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Universitas Hasanuddin

Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Confirm co-authorship of submission to Journal of Drug Delivery Science and Technology

1 message

Journal of Drug Delivery Science and Technology <em@editorialmanager.com> Tue, Sep 29, 2020 at 9:27 AM
Reply-To: Journal of Drug Delivery Science and Technology <jddst@elsevier.com>
To: Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

*This is an automated message. *

Journal: Journal of Drug Delivery Science and Technology
Title: 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
Corresponding Author: Dr. Latifah Rahman
Co-Authors: Reni Sriyani Lembang; Subehan Lallo; Andi Dian Permana
Manuscript Number:

Dear Andi Dian Permana,

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If you did not co-author this submission, please contact the Corresponding Author directly at latifahrahman@unhas.ac.id

Thank you,

Journal of Drug Delivery Science and Technology

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BUKTI REVIEW DARI REVIEWERS

Universitas Hasanuddin

Andi Dian Permana <andi.dian.permana@farmasi.unhas.ac.id>

Fwd: Decision on submission to Journal of Drug Delivery Science and Technology

1 message

Latifah Rahman <latifahrahman@unhas.ac.id> Fri, Apr 7, 2023 at 10:24 AM
To: andi.dian.permana@farmasi.unhas.ac.id

Dear Pak Dian,

Berikut komentar reviewer

---------- Forwarded message ----------
From: **Journal of Drug Delivery Science and Technology** <em@editorialmanager.com>
Date: Friday, February 19, 2021
Subject: Decision on submission to Journal of Drug Delivery Science and Technology
To: Latifah Rahman <latifahrahman@unhas.ac.id>

Manuscript Number: JDDST-D-20-00938R1

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

Dear Dr. Rahman,

Thank you for submitting your manuscript to Journal of Drug Delivery Science and Technology.

I have completed my evaluation of your manuscript. The reviewers recommend reconsideration of your manuscript following minor revision and modification. I invite you to resubmit your manuscript after addressing the comments below. Please resubmit your revised manuscript by Mar 20, 2021.

When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and provide suitable rebuttals for any comments not addressed. Please note that your revised submission may need to be re-reviewed.

To submit your revised manuscript, please log in as an author at https://www.editorialmanager.com/jddst/, and navigate to the "Submissions Needing Revision" folder under the Author Main Menu.

Journal of Drug Delivery Science and Technology values your contribution and I look forward to receiving your revised manuscript.

Please include the following two versions of the manuscript when you submit the revision and ensure that the original version of the manuscript file is removed. Both the versions can be uploaded using the file type 'Manuscript'.

1. Revised Manuscript with changes marked
2. Revised Manuscript with no changes marked

Kind regards,

Michael A Repka

Associate Editor

Journal of Drug Delivery Science and Technology

Editor and Reviewer comments:

Reviewer #1: The research reply was satisfactory and the researcher made considerable changes made in the revised manuscript.

Reviewer #2: Please pay more attention to correctness and clarity in your writing.

*****
Data in Brief (optional):
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BUKTI SUBMIT HASIL REVIEW

Journal of Drug Delivery Science and Technology

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

--Manuscript Draft--

Manuscript Number:	JDDST-D-20-00938R1
Article Type:	Research Paper
Keywords:	Zingiber cassumunar Roxb; bioadhesive; dermal patches; skin infections; antibacterial
Corresponding Author:	Latifah Rahman INDONESIA
First Author:	Latifah Rahman
Order of Authors:	Latifah Rahman
	Reni Sriyani Lembang
	Subehan Lallo
	Sri Resky Handayani
	Usmanengsi Usmanengsi
	Andi Dian Permana
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.
Suggested Reviewers:	Adrian Williams a.c.williams@reading.ac.uk
	Ahmed Faheem ahmed.faheem@sunderland.ac.uk
	Aaron J. Courtenay a.courtenay@ulster.ac.uk
	Majella Lane m.lane@ucl.ac.uk
Opposed Reviewers:	
Response to Reviewers:	Ms. Ref. No.: JDDST-D-20-00938

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

Response to Reviewers
We are very thankful to the expert reviewers for taking the time to kindly review our manuscript and provide helpful comments for improvement and clarification. We have made some changes to the manuscript as a result of these comments. We believe that the manuscript is now substantially improved. We have addressed each of the reviewers' comments in detail below. Importantly, we have made a great effort to improve the English and the discussion parts of our revised manuscript.

Reviewer #1:
In this research work the researchers developed the bio adhesive transdermal patches incorporated with Zingiber Cassumunar Roxb extract. One of the problems in this formulation was estimation of active constitutions present in the formulation. The formulation potential was based on the evaluation report of curcumin present in the Cassumunar Roxb extraction. But the curcumin MIC is approximately above 100 microgram/ml, it is quite high concentration compare with other antibiotics active against Staphylococcus aureus. Further, Cassumunar Roxb extract may contains various phytochemicals. So, the amount of other constitutions presents in this exact to be specified by providing supporting materials with data. The HPLC chromatogram, retention time of various phytochemicals and Rf values are required to support number of phytochemicals present in the formulation. How the researchers obtained the rat skin for in vitro testing? Is it institutional animal ethical committee approval is needed? How the researchers optimized the formulation? No details given about what basis the researchers selected various amount of excipients and extract used to make F1 to F2 formulation.

Response to Reviewer
We are very thankful to the Reviewer for taking the time to review this manuscript and for the expert review, providing helpful comments. We have made a number of key changes to the manuscript as a result of these comments. We believe that the manuscript is now substantially improved. We have addressed each one of the reviewers' comments in detail.

We agree with the Reviewer that MIC of curcumin is higher compared to other antibiotics. However, in this study, one of our main purposes is to develop bioadhesive patches using natural product as alternative of synthetic compounds. Compared to several synthetic compounds, natural compounds are considered to be safer. With respect to HPLC chromatogram, we have provided the HPLC chromatogram of curcumin and the extract in comparison with standard curcumin in the revised manuscript. Importantly, the analytical method was validated as per the International Committee of Harmonization (ICH) 2005.

With respect to the skin for in vitro testing, the skin was obtained from abdominal skin of Male Sprague-Dawley rats, approved and performed in compliance with the health ethical Committee of the Faculty of Medicine, Hasanuddin University. We have now added this into our revised manuscript.

With regards to the optimization of the patch formulation, initially, we performed 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 plasticizer. The patches were too thick and difficult to be bend, 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-90. Regarding the use of the concentration of the bioadhesive polymers used, we also performed 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. We have now explained this in our revised manuscript.

Reviewer #2:
Dear Author, I wish to appreciate the work that you have carried out. It was done well and the results are reported well. But the presentation requires thorough revision, in terms of language, analysis of results and presentation of results. Please put in a little more effort and the result would make you happy.
Response to Reviewer
We are very thankful to the Reviewer for taking the time to review this manuscript and for the expert review, providing helpful comments. We have made a number of key changes to the manuscript as a result of these comments. We believe that the manuscript is now substantially improved.

Reviewer #3:
Review Comments for "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"
In this original paper authors have worked on extraction of Zingiber Cassumunar Roxb and formulating the hydrogel patches using different polymers. The research idea using the natural products extraction and making its formulation is good but the study performed is not that strong. It lacks lot of good experiments which are basics in formulation development. Also, the whole manuscript lacks biostatistics in all the images provided. There are lot of corrections and extra experiments needed to validate this concept.
However, specific comments for this evaluation are given as:
Response to Reviewer
We are very thankful to the Reviewer for taking the time to review this manuscript and for the expert review, providing helpful comments. We have made a number of key changes to the manuscript as a result of these comments. We believe that the manuscript is now substantially improved. We have addressed each one of the reviewers' comments in detail.

Comments:
1. The graphical abstract image is not clear.
Response:
We thank the Reviewer for pointing this out. We have improved the resolution of the graphical abstract image.

2. In the methodology section, authors have taken constant amount of PVP-K10 and Propylene glycol. Why they have not optimized the formulations with different amounts of PVP-K10 and propylene glycol?
Response:
We thank the Reviewer for the comments. Following comments and suggestion from reviewer, we have added some discussions and explanations about our preliminary studies.

With regards to the optimization of the patch formulation, initially, we performed 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 plasticizer. The patches were too thick and difficult to be bend, 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-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. We have now explained this in our revised manuscript.

3. On what basics the amounts of NaCMC, HPMC, chitosan and Carbopol are selected? How they came to know 2% or 0.3% w/w amount of polymer is enough to make these bioadhesive gels?

