.

3. Results and discussion

3.1. Extraction of Zingiber cassumunar Roxb and determination of curcumin content

Zingiber cassumunar Roxb has been reported to have excellent antibacterial activity due to its curcumin content. Curcumin inhibits *S. aureus* growth by disturbing the integrity of the bacterial membrane. In this study, ethanol extract of *Zingiber cassumunar* Roxb showed an extraction yield of 24.12 ± 2.87% w/w. The HPLC chromatograms of standard curcumin and ethanol extract of *Zingiber cassumunar* Roxb are shown in Fig. 1, showing that the retention time of curcumin was 5.43 min. Analyzed using HPLC, the curcumin content of *Zingiber cassumunar* Roxb was found to be 3.98 ± 0.31% w/w.

3.2. Characterization of bioadhesive patches

3.2.1. Uniformity of drug content, weight and thickness

Four different formulations of bioadhesive dermal patches containing *Zingiber cassumunar* Roxb extract were prepared in this study with varied bioadhesive polymer concentrations. With regards to the optimization of the patch formulation, initially, we performed a preliminary study using different amounts of PVP K-90. Following our preliminary study, we found that below 10% w/w, the patches were brittle and did not have adequate mechanical properties. With the concentration above 10%, the properties of the patches were not elastic, despite the use of a plasticizer. The patches were too thick and difficult to be bent, which could potentially cause the difficulty of the application in the skin. Therefore, in this study, 10% w/w was selected as the concentration of PVP K-90. With respect to the use of 0.5% w/w of propylene glycol, we found that the patches prepared from propylene glycol with the concentration below 0.5% w/w did not have sufficient mechanical properties. Therefore, 0.5% w/w was selected as an optimum concentration. Regarding the use of the concentration of the bioadhesive polymers used, we also performed a preliminary study. The patches prepared from the concentration below 2% w/w for NaCMC, HPMC and chitosan; and below 0.3%w/w for Carbopol, did not result in adequate bioadhesive properties. On the other hand, the concentration above 2% w/w for NaCMC, HPMC and chitosan; and the concentration above 0.3% for Carbopol, resulted in similar bioadhesive properties with the formulation containing 2% of polymers. Additionally, the patches became too thick and difficult to bend. Therefore, we used 2% w/w for NaCMC, HPMC, and chitosan, and 0.3% w/w for Carbopol. In this study, all formulations were found to be homogenous and elastic. In an attempt to warrant the reproducibility of patch preparations, the uniformities of drug content, weight and thickness were evaluated. The results revealed that the averages drug content, the average weights and the average thickness were in the range of 97.99 ± 4.02% - 98.67 ± 3.71%, 0.521 ± 0.018 g - 0.545 ± 0.021 g and 0.148 ± 0.003 mm - 0.161 ± 0.005 mm, respectively. The uniformity evaluation results of all formulations are exhibited in Table 2. It is clearly observed that the percentage of relative

