ISSN 2745-455X (Online)
Indonesian Journal of Pharmacology and Therapy

Topical gel preparation from *Staphylococcus epidermidis* isolate extract accelerated healing on incision wound model in rats

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https://doi.org/10.22146/ijpther.11510

Submitted: 28-12-2023
Accepted : 09-01-2024

Keywords:
gel preparation;
Staphylococcus epidermidis;
wound healing;
physical stability;
topical

ABSTRACT

A wound is a disorder of skin integrity caused by physical trauma or disease. Wound healing is a natural restorative response to tissue injury. It is a complex dynamic physiological process involving several overlapping phases. Although wound management has been applied, however, some time it is associated with major drawbak including invasiveness, antibiotic resistance, and high costs. Natural medicines are being developed for wound management to resolve the drawback. Commensal *Staphylococcus epidermidis* was proven to play a role in natural wound healing process. This study aimed to investigate the wound healing activity of a topical gel preparation containing *S. epidermidis* isolate extract on an incision wound model in rats. The methanol extract of *S. epidermidis* isolate was prepared by maceration and used as an active ingredient of the topical gel using NaCMC as a gelling agent. A physical stability test was carried out by assessing organoleptic properties, pH value, homogeneity, dispersion, and viscosity of the gel preparation. The gel preparation was then topically applied once-daily for 11 d on incision wound model of male white rats (*Rattus norvegicus*). The wound healing was observed daily and calculated using Morton's formula. No significant change in the physical stability parameters of the gel preparation was observed during observation on day 1, 7, and 14 ($p>0.05$). A significant increase in wound healing after the application of the gel preparation compared to negative control was observed ($p<0.05$). In contrast, no significant difference in wound healing between the gel preparation and the positive control group was observed ($p>0.05$). In conclusion, the topical gel preparation from *S. epidermidis* isolate extract accelerates healing on incision wound models in rats.

ABSTRAK

Luka adalah gangguan integritas kulit yang disebabkan oleh trauma fisik atau penyakit. Penyembuhan luka merupakan respon restoratif alami terhadap cedera jaringan. Ini adalah proses fisiologis dinamis yang kompleks yang melibatkan beberapa fase yang tumpang tindih. Meskipun manajemen luka telah diterapkan, namun terkadang hal ini dikaitkan dengan kekurangan besar termasuk sifat invasifnya, terjadinya resistensi antibiotik, dan biaya tinggi. Obat alami sedang dikembangkan untuk manajemen luka guna mengatasi kekurangan tersebut. Commensal *Staphylococcus epidermidis* terbukti berperan dalam proses penyembuhan luka secara alami. Penelitian ini bertujuan untuk mengetahui aktivitas penyembuhan luka dari sediaan gel topikal yang mengandung ekstrak isolat *S. epidermidis* pada model luka sayatan pada tikus. Ekstrak metanol isolat *S. epidermidis* dibuat dengan cara maserasi dan digunakan sebagai bahan aktif gel topikal menggunakan NaCMC sebagai bahan pembentuk gel. Uji stabilitas fisik dilakukan dengan menilai sifat organoleptik, nilai pH, homogenitas, dispersi,

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dan viskositas sediaan gel. Sediaan gel tersebut kemudian dioleskan pada model luka sayatan tikus putih jantan (*Rattus norvegicus*). Sediaan gel dioleskan sekali sehari selama 11 hari. Penyembuhan luka diamati setiap hari dan dihitung menggunakan rumus Morton. Tidak ada perubahan signifikan pada parameter stabilitas fisik sediaan gel yang diamati selama pengamatan pada hari ke-1, 7, dan 14 ($p>0,05$). Peningkatan yang signifikan dalam penyembuhan luka setelah penerapan sediaan gel dibandingkan dengan kontrol negatif diamati ($p<0,05$). Sebaliknya, tidak ada perbedaan signifikan dalam penyembuhan luka antara sediaan gel dan kelompok kontrol positif ($p>0,05$). Kesimpulannya, sediaan gel topikal dari ekstrak isolat *S. epidermidis* mempercepat penyembuhan model luka sayatan pada tikus.

INTRODUCTION

A wound is a disorder of skin integrity caused by physical trauma or disease. Wound healing is a natural restorative response to tissue injury.[1] Wound healing is a complex dynamic physiological process involving several overlapping phases that include 1) inflammation phase characterized by hemostasis, chemotaxis, and increased vascular permeability, limiting further damage, closing the wound, removing cellular debris and bacteria, and fostering cellular migration; 2) proliferative phase characterized by hemostasis, chemotaxis, and increased vascular permeability, limiting further damage, closing the wound, removing cellular debris and bacteria, and fostering cellular migration; 3) maturation and remodeling phase characterized by reformulations and improvement in the components of the collagen fiber that increases the tensile strength.[2-3]