Response:

	We thank the Reviewer for the comments. Following comments and suggestion from reviewer, we have added some discussions and explanations about our preliminary studies. Regarding the use of the concentration of the bioadhesive polymers used, we also performed 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. We have now explained this in our revised manuscript. 4. What amount of triethanolamine was used to neutralize the pH in Carbopol containing gels and moreover what was final pH of these formulations? Response: We thank the Reviewer for the comments. The concentration of triethanolamine was 1.5 times the concentration of Carbopol and the pH achieved was 7.01 ± 0.03. We have now added this in our revised manuscript. 5. Can authors produce HPTLC graph for the extracts they have obtained after extraction of Zingiber cassumunar Roxb? HPTLC graph can also be compared with standard curcumin. Also, the authors have not provided any HPLC graphs as well. So, it is difficult to say what exact extract they have obtained. Also, HPTLC study can also be used to calculate the percentage of curcumin and other components of interest. Response: We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have provided the HPLC chromatogram of the extract in comparison with standard curcumin in the revised manuscript. Importantly, the analytical method using HPLC was validated as per the International Committee of Harmonization (ICH) 2005. Therefore, we believe that the validated analytical method was sufficient to quantify curcumin in the extract and all experiments in this study. 6. Can authors provide images or videos for folding endurance test? They will be helpful to exactly see the nature of the patch obtained. Response: We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have provided the images for folding endurance test in the revised manuscript. 7. As authors mentioned these patches as hydrogels, it will be very helpful if they provide data for hydrogel characterization such as rheological studies, physiochemical properties such as swelling properties, gel fraction, SEM images etc. Response: We thank the Reviewer for the suggestions. We agree that the patches were made from hydrogel formulation. However, the final form of our formulation was solid dosage form. Therefore, we only evaluated and characterized the final patch formulation, which have been explained in our manuscript. 8. Can authors compare the bioadhesive properties of these gels with any marketed formulation? Response: We thank the Reviewer for the question and suggestion. As previously explained, the final form of our formulation was solid dosage form. Therefore, we did not compare the bioadhesive properties with semisolid dosage forms. Furthermore, to the best our knowledge, there has no marketed formulation of bioadhesive patch. Thus, we only compared the bioadhesive properties of the formulations developed in our study. 9. In all the figures, no biostatistics is used. Need to perform statistics to understand the exact difference in all the groups. Response: We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have provided all figures with statistic results. Importantly, we have also discussed all results according to biostatistical results.

ADDITIONAL RESPONSE
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 Rahman1* , Reni Sriyani Lembang1 , Subehan Lallo2 , Andi Dian Permana1**
Report on the above paper:
5.12.2020
Comments on the research work:
The work reported in this research paper is of good quality, it was carried out as per standard procedures and definitely merits publication in a reputed journal. They have established that a natural compound, curcumin, possesses antibacterial activity in infections that may arise from burn injuries; this is also a good achievement. Literature review was carried out in an appropriate manner. Procedures adopted in the collection and characterisation of the herb, analytical procedures, preparation of bio-adhesive patches and their characterisation are all good standard procedures. Analysis of the results was done appropriately and proper conclusions were also drawn after a good discussion.
Response:
We are very thankful to the expert reviewers for taking the time to kindly review our manuscript and provide helpful comments for improvement and clarification. We are grateful to hear that our research paper is a good quality and the experiments were carried out as per standard procedures.
Comments on presentation of the work:
The presentation of the work was done in a very poor manner.
Language: There are many language errors throughout the write up of the paper. I think almost every sentence has a grammatical error or a presentation (wrong way of writing a sentence) error. I initially started to make a Table so that I can point out the errors and suggest their corrections. I started with the abstract given in the first page. I later noticed that some of these errors are not there in the actual paper. I stopped that effort as the errors are just too many in number. I will give that table as an attachment. Nevertheless, this paper is full of mistakes in its language and it needs a thorough revision. I will present some errors below:
Response:
We thank the Reviewer for the comments. We have made some changes to the manuscript as a result of these comments. We believe that the manuscript is now substantially improved. We have addressed each of the reviewers' comments in detail below. Importantly, we have made a great effort to improve the English and the discussion parts of our revised manuscript.
Grammatical errors:
1.In comparison with the topical standard dosage forms, such as ointments, creams, lotions and emulsions, patch dosage forms are more comfortable for utilization and can avoid active ingredients from being wiped out (Bian et al., 2003; El-Gendy et al., 2009; Mir et al., 2019).

Correction: When patch dosage forms are compared with standard topical dosage forms, such as, ointments, creams, lotions and ointments, 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.
Response:
Needful is done
2.Same para—8th line: infection wound skin
Correction: Infected skin or wounded and infected skin
Response:
Needful is done
3.2.3: first line -- as analyte of interest
Correction: as the analyte of interest
Response:
Needful is done
4.2.4: procedures can be written in a clearer manner.
Response:
Needful is done

	5.2.4: 3rd line from bottom: in room temperature- correction: at room temperature. Response: Needful is done 6.2.4.1 first line: uniformity content of curcumin- correction: uniformity of content of curcumin. Response: Needful is done 7.2.4.2- averages weight were calculated- correction: average weight was calculated. Response: Needful is done 8.For the thickness uniformity, a calibrated digital caliper was used to measure the thickness of five different points of six different patches and the averages thickness were calculated (El-Gendy et al., 2009; S. Pendekal and K. Tegginamat, 2012). Correction: 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. Average thickness of the patch was calculated. This procedure was carried out for six patches of each variety of curcumin patches prepared. Response: Needful is done 9.2.4.4- first line- as per method—correction: as per the method. Response: Needful is done 10.2.4.5_ first line: ability of patch- correction: the ability of the prepared patches Response: Needful is done Comments on write up: 1.Explain each procedure with greater clarity, give more details. Response: We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have provided all details in the methods. 2.2.5: Explain how the rats were procured, and stored. Give details of Ethics Committee permission. Explain that the animals were sacrificed and then the skins were taken and then further procedures were carried out. Response: We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have included the details of Ethics Committee permission in the revised manuscript. 3.Give the exact number of foldings instead of saying >300- this is vague. Response: Needful is done 4.3.2.4- results of the evaluation are shown in -----., not exhibited in. Response: Needful is done 5.Moisture absorption studies: we can do analysis for kinetics and report on whether the absorption is following zero order or first order. Response: We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have analyzed the kinetics and explained the results in the revised manuscript. “Furthermore, we analyzed the kinetic profiles of the moisture absorption. The results showed that the kinetic profiles of all formulations followed first order kinetic. Therefore, the moisture absorption ability of all patches depends on the moisture content of the environment.” 6.Dermal delivery: The graphs seem to follow one phase upto 8 hours and then another phase from 8 hours to 24 hours. There are probably no observations between 8 hours and 24 hours. If that is true, if analysis for kinetics is carried out for data from 0 time to 8 hours, we will get a good picture of rate and type of delivery- first order or zero order. It will be interesting to see, whether there is any correlation between moisture uptake behaviour and dermal delivery behaviour of the patches. Each rate can be converted into a rate constant and the rate constants of the four varieties of

	patches for moisture uptake and dermal delivery may be tested for correlation. Response: We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have analyzed the kinetics and explained the results in the revised manuscript. We have also included the relation between moisture uptake behaviour and dermal delivery behaviour of the patches. “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 (Ramadon et al., 2021). Furthermore, we evaluated the kinetic profiles of the dermal delivery of curcumin from the patch formulations. Figure 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 kinetic profile from 0 h to 8 h. Following the analysis, we found that that the kinetic profiles of dermal delivery of all formulations followed first order kinetic. Therefore, the delivery of curcumin from the bioadhesive patches depends on the concentration of the curcumin in the formulation (Permana et al., 2019a).” 7.Kinetics may be calculated for bacterial viability. One may again write the values of the average rate of dermal delivery of the four varieties of patches in one column and the corresponding rate of antibacterial activity in another column and correlation may be discussed. This relationship was observed by the authors, it was recorded and was discussed in Section 3.4, but if the values are presented in a table the effect on the reader would be much better. Response: We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have included the relation between dermal delivery and antibacterial activity of all formulations. 8.Over all, this work is good but it can be presented in a much better manner. It requires a thorough revision. Response: We thank the Reviewer for the comments. We have made a great effort to improve the English and the discussion parts of our revised manuscript. We believe that all the data are now presented in a much better manner.

MINISTRY OF EDUCATION CULTURE OF INDONESIA
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The Editor

Journal of Drug Delivery Science and Technology

21st September 2021

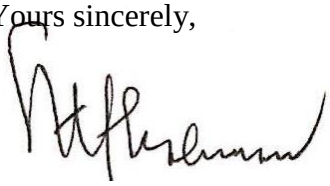
Dear Sir/Madam,

I wish you to re-consider our manuscript entitled: **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'** for publication in Journal of Drug Delivery Science and Technology.

We have addressed all of the comments raised by the Reviewer, substantially re-writing much of the manuscript, especially the Methods, Results and Discussion. We believe that the manuscript is now greatly improved. We have also made an effort to improve the scientific English of the manuscript.

We hope that you now consider our study worthy of publication in Journal of Drug Delivery Science and Technology and look forward to hearing from you in due course.

Yours sincerely,



Dr. Latifah rahman (on behalf of all authors)

Department of Pharmaceutics, Faculty of Pharmacy, Hasanuddin University, Makassar, Indonesia

Email: **latifahrahman@unhas.ac.id**

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[1*], Reni Sriyani Lembang[1], Subehan Lallo[2], Sri Resky Handayani[1], Usmanengsi[1], Andi Dian Permana[1]**

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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.