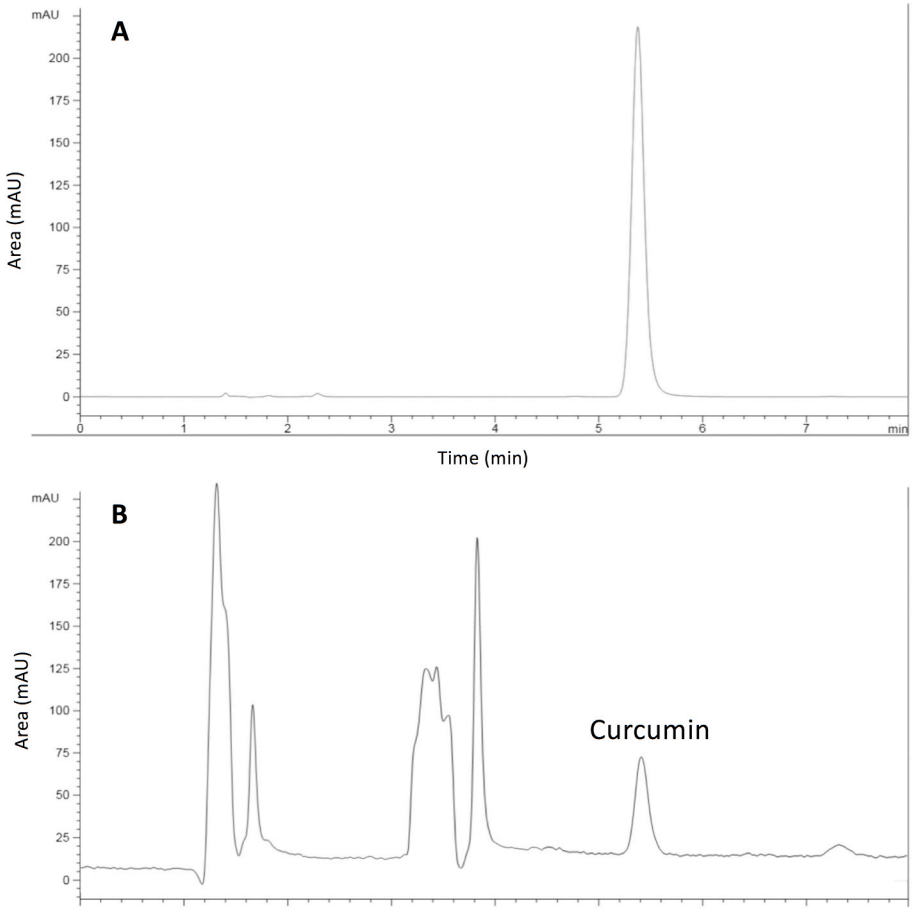

Fig. 1. The HPLC chromatograms of standard curcumin and ethanol extract of *Zingiber cassumunar* Roxb.

Table 2
Physicochemical properties of the prepared bioadhesive patches (means ± SD).

	Drug content (%)	Relative Standard Deviation	Average weight (g)	Relative Standard Deviation	Average thickness (mm)	Relative Standard Deviation
F1	98.07 ± 4.78	4.87	0.521 ± 0.018	3.45	0.152 ± 0.006	3.95
F2	98.34 ± 3.92	3.98	0.545 ± 0.021	3.85	0.161 ± 0.005	3.11
F3	97.99 ± 4.02	4.10	0.528 ± 0.012	3.97	0.148 ± 0.003	2.03
F4	98.67 ± 3.71	3.76	0.537 ± 0.016	2.98	0.153 ± 0.005	3.27

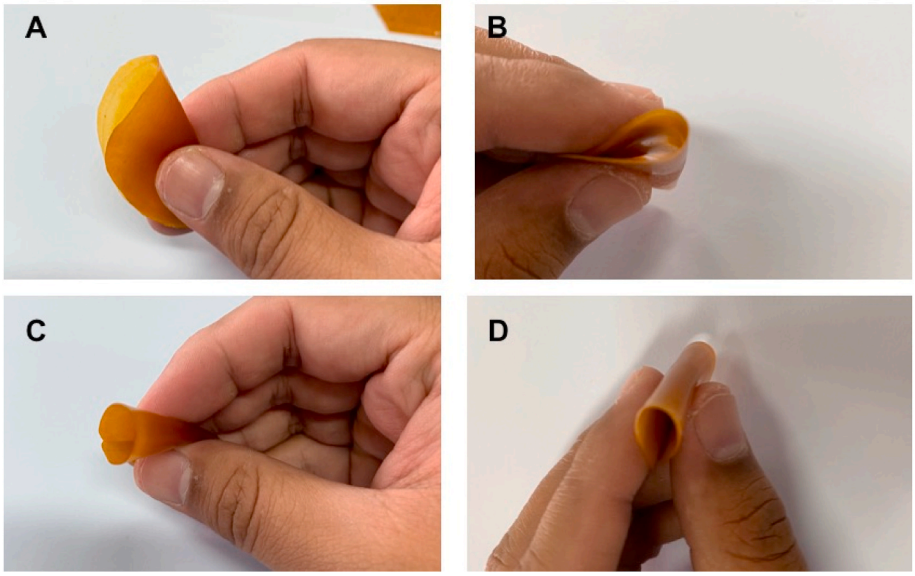

Fig. 2. Representative images of folding endurance evaluation of F1 (**A**), F2 (**B**), F3 (**C**) and F4 (**D**).