Wound often causes complications associated with health and need high costs for treatment. Although wound management including irrigation, debridement, antibiotics administration, proteolytic enzymes, and tissue grafts is applied, however, it is associated with major drawbacks such as invasiveness, antibiotic resistance, and high costs.[4] Natural medicines are being developed for wound management to resolve costs, adverse effects, and antibiotic resistance. However, scientific evidence, formulation development, standardization, and safety assessment should be confirmed before they are used in conventional medicine.[5]

Staphylococcus epidermidis is usually a commensal inhabitant of healthy human skin and mucosa. However, it could be a common nosocomial pathogen in immunocompromised patients, neonates, and patients with medical equipment installed.[6,7] Recent studies reported that *S. epidermidis* has an important role in improving the wound healing process and potential to be developed as a wound healing promoting agent. Linehan *et al.*[8] reported that *S. epidermidis* can induce a highly physiological and pleiotropic form of adaptive immunity by the accumulation of CD8+ T lymphocytes cells that work in repairing tissues leading to improving wound healing process. In addition, lipoteichoic acid (LTA) produced by *S. epidermidis* inhibits both inflammatory cytokine release from keratinocytes and inflammation triggered by injury through a TLR2-dependent mechanism.[9] *Staphylococcus epidermidis* also produced lipopeptide 78 which activates β-catenin to inhibit skin inflammation.[10] In this study, a topical gel preparation containing *S. epidermidis* isolate extract was developed and evaluated its activity in acelarate wound healing in rats.

MATERIALS AND METHOD

Ethical clearance of research

This study has been approved by

the Research Ethics Committee, Faculty of Medicine and Health Sciences, Universitas Islam Negeri Maulana Malik Ibrahim, Malang (ref. no. 60/40/EC/KEPK-FKIK/11/2023)

Extraction preparation from *S. epidermidis* isolate

Two *S. epidermidis* strains were used to prepare the extract in this study i.e. the standard strain of *S. epidermidis* ATCC 12228 obtained from the collection of the Department of Microbiology, the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, and the commensal strains of *S.epidermidis* obtained from the collection of the Microbiology Laboratory, Universitas Muhammadiyah, East Kalimantan. Each *S. epidermidis* isolate was grown in 3% tryptic soy broth (TSB) at 37°C overnight. Followed after overnight incubation, the bacterial culture was diluted with fresh TSB (1 : 100) and then continued with culture for 16 hr. The bacterial culture medium was centrifuged at 10,000 × g for 30 min to remove the bacteria. The supernatant was filtered with 0.22 μm Stericup (Millipore, Shanghai). The sterile supernatant was set to pH 2 and then set at 4°C for overnight precipitation. The next day, the precipitate was collected with a centrifuge (10,000 × g) at 4°C for 30 min, and macerated with methanol overnight. The macerates obtained were then evaporated using a rotary evaporator to obtain a dry extract.

Preparation of gel formula

The formula of this gel is based on Maswadeh *et al.*[11] by using a standard gel base of sodium carboxymethylcellulose (Na-CMC) based on the following components: Na-CMC 5%, glycerin 10%, propyleneglycol 5% and aquadest ad 100 mL. In this study the gel preparations were prepared at extract concentrations of 100%, 50%, and 25%. Each gel preparation was made as much as 25 g for used 14 times application for 14 d of observation. A certain amount of extract was weighed and dissolved with the required volume of water and then added to 5% of its base. A predetermined amount of glycerol and propilenglikol were then added by continuous stirring at room temperature for 15 min using a mechanical stirrer. Each gel was stored separately in a cool, cool place overnight.

Physical quality test

Organoleptic test

Organoleptic tests covering the shape, color, and smell of the gel were performed visually.[12]

pH measurement test

The pH value check was carried out using a pH meter. The pH value of the preparation was set close to the pH of the skin between 4.5-6.5 or ideally the same, this aims to avoid irritation.[13]

Homogeneity test

The homogeneity of the gel was visually observed by applying the gel on the glass surface of the object. It was observed whether some coarse grains or parts were not mixed properly. If it was not found, it means homogeneous.[14]

Viscosity test

Observation of gel viscosity was carried out using a Brookfield viscometer. The standard viscosity value of a good gel preparation was in the range of 2000 – 4000 Cps.[15]

Spreadability test

A gel of 0.5 g was placed in the center of a round glass glass with a diameter of 15 cm and then covered with another

glass. Next, an additional load of 150 g was given and left for 1 min, and then measured the diameter of the gel spread.[16,17]

Stability test

The stability test that has been carried out using the cycling test method was 3 cycles. Each cycle was stored for 72 hr at 4°C and then transferred to the oven at 40°C for 72 hr. Every cycle was tested for organoleptic, pH, homogeneity, viscosity, and dispersion.

Animal study

The test animals that have been used in this study are male white rats of the Wistar strain (*Rattus norvegicus* L) as many as 24 adults. The mice had been given the adaptation for a week before testing began.