Keywords: *Zingiber cassumunar* Roxb; bioadhesive; dermal patches; skin infections; antibacterial

1. INTRODUCTION

Skin is the principal body part that defends the body from the entry of microorganism. However, burn wound injury affects the physiology of the skin by harming the local networks (Li et al., 2018). 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 skin main functions owing to damage of tissue and fluid, as well as the loss of water (Ahmad et al., 2018; Madaghiele et al., 2014). In normal conditions, skin infection is mainly triggered by Gram- positive bacteria named as *Staphylococcus aureus* (*S. aureus*) (Devices and Lafayette, 2000; Li et al., 2018). Additionally, more than 80% of chronic wounds are expected to be associated with biofilm development of bacteria (Permana et al., 2020a). The main target in the skin infections caused by burning is skin wound closure to decrease the rate of mortality (Atiyeh et al., 2005).

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 (Bian et al., 2003; El-Gendy et al., 2009; Mir et al., 2019). 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 (Mohamed et al., 2020; S. Pendekal and K. Tegginamat, 2012; Souza de Araujo et al., 2020), particularly in the condition of wounded and infected skin. Importantly, since the patch delivery system maintains a solidified dimension, hence, no differences in its thickness or the employed footprint can be found. 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 (El-Gendy et al., 2009; Souza de Araujo et al., 2020).

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 (Mir et al., 2019; Tatiya-aphiradee et al., 2016). However, numerous strains of *S. aureus* have been found to be resistant to those antibacterial agents (Haddadin et al., 2002; Kaur and Chate, 2015). 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 (Teow et al., 2016; Tyagi et al., 2015; Zorofchian Moghadamtousi et al., 2014). Importantly, one of the Gram-positive strains that is sensitive to curcumin-mediated inhibition is *S. aureus* (Tong et al., 2015). Curcumin exhibited the minimal inhibitory concentrations (MICs) of 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) (Mun et al., 2013). Also, Wang et al reported that the MIC of curcumin against MSSA was 256 μg/mL (Wang et al., 2016). *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 bacterial, including *S. aureus* (Jitendra Sharma et al., 2005; Li et al., 2019; Taechowisan et al., 2018).

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 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 and 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^{o}C for 48 days. The dried samples were then ground into fine powder with a mesh number of 20. The fine powders were extracted using maceration technique with 70% ethanol

as a solvent. The extraction was carried out for 3 days in a room with no direct sunlight. The ethanol was evaporated by subjecting the extract obtained from 70% ethanol to 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 (*v*er. 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 (Permana et al., 2019b).

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 of patches. Different bioadhesive polymers were also used, namely NaCMC, HPMC, chitosan and Carbopol. The compositions of each formulation 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 achieved 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 the dark in sealed vials at 4^0C until further investigations. The final mixtures were finally poured into glass petri dishes and dried in room temperature for 48 h.

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

2.4. Characterization of Bioadhesive Patches

2.4.1. 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 (El-Gendy et al., 2009).

2.4.2. Uniformity of Weight and Thickness Evaluation

The weight uniformity of patches was carried out by weighing six patches randomly and the averages weight were calculated. For the thickness uniformity, a calibrated digital caliper was used for the purpose of determining the uniformity of thickness. The calipers were 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 (El-Gendy et al., 2009; S. Pendekal and K. Tegginamat, 2012).

2.4.3. 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 folding times needed to rupture the patches (S. Pendekal and K. Tegginamat, 2012).

2.4.4. Surface pH Measurement

The pH of the surface was evaluated as per the method described previously (Miksusanti et al., 2020), with slight modification. Briefly, the patches were soaked in 10 mL of phosphate buffer for 2 hours and the surface pH was determined using a digital pH meter.

2.4.5. 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 minor modifications. Initially, patches were placed in a desiccator containing anhydrous calcium chloride for 24 hours 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 patches was determined every 48 days for 14 days. Finally, the percentage of moisture absorption was determined using the following calculation:

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

2.4.6. In vitro Bioadhesive Evaluation

The bioadhesive property of patches was evaluated using a modified physical balance, as previously described (Gupta et al., 1993), 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 pH 5.5 at 37^0C. Patches were fixed to the lower part of rubber stoppers with glue. The patches were then attached to the skin and the mass (g) required to separate the patches from the skin surface was denoted as the bioadhesive strength (shear stress). The force of adhesion and the bond strength was finally calculated using the following equations:

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

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

2.5. *Ex Vitro* Dermal Delivery Studies

Ex-vivo dermal delivery studies of bioadhesive patches were performed using 25 mL Franz diffusion cell, as previously described (Permana et al., 2020c, 2019c, 2019a). In this study, PBS (pH 7.4) containing 1% *w/v* Tween 80 was used as a medium prior. The skin was obtained from abdominal skin of Male Sprague-Dawley rats, approved and performed in compliance with the health ethical Committee of the Faculty of Medicine, Hasanuddin University. 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 x 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.

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

2.6.1. 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.

2.6.2. 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 (Permana et al., 2020a). 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 5 days.

2.6.3. 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 (Mir et al., 2020; Permana et al., 2020a, 2020b), with 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 (Permana et al., 2021). As control, infected skin without patch application was used.

2.7. 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 Figure 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.

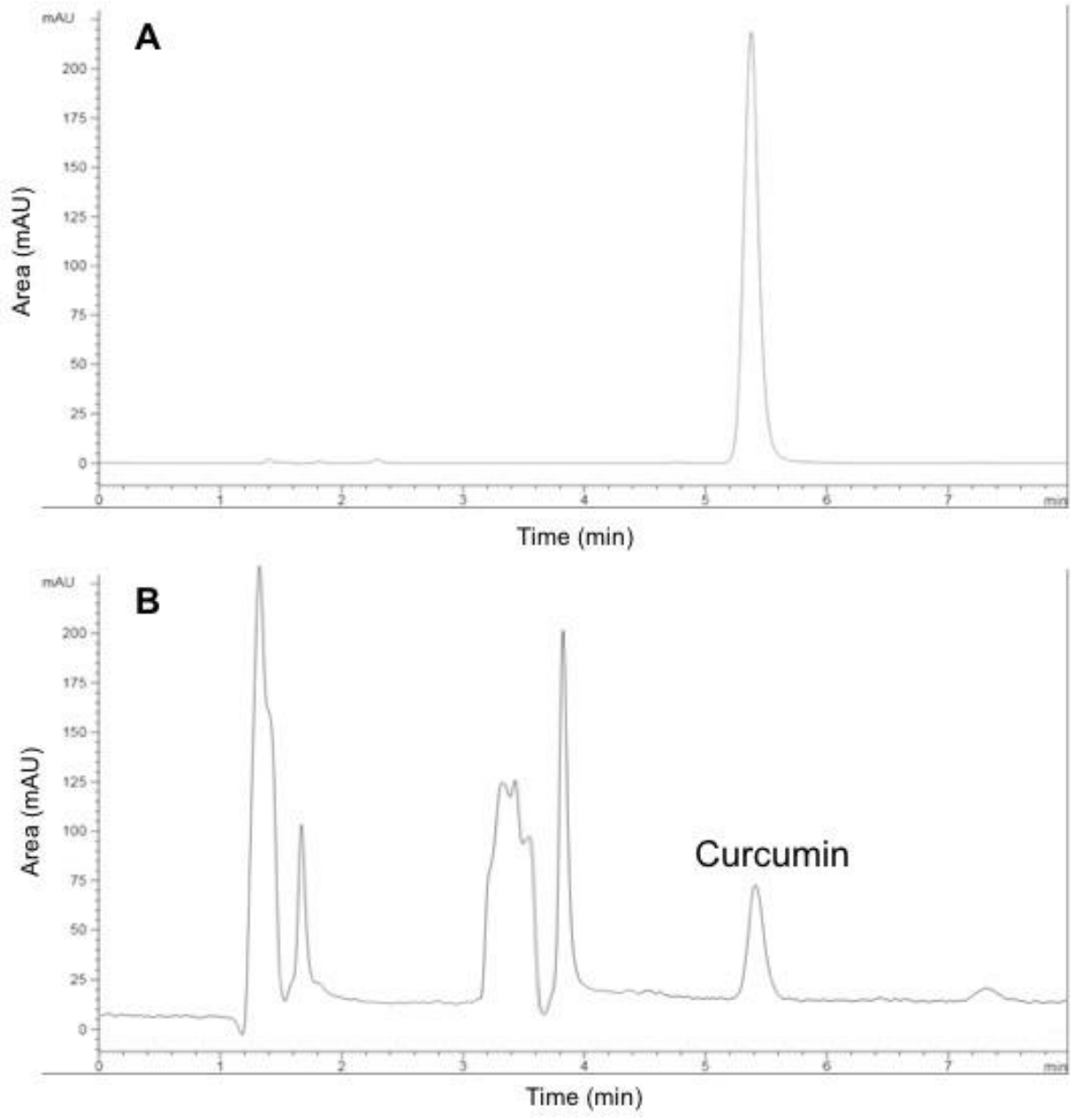

Figure 1. The HPLC chromatograms of standard curcumin and ethanol extract of *Zingiber cassumunar* Roxb

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 bend, 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 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.

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

	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

3.2.2. Folding Endurance Test

In order to evaluate the resistance capability of the patches, the folding endurance test was carried out (Figure 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 300 times (Miksusanti et al., 2020). 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 after the plasticizer binds to the polymer matrix, 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.