Table 3
Folding endurance and surface pH of the prepared bioadhesive patches (means ± SD).

	Folding endurance (times)	Surface pH
F1	387 ± 8	5.61 ± 0.08
F2	381 ± 9	5.75 ± 0.07
F3	379 ± 12	5.68 ± 0.08
F4	383 ± 11	5.67 ± 0.08

standard deviation (%RSD) of the averages drug content, the average weights and the average thickness were less than 5%, showing acceptable uniformity. Therefore, the preparation method of the patches was able to form uniform dermal patches.

3.2.2. Folding endurance test

In order to evaluate the resistance capability of the patches, the folding endurance test was carried out (Fig. 2). The results showed that the folding endurance of all formulations was found to be more than 300 times (Table 3). It has been previously reported that a good patch should possess folding endurance of at least 300 times [15]. In our preliminary study, an attempt to prepare the patches without propylene glycol was conducted, however, the patches obtained were extremely brittle (data not shown). This finding implied that the use of plasticizer was able to improve the flexibility of patch formulations. It might be caused by the binding of the plasticizer to the polymer matrix. Therefore, the plasticizer could potentially enhance the void volume amongst the chains of polymers, thus permitting the chain section to transfer easily. Therefore, the increase of the movement of the polymer could potentially increase the flexibility and elasticity of the patches.

3.2.3. Surface pH measurement

The surface pH of the topical dosage forms is one of the critical parameters. The inappropriate pH may produce irritation to the mucous membrane of the skin. Additionally, this may also affect the degree of hydration of polymers in the skin. Therefore, it is important to produce a dermal patch with a pH value close to the pH of the skin, which is around 5.8 [15]. The results showed that the surface pH of the patches was found to be between 5.61 ± 0.08 and 5.75 ± 0.07 (Table 3). Accordingly, it may be hypothesized that the application of these bioadehsive patches will not produce any irritation to the mucous membrane of the skin.

3.2.4. Moisture absorption ability of the patches

The ability of bioadhesive patches to absorb moisture is a critical parameter as it could potentially influence the release profile and mechanical properties. In this study, we evaluated the moisture absorption ability in three different RH values. The absorption capacity of polymers used in this study may be due to the presence of hydrophilic structure. The results of this evaluation are shown in Fig. 3.

As shown in Fig. 1, the increase in moisture absorption ability followed the increase in humidity. After 14 days, the highest moisture absorption ability was shown by patches containing NaCMC (3.54 ± 0.31% in 33% RH, 6.43 ± 0.58% in 65% RH and 9.71 ± 0.81% in 97% RH). This may be due to the hygroscopic properties of NaCMC, increasing its ability to absorb and retain water. On the other hand, the lowest moisture absorption ability was exhibited by patches containing HPMC (1.65 ± 0.11% in 33% RH, 3.67 ± 0.28% in 65% RH and 4.65 ± 0.33% in 97% RH). Adhikari et al., also reported that the patches containing HPMC resulted in lower moisture absorption ability compared to those prepared from NaCMC [1]. Michailova et al., have reported that the presence of the elastic structure of HPMC leads to the low velocity of water uptake [14]. Furthermore, we analyzed the kinetic profiles of the

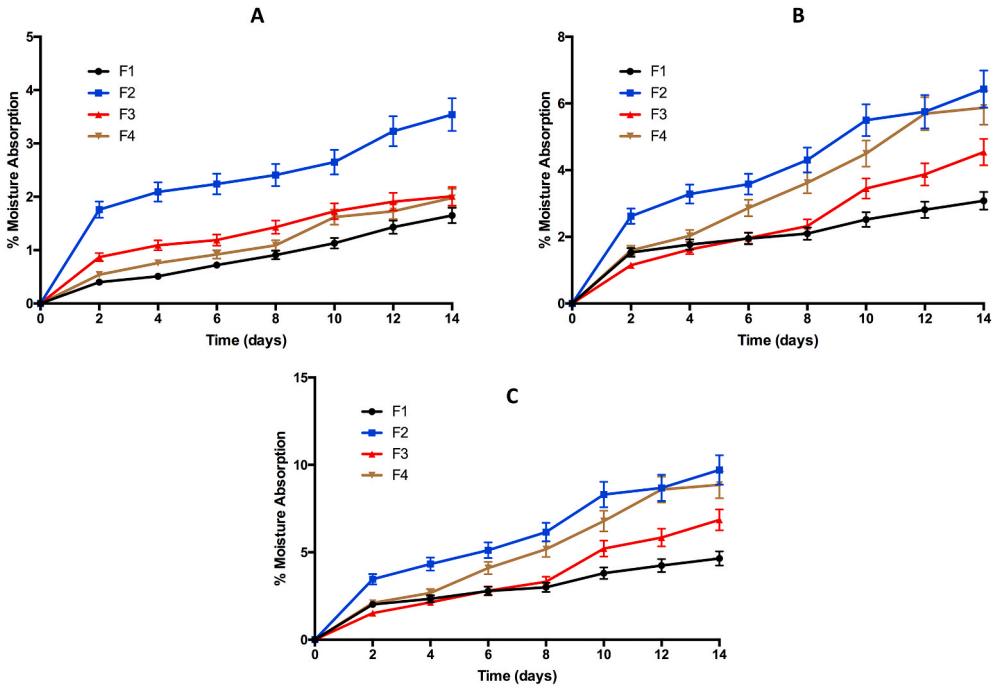

Fig. 3. Moisture absorption ability of bioadhesive patches at 33% RH (**A**), 65% RH (**B**) and 97% RH (**C**) (means ± S.D). *$p < 0.05$.

Table 4
Bioadhesive properties of the prepared bioadhesive patches (means ± S.D).

	F1	F2	F3	F4
Bioadhesion strength (g)	17.98 ± 1.98	5.76 ± 0.48	18.16 ± 1.76	18.65 ± 1.43
Force of adhesion (N)	0.176 ± 0.019	0.05 ± 0.004	0.178 ± 0.017	0.183 ± 0.015
Bond strength (N/m^2)	98.32 ± 10.61	27.93 ± 2.23	99.44 ± 9.49	102.23 ± 8.34

moisture absorption. The results showed that the kinetic profiles of all formulations followed first order kinetic. The absorption capacity shown by the polymers, in this study, may be, because they have a hydrophilic structure.

3.2.5. *In vitro bioadhesive evaluation*

One of the most advantages of bioadhesive formulations is that the formulation is able to stay longer in the site of action. It thus enhances the bioavailability of the drugs. Additionally, the improved residence times may also extend the local action of topical drugs. After being hydrated in the skin, the polymers could form hydrogen bonding and/or electrostatic interaction with the mucous network. Accordingly, it is important to evaluate the bioadhesive properties of the patches as a critical physicochemical property.

The results showed the highest bioadhesive property was shown by patches prepared from Carbopol, followed by chitosan, NaCMC and HPMC, respectively (Table 4). It was postulated that the presence of strong anionic charge in the polymer increases the bioadhesive properties [28]. Therefore, as anionic polymers, Carbopol and NaCMC exhibited excellent bioadhesive characteristics. Chitosan, a cationic polymer, has been reported to have the ability to form an electrostatic attraction to mucin, increasing their bioadhesive properties [28]. The differences observed among the three varieties of patches, with respect to their bioadhesive property, were analyzed by one-way analysis of variance technique and it was found that, there were no significant differences ($p > 0.05$) on bioadhesive properties from Carbopol, chitosan and NaCMC patches. On the other hand, as a non-ionic polymer, HPMC is not able to interact electrostatically with the mucin [28,30], resulting in poor bioadhesive properties.