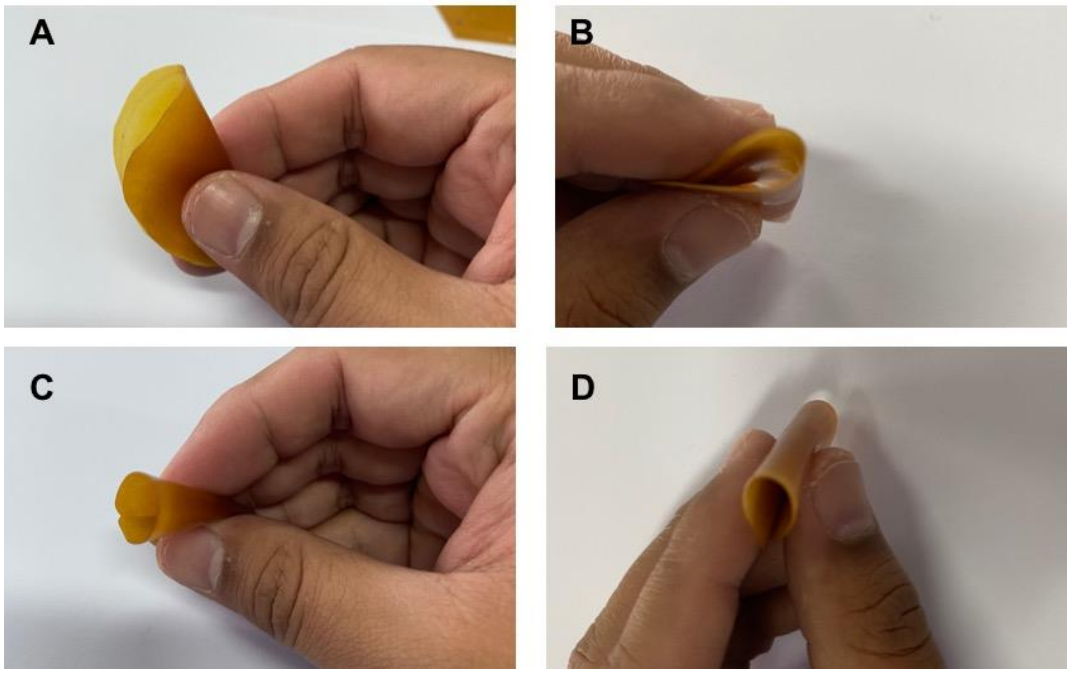

Figure 2. Representative images of folding endurance evaluation of F1 (**A**), F2 (**B**), F3 (**C**) and F4 (**D**).

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 (Miksusanti et al., 2020). 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.

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

	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

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 Figure 3.

As shown in Figure 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 (Adhikari et al., 2010). Michailova *et al.*, have reported that the presence of the elastic structure of HPMC leads to the low velocity of water uptake (Michailova et al., 2000). Furthermore, we analyzed the kinetic profiles of the moisture absorption. The results showed that the kinetic profiles of all

formulations followed first order kinetic. Therefore, the moisture absorption ability of all patches depends on the moisture content of the environment.

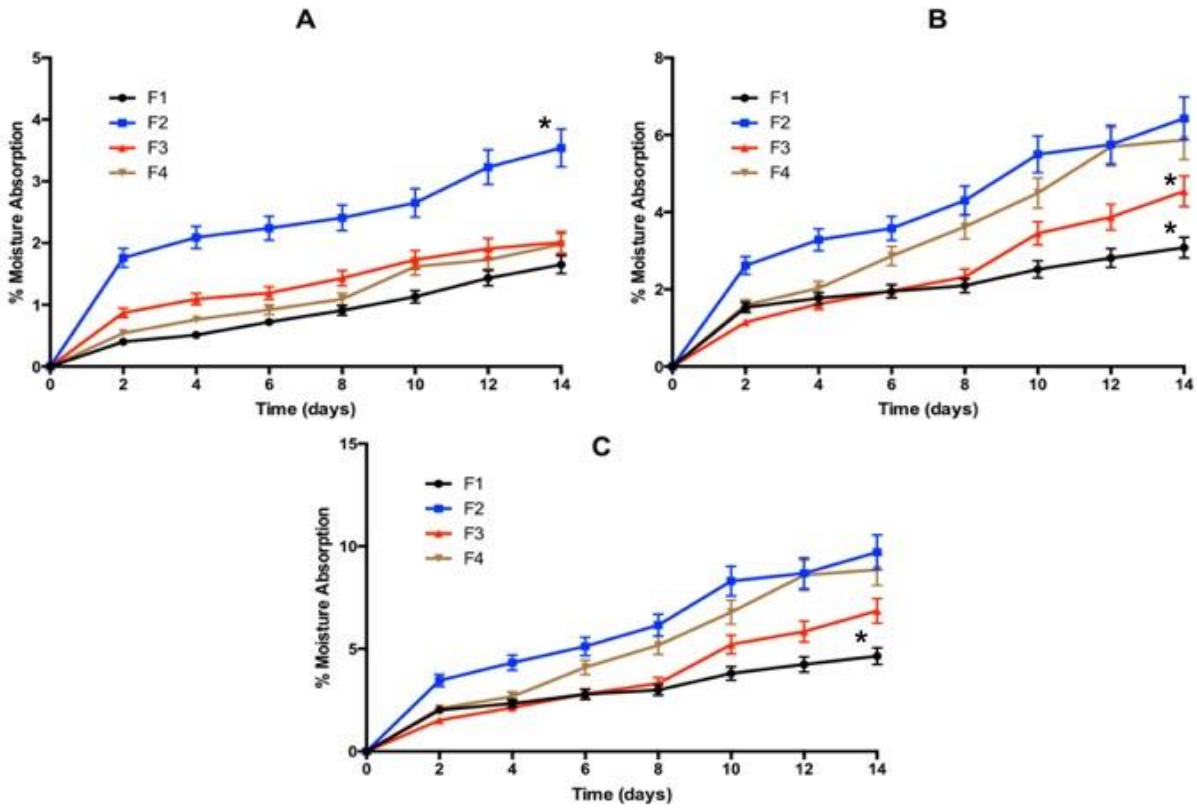

Figure 3. Moisture absorption ability of bioadhesive patches at 33 % RH (**A**), 65% RH (**B**) and 97% RH (**C**) (means ± S.D). * $p < 0.05$

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, enhancing 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. As such, we evaluated 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 (Refai and Tag, 2011). 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 (Refai and Tag, 2011). Analyzed statistically, 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 (Refai and Tag, 2011; Sankalia et al., 2008), resulting in poor bioadhesive properties.

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

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 Figure 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 expansively 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 (Ramadon et al., 2021). Furthermore, we evaluated the kinetic profiles of the dermal delivery of curcumin from the patch formulations. Figure 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 kinetic profile from 0 h to 8 h. Following the analysis, we found that that the kinetic profiles of dermal delivery of all formulations followed first order kinetic. Therefore, the delivery of curcumin from the bioadhesive patches depends on the concentration of the curcumin in the formulation (Permana et al., 2019a).

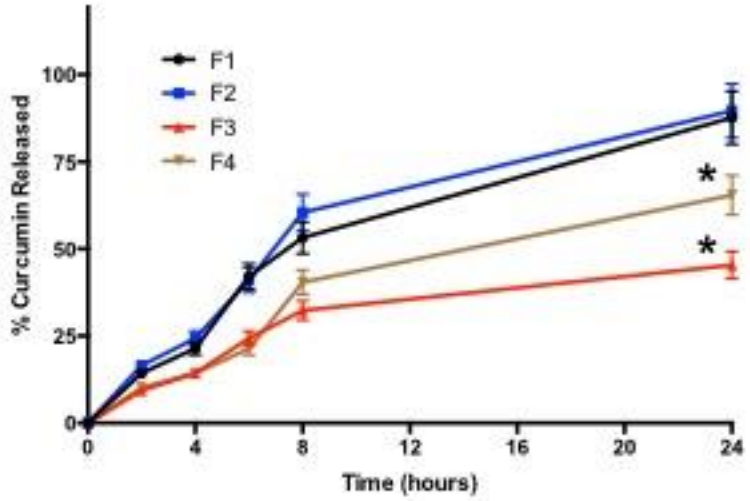

Figure 4. Dermal delivery of bioadhesive patches in excised rat skin (means ± S.D). * $p < 0.05$

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

In an attempt to evaluate the efficacy of our approach, we investigate 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 Figure 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. 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, 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.

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

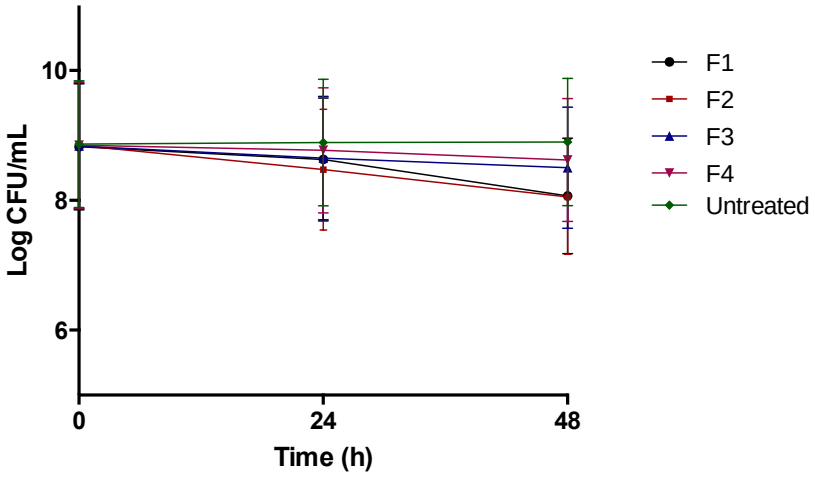

Figure 5. Bacterial viability (log CFU/mL) on in an *ex vivo S. aureus* infection model in rat skin following the application of different formulations of bioadhesive patches (means ± S.D).