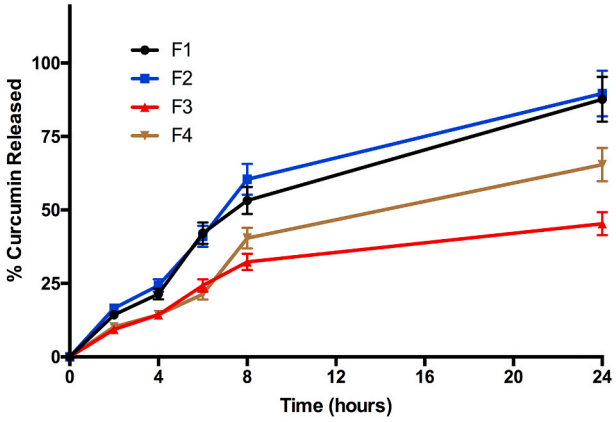

Fig. 4. Dermal delivery of bioadhesive patches in excised rat skin (means ± S.D). *$p < 0.05$.

3.3. *Ex vivo dermal delivery studies*

The main purpose of this study was to deliver the active compound of *Zingiber cassumunar* Roxb extract, curcumin, into the skin, where *S. aureus* colonizes and infects the skin. Therefore, it is crucial to investigate the dermal delivery of the active compound delivered by patch formulations.

As shown in Fig. 4, the delivery studies revealed that after 24 h, the patches containing HPMC and NaCMC resulted in the highest dermal delivery, with 87.65 ± 7.87% and 89.71 ± 8.02% of active compound being delivered, respectively. This may be caused by the high-water solubility of these polymers. On the other hand, only 65.32 ± 5.98% and 47.61 ± 3.87% of curcumin were delivered following the application of patches prepared from Carbopol and chitosan, respectively. This could be explained due to the fact that chitosan is broadly utilized for sustained delivery, decreasing the ability of curcumin to penetrate the

skin layer. Interestingly, we found that NaCMC with the highest moisture absorption ability showed the highest dermal delivery ability. This shows that when patches prepared from NaCMC absorbed moisture from the environment, it could potentially increase the hydration of the skin, increasing the delivery of curcumin penetration the skin [27]. Furthermore, we evaluated the kinetics profiles of the dermal delivery of curcumin from the patch formulations. Fig. 4 shows that the release profile from 0 h to 8 h follows one phase and the profile from 8 h to 24 h follows another phase. Because we did not perform any observations between 8 h and 24 h, we only analyzed the kinetics profile from 0 h to 8 h. Following the analysis, we found that the kinetics profiles of dermal delivery of all formulations followed first order kinetics. Therefore, the delivery of curcumin from the bioadhesive patches depends on the concentration of the curcumin in the formulation [21].

3.4. Antibacterial activity in an ex vivo infection model in rat skin

In an attempt to evaluate the efficacy of our approach, we investigated the antibacterial activity of all patches in an *ex vivo S. aureus* infection model in rat skin. In this evaluation, we compared the viable cell counts of infected skins after the application of different formulations of bioadhesive patches. The result of this study is shown in Fig. 5. Without any treatment, the bacterial burden increased from 8.92 log CFU to 8.95 log CFU after 48 h, indicating the successful skin infection model developed. The antibacterial activity of dermal patches was in a good agreement with the dermal delivery studies. It was found that the higher curcumin delivered to the skin, the higher antibacterial activity of the formulation. It was found that the highest antibacterial activity was achieved by the patched prepared from NaCMC and HPMC, with around 82.45 ± 7.98% and 83.53 ± 9.21% of *S. aureus* killed after 48 h of application. Analyzed statistically using one-way anova, there was no significant difference ($p > 0.05$) in the decrease of bacterial burden in the patches prepared from NaCMC and HPMC. On the other hand, the application of patches prepared from Carbopol was only able to kill 40.81 ± 5.09% of bacterial burden after 48 h. Although the curcumin delivered from chitosan formulation was significantly lower ($p < 0.05$) than those prepared from Carbopol, interestingly, the decrease of bacterial burden following chitosan patches was not statistically lower than Carbopol patches, which was around 53.87 ± 6.19% killing of bacterial burden. This may be due to the fact that chitosan has an antibacterial activity which improved the antibacterial activity of patch formulations. However, this value was significantly lower ($p < 0.05$) compared to the antibacterial activities of NaCMC and HPMC patches. The relationship between curcumin delivered and antibacterial activity are shown in Table 5. Accordingly, these results exhibited the successful development of formulations containing *Zingiber cassumunar* Roxb extract for improved antibacterial activity in *ex vivo* infection wound model. However, although patches prepared from NaCMC and HPMC showed non-significant different in terms of their dermal delivery and antibacterial activity, considering the clinical application, as NaCMC patches showed higher bioadhesive properties, this formation could be considered as the promising system for the delivery of active compound in *Zingiber cassumunar* Roxb extract.