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. Importantly, the obtained surface pH was around the pH of the skin, indicating that the use of the dermal patches would avoid any irritations. 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 prerequisite, including irritation study, toxicity and *in vivo* pharmacodynamic studies in a suitable model system.

ACKNOWLEDGEMENT

The authors thank Prof. Ryan Donnelly from the School of Pharmacy, Queen's University Belfast for providing HPLC column for analysis.

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Ms. Ref. No.: JDDST-D-20-00938

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

Response to Reviewers

We are very thankful to the expert reviewers for taking the time to kindly review our manuscript and provide helpful comments for improvement and clarification. We have made some changes to the manuscript as a result of these comments. We believe that the manuscript is now substantially improved. We have addressed each of the reviewers' comments in detail below. Importantly, we have made a great effort to improve the English and the discussion parts of our revised manuscript.

Reviewer #1:

In this research work the researchers developed the bio adhesive transdermal patches incorporated with Zingiber Cassumunar Roxb extract. One of the problems in this formulation was estimation of active constitutions present in the formulation. The formulation potential was based on the evaluation report of curcumin present in the Cassumunar Roxb extraction. But the curcumin MIC is approximately above 100 microgram/ml, it is quite high concentration compare with other antibiotics active against Staphylococcus aureus. Further, Cassumunar Roxb extract may contains various phytochemicals. So, the amount of other constitutions presents in this exact to be specified by providing supporting materials with data. The HPLC chromatogram, retention time of various phytochemicals and Rf values are required to support number of phytochemicals present in the formulation. How the researchers obtained the rat skin for in vitro testing? Is it institutional animal ethical committee approval is needed? How the researchers optimized the formulation? No details given about what basis the researchers selected various amount of excipients and extract used to make F1 to F2 formulation.

Response to Reviewer

We are very thankful to the Reviewer for taking the time to review this manuscript and for the expert review, providing helpful comments. We have made a number of key changes to the manuscript as a result of these comments. We believe that the manuscript is now substantially improved. We have addressed each one of the reviewers' comments in detail.

We agree with the Reviewer that MIC of curcumin is higher compared to other antibiotics. However, in this study, one of our main purposes is to develop bioadhesive patches using natural product as alternative of synthetic compounds. Compared to several synthetic compounds, natural compounds are considered to be safer. With respect to HPLC chromatogram, we have provided the HPLC chromatogram of curcumin and the extract in comparison with standard curcumin in the revised manuscript. Importantly, the analytical method was validated as per the International Committee of Harmonization (ICH) 2005.

With respect to the skin for in vitro testing, the skin was obtained from abdominal skin of Male Sprague-Dawley rats, approved and performed in compliance with the health ethical Committee of the Faculty of Medicine, Hasanuddin University. We have now added this into our revised manuscript.

With regards to the optimization of the patch formulation, initially, we performed 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 plasticizer. The patches were too thick and difficult to be bend, 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-90. Regarding the use of the concentration of the bioadhesive polymers used, we also performed 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. We have now explained this in our revised manuscript.

Reviewer #2:

Dear Author, I wish to appreciate the work that you have carried out. It was done well and the results are reported well. But the presentation requires thorough revision, in terms of language, analysis of results and presentation of results. Please put in a little more effort and the result would make you happy.

Response to Reviewer

We are very thankful to the Reviewer for taking the time to review this manuscript and for the expert review, providing helpful comments. We have made a number of key changes to the manuscript as a result of these comments. We believe that the manuscript is now substantially improved.

Reviewer #3:

Review Comments for "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"

In this original paper authors have worked on extraction of Zingiber Cassumunar Roxb and formulating the hydrogel patches using different polymers. The research idea using the natural products extraction and making its formulation is good but the study performed is not that strong. It lacks lot of good experiments which are basics in formulation development. Also, the whole manuscript lacks biostatistics in all the images provided. There are lot of corrections and extra experiments needed to validate this concept. However, specific comments for this evaluation are given as:

Response to Reviewer

We are very thankful to the Reviewer for taking the time to review this manuscript and for the expert review, providing helpful comments. We have made a number of key changes to

the manuscript as a result of these comments. We believe that the manuscript is now substantially improved. We have addressed each one of the reviewers' comments in detail.

Comments:

1. The graphical abstract image is not clear.

Response:

We thank the Reviewer for pointing this out. We have improved the resolution of the graphical abstract image.

2. In the methodology section, authors have taken constant amount of PVP-K10 and Propylene glycol. Why they have not optimized the formulations with different amounts of PVP-K10 and propylene glycol?

Response:

We thank the Reviewer for the comments. Following comments and suggestion from reviewer, we have added some discussions and explanations about our preliminary studies.

With regards to the optimization of the patch formulation, initially, we performed 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 plasticizer. The patches were too thick and difficult to be bend, 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-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. We have now explained this in our revised manuscript.

3. On what basics the amounts of NaCMC, HPMC, chitosan and Carbopol are selected? How they came to know 2% or 0.3% w/w amount of polymer is enough to make these bioadhesive gels?

Response:

We thank the Reviewer for the comments. Following comments and suggestion from reviewer, we have added some discussions and explanations about our preliminary studies.

Regarding the use of the concentration of the bioadhesive polymers used, we also performed 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. We have now explained this in our revised manuscript.

4. What amount of triethanolamine was used to neutralize the pH in Carbopol containing gels and moreover what was final pH of these formulations?

Response:

We thank the Reviewer for the comments. The concentration of triethanolamine was 1.5 times the concentration of Carbopol and the pH achieved was 7.01 ± 0.03. We have now added this in our revised manuscript.

5. Can authors produce HPTLC graph for the extracts they have obtained after extraction of Zingiber cassumunar Roxb? HPTLC graph can also be compared with standard curcumin. Also, the authors have not provided any HPLC graphs as well. So, it is difficult to say what exact extract they have obtained. Also, HPTLC study can also be used to calculate the percentage of curcumin and other components of interest.

Response:

We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have provided the HPLC chromatogram of the extract in comparison with standard curcumin in the revised manuscript. Importantly, the analytical method using HPLC was validated as per the International Committee of Harmonization (ICH) 2005. Therefore, we believe that the validated analytical method was sufficient to quantify curcumin in the extract and all experiments in this study.

6. Can authors provide images or videos for folding endurance test? They will be helpful to exactly see the nature of the patch obtained.

Response:

We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have provided the images for folding endurance test in the revised manuscript.

7. As authors mentioned these patches as hydrogels, it will be very helpful if they provide data for hydrogel characterization such as rheological studies, physiochemical properties such as swelling properties, gel fraction, SEM images etc.

Response:

We thank the Reviewer for the suggestions. We agree that the patches were made from hydrogel formulation. However, the final form of our formulation was solid dosage form. Therefore, we only evaluated and characterized the final patch formulation, which have been explained in our manuscript.

8. Can authors compare the bioadhesive properties of these gels with any marketed formulation?

Response:

We thank the Reviewer for the question and suggestion. As previously explained, the final form of our formulation was solid dosage form. Therefore, we did not compare the bioadhesive properties with semisolid dosage forms. Furthermore, to the best our knowledge, there has no marketed formulation of bioadhesive patch. Thus, we only compared the bioadhesive properties of the formulations developed in our study.

9. In all the figures, no biostatistics is used. Need to perform statistics to understand the exact difference in all the groups.

Response:

We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have provided all figures with statistic results. Importantly, we have also discussed all results according to biostatistical results.

ADDITIONAL RESPONSE

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 Rahman1* , Reni Sriyani Lembang1 , Subehan Lallo2 , Andi Dian Permana1**

Report on the above paper:

5.12.2020

Comments on the research work:

The work reported in this research paper is of good quality, it was carried out as per standard procedures and definitely merits publication in a reputed journal. They have established that a natural compound, curcumin, possesses antibacterial activity in infections that may arise from burn injuries; this is also a good achievement. Literature review was carried out in an appropriate manner. Procedures adopted in the collection and characterisation of the herb, analytical procedures, preparation of bio-adhesive patches and their characterisation are all good standard procedures. Analysis of the results was done appropriately and proper conclusions were also drawn after a good discussion.

Response:

We are very thankful to the expert reviewers for taking the time to kindly review our manuscript and provide helpful comments for improvement and clarification. We are grateful to hear that our research paper is a good quality and the experiments were carried out as per standard procedures.

Comments on presentation of the work:

The presentation of the work was done in a very poor manner.

Language: There are many language errors throughout the write up of the paper. I think almost every sentence has a grammatical error or a presentation (wrong way of writing a sentence) error. I initially started to make a Table so that I can point out the errors and suggest their corrections. I started with the abstract given in the first page. I later noticed that some of these errors are not there in the actual paper. I stopped that effort as the errors are just too many in number. I will give that table as an attachment. Nevertheless, this paper is full of mistakes in its language and it needs a thorough revision. I will present some errors below:

Response:

We thank the Reviewer for the comments. We have made some changes to the manuscript as a result of these comments. We believe that the manuscript is now substantially improved. We have addressed each of the reviewers' comments in detail below. Importantly, we have made a great effort to improve the English and the discussion parts of our revised manuscript.

Grammatical errors:

1. In comparison with the topical standard dosage forms, such as ointments, creams, lotions and emulsions, patch dosage forms are more comfortable for utilization and can avoid active ingredients from being wiped out (Bian et al., 2003; El-Gendy et al., 2009; Mir et al., 2019).

   Correction: When patch dosage forms are compared with standard topical dosage forms, such as, ointments, creams, lotions and ointments, 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.

   Response:

   Needful is done

2. Same para—8th line: infection wound skin

   Correction: Infected skin or wounded and infected skin

   Response:

   Needful is done

3. 2.3: first line -- as analyte of interest

   Correction: as the analyte of interest

   Response:

   Needful is done

4. 2.4: procedures can be written in a clearer manner.

   Response:

   Needful is done

5. 2.4: 3rd line from bottom: in room temperature- correction: at room temperature.

Response:

Needful is done

6. 2.4.1 first line: uniformity content of curcumin- correction: uniformity of content of curcumin.

   Response:

   Needful is done

7. 2.4.2- averages weight were calculated- correction: average weight was calculated.

   Response:

   Needful is done

8. For the thickness uniformity, a calibrated digital caliper was used to measure the thickness of five different points of six different patches and the averages thickness were calculated (El-Gendy et al., 2009; S. Pendekal and K. Tegginamat, 2012).

   Correction: 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. Average thickness of the patch was calculated. This procedure was carried out for six patches of each variety of curcumin patches prepared.

   Response:

   Needful is done

9. 2.4.4- first line- as per method—correction: as per the method.

   Response:

   Needful is done

10. 2.4.5_ first line: ability of patch- correction: the ability of the prepared patches

    Response:

    Needful is done

Comments on write up:

1. Explain each procedure with greater clarity, give more details.

   Response:

   We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have provided all details in the methods.

2. 2.5: Explain how the rats were procured, and stored. Give details of Ethics Committee permission. Explain that the animals were sacrificed and then the skins were taken and then further procedures were carried out.

   Response:

   We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have included the details of Ethics Committee permission in the revised manuscript.

3. Give the exact number of foldings instead of saying >300- this is vague.

   Response:

   Needful is done

4. 3.2.4- results of the evaluation are shown in -----., not exhibited in.

   Response:

   Needful is done

5. Moisture absorption studies: we can do analysis for kinetics and report on whether the absorption is following zero order or first order.

   Response:

   We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have analyzed the kinetics and explained the results in the revised manuscript.

   "Furthermore, we analyzed the kinetic profiles of the moisture absorption. The results showed that the kinetic profiles of all formulations followed first order kinetic. Therefore, the moisture absorption ability of all patches depends on the moisture content of the environment."

6. Dermal delivery: The graphs seem to follow one phase upto 8 hours and then another phase from 8 hours to 24 hours. There are probably no observations between 8 hours and 24 hours. If that is true, if analysis for kinetics is carried out for data from 0 time to 8 hours, we will get a good picture of rate and type of delivery- first order or zero order. It will be interesting to see, whether there is any correlation between moisture uptake behaviour and dermal delivery behaviour of the patches. Each rate can be converted into a rate constant and the rate constants of the four varieties of patches for moisture uptake and dermal delivery may be tested for correlation.

Response:

We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have analyzed the kinetics and explained the results in the revised manuscript. We have also included the relation between moisture uptake behaviour and dermal delivery behaviour of the patches.

"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 (Ramadon et al., 2021). Furthermore, we evaluated the kinetic profiles of the dermal delivery of curcumin from the patch formulations. Figure 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 kinetic profile from 0 h to 8 h. Following the analysis, we found that that the kinetic profiles of dermal delivery of all formulations followed first order kinetic. Therefore, the delivery of curcumin from the bioadhesive patches depends on the concentration of the curcumin in the formulation (Permana et al., 2019a)."

7. Kinetics may be calculated for bacterial viability. One may again write the values of the average rate of dermal delivery of the four varieties of patches in one column and the corresponding rate of antibacterial activity in another column and correlation may be discussed. This relationship was observed by the authors, it was recorded and was discussed in Section 3.4, but if the values are presented in a table the effect on the reader would be much better.

   Response:

   We thank the Reviewer for the suggestions. Following the suggestion from the Reviewer, we have included the relation between dermal delivery and antibacterial activity of all formulations.

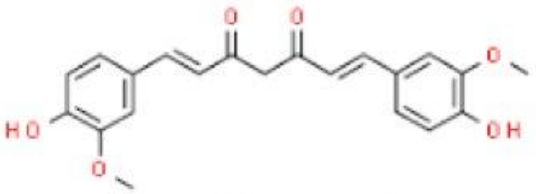
8. Over all, this work is good but it can be presented in a much better manner. It requires a thorough revision.

Response:

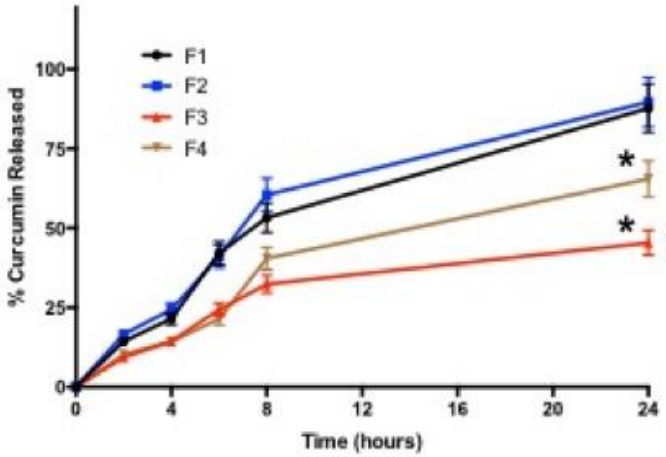
We thank the Reviewer for the comments. We have made a great effort to improve the English and the discussion parts of our revised manuscript. We believe that all the data are now presented in a much better manner.

Graphical Abstract

Zingiber cassumunar Roxb.

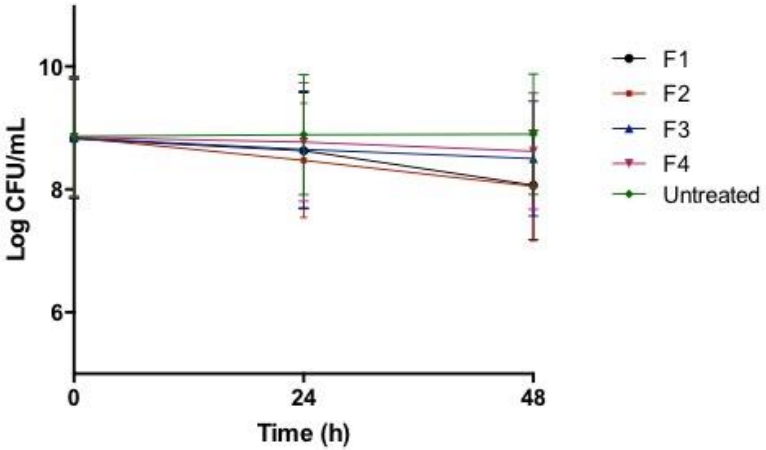


Curcumin



Ex Vivo Dermal Delivery

Bioadhesive dermal patch



Ex Vivo Antibacterial Activity

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author Contribution Statement

Latifah Rahman: Conceptualization, Review & Editing, Project Administration, Funding Acquisition, Validation, Supervision; **Reni Sriyani Lembang**: Methodology, Investigation, Data Curation; **Subehan Lallo**: Review & Editing, Project Administration; **Sri Resky Handayani**: Methodology, Investigation, Data Curation, Formal Analysis; **Usmanengis**: Methodology, Investigation, Data Curation**; Andi Dian Permana:** Conceptualization, Methodology, Writing - Original Draft, Investigation, Data Curation., Visualization.

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

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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.

Keywords: *Zingiber cassumunar* Roxb; bioadhesive; dermal patches; skin infections; antibacterial

1. INTRODUCTION

Skin is the principal body part that defends the body from the entry of microorganism. However, burn wound injury affects the physiology of the skin by harming the local networks (Li et al., 2018). 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 skin main functions owing to damage of tissue and fluid, as well as the loss of water (Ahmad et al., 2018; Madaghiele et al., 2014). In normal conditions, skin infection is mainly triggered by Gram- positive bacteria named as *Staphylococcus aureus* (*S. aureus*) (Devices and Lafayette, 2000; Li et al., 2018). Additionally, more than 80% of chronic wounds are expected to be associated with biofilm development of bacteria (Permana et al., 2020a). The main target in the skin infections caused by burning is skin wound closure to decrease the rate of mortality (Atiyeh et al., 2005).

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 (Bian et al., 2003; El-Gendy et al., 2009; Mir et al., 2019). 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 (Mohamed et al., 2020; S. Pendekal and K. Tegginamat, 2012; Souza de Araujo et al., 2020), particularly in the condition of wounded and infected skin. Importantly, since the patch delivery system maintains a solidified dimension, hence, no differences in its thickness or the employed footprint can be found. 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 (El-Gendy et al., 2009; Souza de Araujo et al., 2020).