4. Conclusion

This study explored the potential of a bioadhesive dermal patch delivery system to deliver the main compound of *Zingiber cassumunar* Roxb extract, curcumin, to the skin. By using simple maceration technique with 70% ethanol, the extract obtained was found to contain 3.98 ± 0.31% of curcumin. Several bioadhesive polymers were then optimized to develop the dermal patch formulation, namely HPMC, NaCMC, chitosan and Carbopol. Several characterizations were conducted with the main results indicated that the percentage of the relative standard deviation of the averages drug content, the average weights and the average thickness were <5%, presenting satisfactory uniformity.

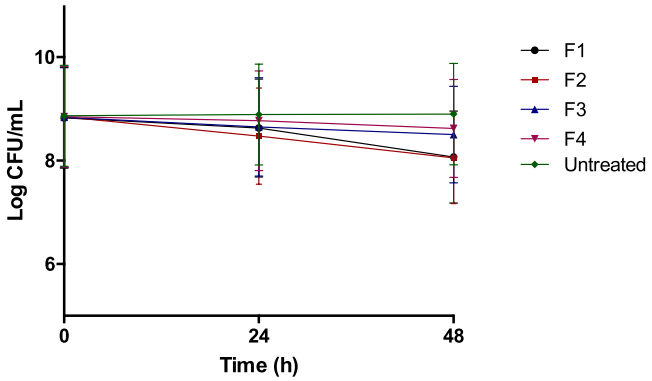

Fig. 5. Bacterial viability (log CFU/mL) on in an *ex vivo S. aureus* infection model in rat skin following the application of different formulations of bioadhesive patches (means ± S.D).

Table 5
The relationship between curcumin delivered and antibacterial activity (means ± S.D).

	% Curcumin Released	% Antibacterial activity
F1	87.65 ± 7.87	82.45 ± 7.98
F2	89.71 ± 8.02	83.53 ± 9.21
F3	47.61 ± 3.87	53.87 ± 6.19
F4	65.32 ± 5.98	40.81 ± 5.09

Importantly, a significant feature of the dermal patches, prepared in this study, is that they show a pH of around 5.6, which is very near to the pH of the skin. Hence, the possibility of irritation of skin is not there. With respect to the mechanical property, all formulations exhibited >300 times folding endurance. The bioadhesion ability of the patches was also evaluated, showing that chitosan, Carbopol and NaCMC showed significant higher bioadhesive property compared to HPMC patches. Finally, supported with bioadhesion property, patches prepared from NaCMC was selected as the most optimum formulation with more than 80% killing percentage in an *ex vivo Staphylococcus aureus* infection model in rat skin. However, further extensive investigations are required, including irritation study, toxicity and *in vivo* pharmacodynamic studies in a suitable model system.

CRediT authorship contribution statement

Latifah Rahman: Conceptualization, Writing – review & editing, Project administration, Funding acquisition, Validation, Supervision. **Reni Sriyani Lembang:** Methodology, Investigation, Data curation. **Subehan Lallo:** Writing – review & editing, Project administration. **Sri Resky Handayani:** Methodology, Investigation, Data curation, Formal analysis. **Usmanengsi:** Methodology, Investigation, Data curation. **Andi Dian Permana:** Conceptualization, Methodology, Writing – original draft, Investigation, Data curation, Visualization.

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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