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 (Mir et al., 2019; Tatiya-aphiradee et al., 2016). However, numerous strains of *S. aureus* have been found to be resistant to those antibacterial agents (Haddadin et al., 2002; Kaur and Chate, 2015). 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 (Teow et al., 2016; Tyagi et al., 2015; Zorofchian Moghadamtousi et al., 2014). Importantly, one of the Gram-positive strains that is sensitive to curcumin-mediated inhibition is *S. aureus* (Tong et al., 2015). Curcumin exhibited the minimal inhibitory concentrations (MICs) of 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) (Mun et al., 2013). Also, Wang et al reported that the MIC of curcumin against MSSA was 256 μg/mL (Wang et al., 2016). *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 bacterial, including *S. aureus* (Jitendra Sharma et al., 2005; Li et al., 2019; Taechowisan et al., 2018).

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 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 and 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^{o}C for 48 days. The dried samples were then ground into fine powder with a mesh number of 20. The fine powders were extracted using maceration technique with 70% ethanol

as a solvent. The extraction was carried out for 3 days in a room with no direct sunlight. The ethanol was evaporated by subjecting the extract obtained from 70% ethanol to 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 (*v*er. 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 (Permana et al., 2019b).

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 of patches. Different bioadhesive polymers were also used, namely NaCMC, HPMC, chitosan and Carbopol. The compositions of each formulation 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 achieved 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 the dark in sealed vials at 4^0C until further investigations. The final mixtures were finally poured into glass petri dishes and dried in room temperature for 48 h.

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

2.4. Characterization of Bioadhesive Patches

2.4.1. 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 (El-Gendy et al., 2009).

2.4.2. Uniformity of Weight and Thickness Evaluation

The weight uniformity of patches was carried out by weighing six patches randomly and the averages weight were calculated. For the thickness uniformity, a calibrated digital caliper was used for the purpose of determining the uniformity of thickness. The calipers were 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 (El-Gendy et al., 2009; S. Pendekal and K. Tegginamat, 2012).

2.4.3. 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 folding times needed to rupture the patches (S. Pendekal and K. Tegginamat, 2012).

2.4.4. Surface pH Measurement

The pH of the surface was evaluated as per the method described previously (Miksusanti et al., 2020), with slight modification. Briefly, the patches were soaked in 10 mL of phosphate buffer for 2 hours and the surface pH was determined using a digital pH meter.

2.4.5. 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 minor modifications. Initially, patches were placed in a desiccator containing anhydrous calcium chloride for 24 hours 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 patches was determined every 48 days for 14 days. Finally, the percentage of moisture absorption was determined using the following calculation:

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

2.4.6. In vitro Bioadhesive Evaluation

The bioadhesive property of patches was evaluated using a modified physical balance, as previously described (Gupta et al., 1993), 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 pH 5.5 at 37^0C. Patches were fixed to the lower part of rubber stoppers with glue. The patches were then attached to the skin and the mass (g) required to separate the patches from the skin surface was denoted as the bioadhesive strength (shear stress). The force of adhesion and the bond strength was finally calculated using the following equations:

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

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

2.5. *Ex Vitro* Dermal Delivery Studies

Ex-vivo dermal delivery studies of bioadhesive patches were performed using 25 mL Franz diffusion cell, as previously described (Permana et al., 2020c, 2019c, 2019a). In this study, PBS (pH 7.4) containing 1% *w*/*v* Tween 80 was used as a medium prior. The skin was obtained from abdominal skin of Male Sprague-Dawley rats, approved and performed in compliance with the health ethical Committee of the Faculty of Medicine, Hasanuddin University. 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 x 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.

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

2.6.1. 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.

2.6.2. 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 (Permana et al., 2020a). 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 5 days.

2.6.3. 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 (Mir et al., 2020; Permana et al., 2020a, 2020b), with 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 (Permana et al., 2021). As control, infected skin without patch application was used.

2.7. 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 Figure 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.

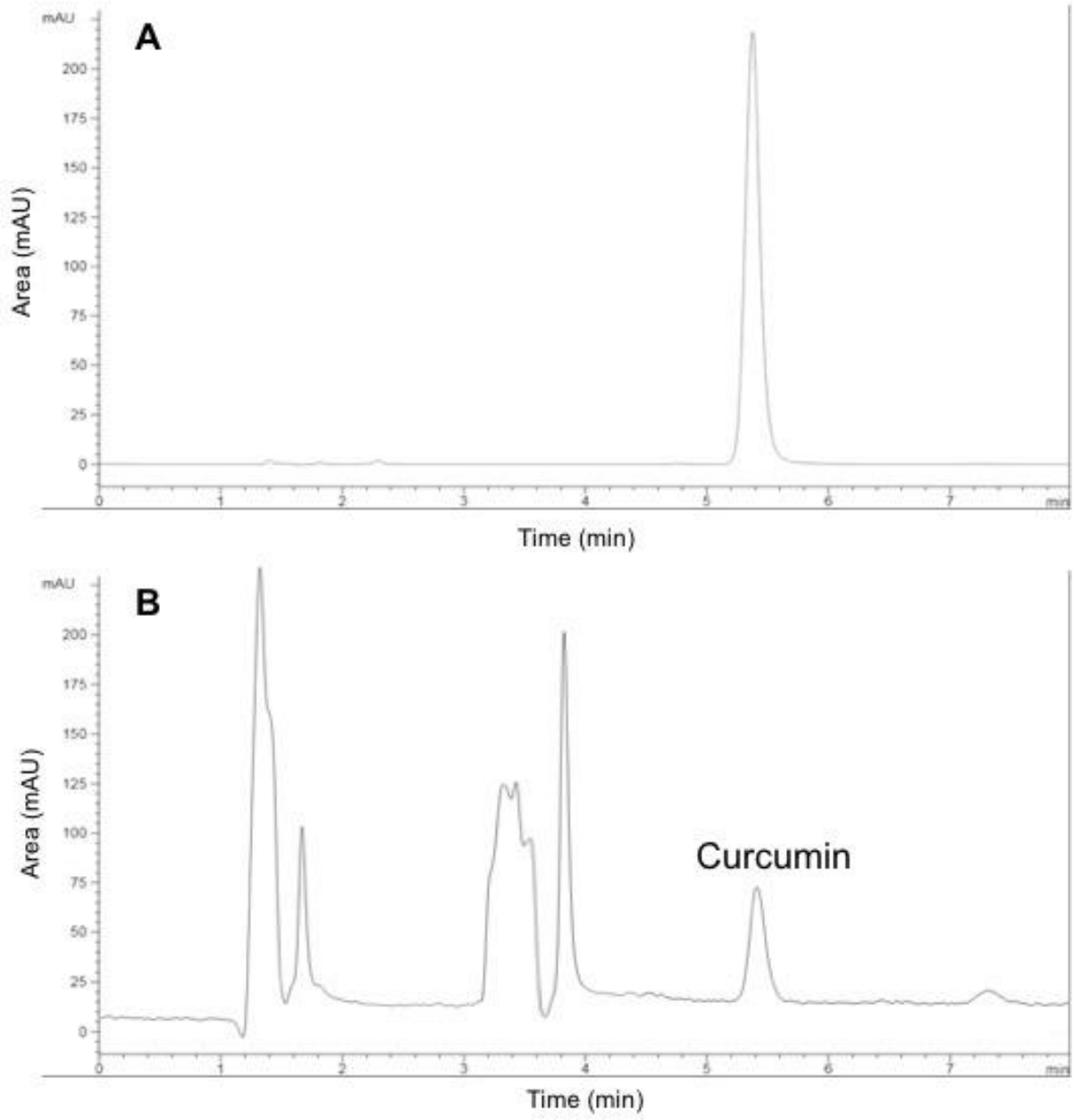

Figure 1. The HPLC chromatograms of standard curcumin and ethanol extract of *Zingiber cassumunar* Roxb

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 bend, 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 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.

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

	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

3.2.2. Folding Endurance Test

In order to evaluate the resistance capability of the patches, the folding endurance test was carried out (Figure 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 300 times (Miksusanti et al., 2020). 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 after the plasticizer binds to the polymer matrix, 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.

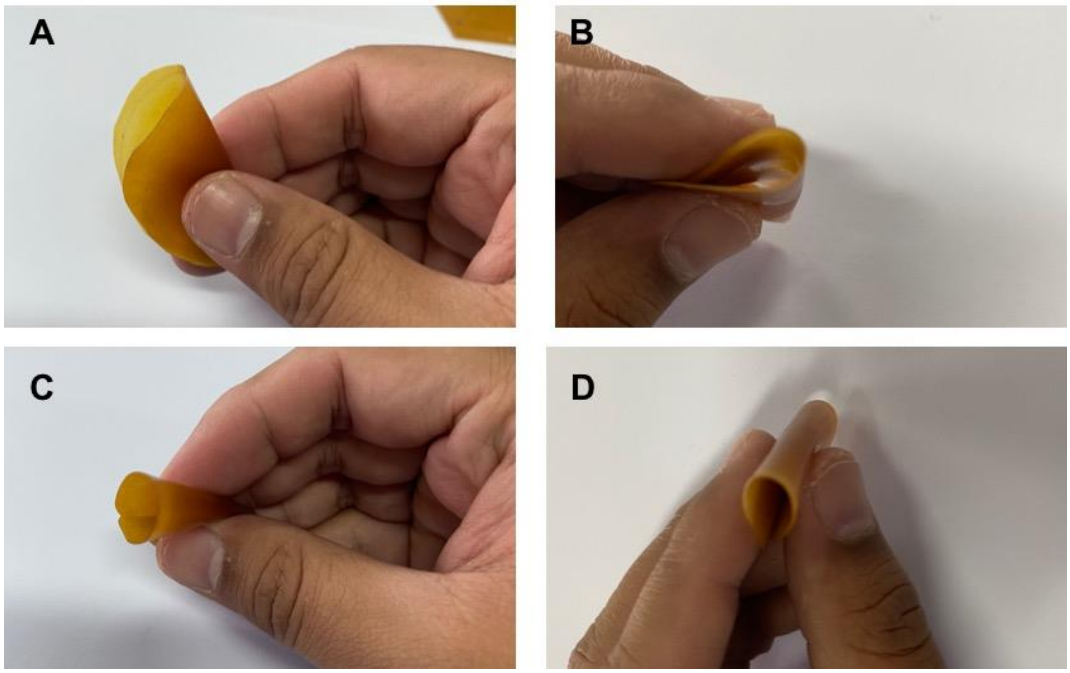

Figure 2. Representative images of folding endurance evaluation of F1 (**A**), F2 (**B**), F3 (**C**) and F4 (**D**).

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 (Miksusanti et al., 2020). 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.

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

	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

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 Figure 3.

As shown in Figure 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 (Adhikari et al., 2010). Michailova *et al.*, have reported that the presence of the elastic structure of HPMC leads to the low velocity of water uptake (Michailova et al., 2000). Furthermore, we analyzed the kinetic profiles of the moisture absorption. The results showed that the kinetic profiles of all

formulations followed first order kinetic. Therefore, the moisture absorption ability of all patches depends on the moisture content of the environment.

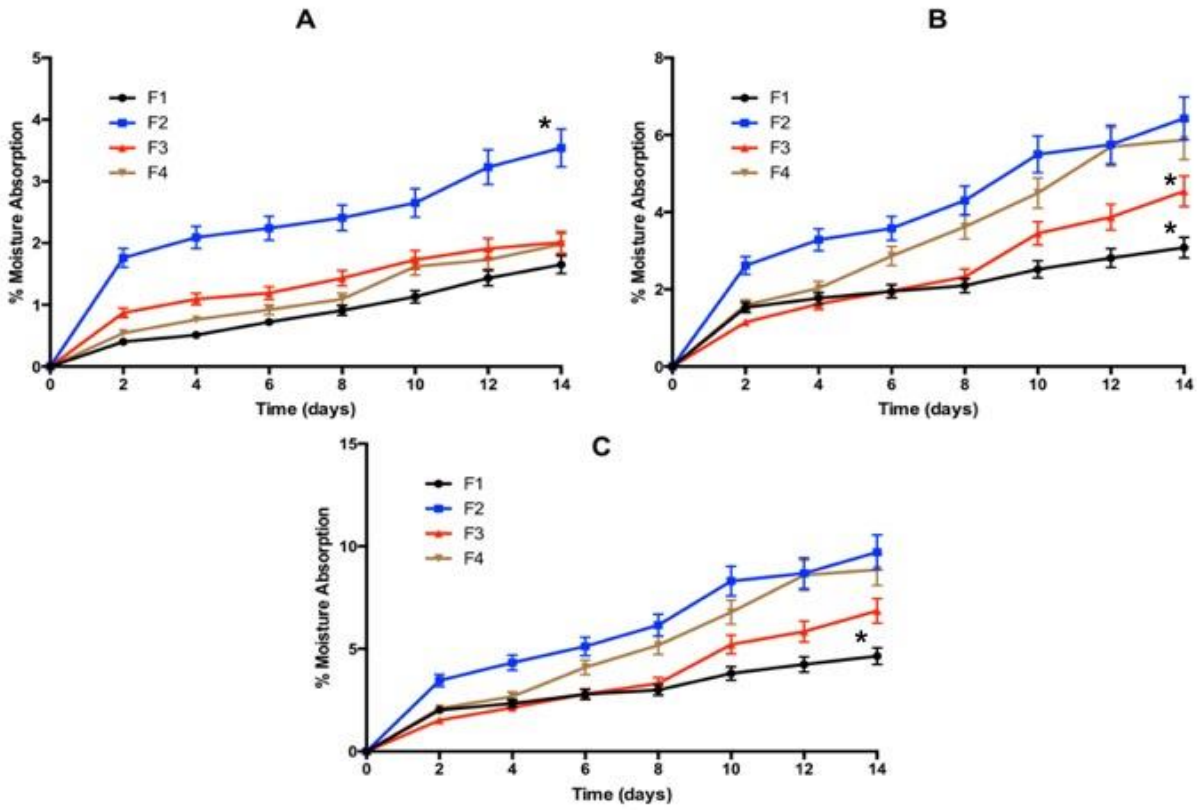

Figure 3. Moisture absorption ability of bioadhesive patches at 33 % RH (**A**), 65% RH (**B**) and 97% RH (**C**) (means ± S.D). * $p < 0.05$

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, enhancing 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. As such, we evaluated 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 (Refai and Tag, 2011). 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 (Refai and Tag, 2011). Analyzed statistically, 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 (Refai and Tag, 2011; Sankalia et al., 2008), resulting in poor bioadhesive properties.

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

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 Figure 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 expansively 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 (Ramadon et al., 2021). Furthermore, we evaluated the kinetic profiles of the dermal delivery of curcumin from the patch formulations. Figure 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 kinetic profile from 0 h to 8 h. Following the analysis, we found that that the kinetic profiles of dermal delivery of all formulations followed first order kinetic. Therefore, the delivery of curcumin from the bioadhesive patches depends on the concentration of the curcumin in the formulation (Permana et al., 2019a).

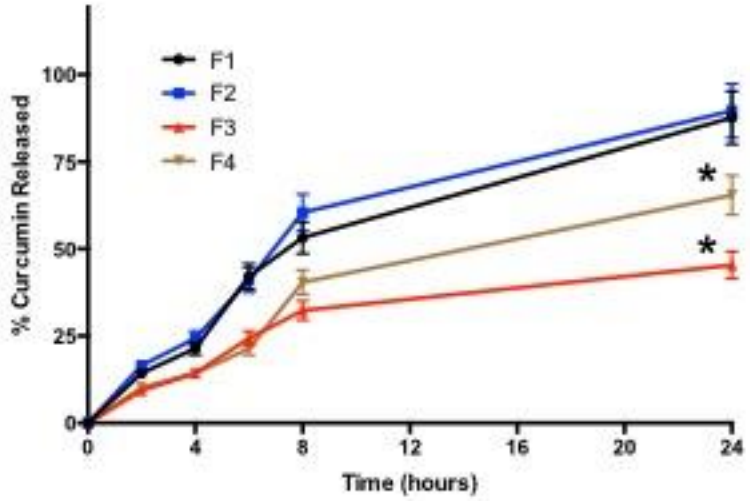

Figure 4. Dermal delivery of bioadhesive patches in excised rat skin (means ± S.D). * $p < 0.05$

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

In an attempt to evaluate the efficacy of our approach, we investigate 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 Figure 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. 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, 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.

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

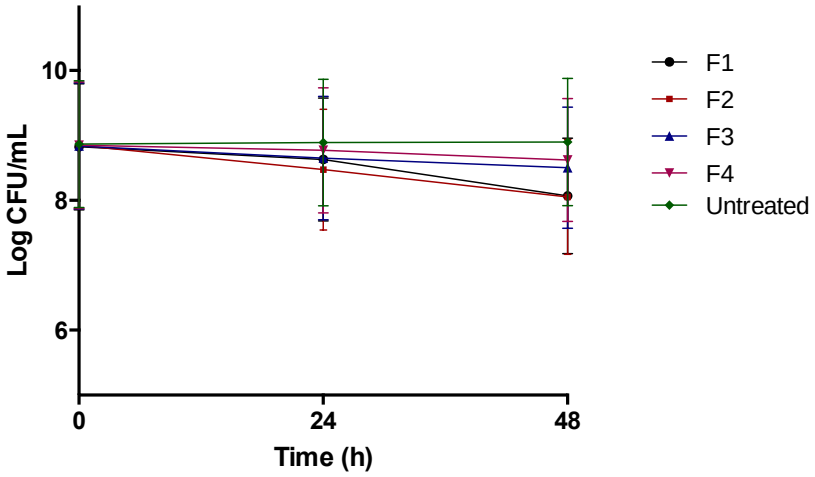

Figure 5. Bacterial viability (log CFU/mL) on in an *ex vivo S. aureus* infection model in rat skin following the application of different formulations of bioadhesive patches (means ± S.D).

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. Importantly, the obtained surface pH was around the pH of the skin, indicating that the use of the dermal patches would avoid any irritations. 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 prerequisite, including irritation study, toxicity and *in vivo* pharmacodynamic studies in a suitable model system.

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