Wound healing activity test

The wound-healing activity the topical gel of *S. epidermidis* extract has been evaluated using the incision wound model method in rats.[18,19] Rats were randomized into 8 groups, negative control group, positive control group (Bioplacenton® gel), 3 gel groups of *S. epidermidis extract* strain ATCC 12228 (code: standard strain - SS) with concentration variations, and 3 gel groups of *S. epidermidis extract* commensal strain (code: commensal strain - CS) with concentration variations. The back of the test rat has been cleaned of hair and anesthetized which was then made an incision wound with a length of 1 cm and a depth of 2 mm using a scalpel. The gel was applied to the incision wound once every 24 hr which was first cleaned in the incision wound area with an alcohol swab. Observations on the wound healing process have been carried out for 11 d by measuring the length of the wound every day. Wound healing has been calculated using and using Morton's formula :

$$Wound\ Healing\ (\%) = \frac{W1 - Wx}{W1} x100\%$$

Descriptions :
Wound healing (%) : percentage of wound healing
W1 : wound length day 1
Wx : wound length on day x

Statistical analysis

Data were analyzed statistically using One way analysis of variant (ANOVA) followed by a post hoc test. A p-value of <0.05 was considered significant.

RESULTS

Physical quality of gel

Organoleptic

The organoleptic of gel preparation was observed based on shape, color, and odor (TABLE 1). The resulting gel gave it a viscous shape. The entire gel formula changed the appearance of clarity produced on day 14, where the appearance was from clear to cloudy. The smell that has been produced from the entire gel formula had no change (odorless).

pH of gel

The pH value that has been produced from the whole formula had the same pH (FIGURE 1). The three gel formulas that have been measured experienced an increase in acidity in the second observation (day 7) and third observation (day 14), but the increase that occurs was still by the pH standard of human skin, namely pH: 4.5 – 6.5.[20]

TABEL 1. Organoleptic observation of gel preparation of *S. epidermidis* extract

Formula	Observation	Observation result (d)		
		1	7	14
Negative control	Shape	V	V	V
	Colors	YC	YC	YCL
	Odors	OD	OD	OD
SS 100%	Shape	V	V	V
	Colors	YC	YC	YCL
	Odors	OD	OD	OD
SS 50%	Shape	V	V	V
	Colors	YC	YC	YCL
	Odors	OD	OD	OD
SS 25%	Shape	V	V	V
	Colors	YC	YC	YC
	Odors	OD	OD	OD
CS 100%	Shape	V	V	V
	Colors	LGC	LGC	LGCL
	Odors	OD	OD	OD
CS 50%	Shape	V	V	V
	Colors	WGC	WGC	WGCL
	Odors	OD	OD	OD
CS 25%	Shape	V	V	V
	Colors	WGC	WGC	WGCL
	Odors	OD	OD	OD

Note: V (viscous); YC (yellowish clear); LGC (light green clear); WGC (whitish green clear); YCL (yellowish cloudy); LGCL (light green cloudy); WGCL (whitish green cloudy); OD (odorless)

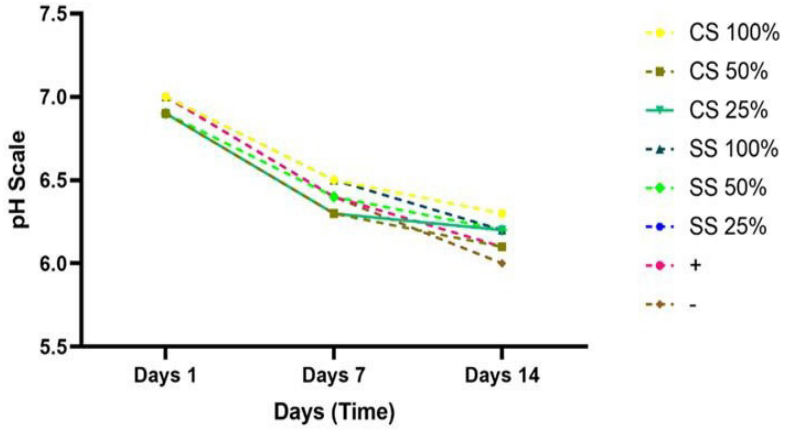

FIGURE 1. pH of the topical gel preparation of *S. epidermidis* extract

Spreadability of gel

The FIGURE 2 shows that on day 14 there had been a widening of the dispersion of each gel formula. The dispersion ability of the bioactive gel preparation of *S.epidermidis bacteria* extract was greater than that of negative controls.

Homogeneity of gel

The homogeneity of testing results have shown that the entire gel formula has good homogeneity. After three observations on day 1, day 7, and day 14, no visually change in homogeneity of the entire gel formula had been observed.

Viscosity of gel

The viscosity of each gel preparation test results of the entire gel formula have been performed which can be observed in FIGURE 3. The overall formula of the gel has increased viscosity value with each observation made. In the negative control, there is a difference in viscosity values that are different from the formula.

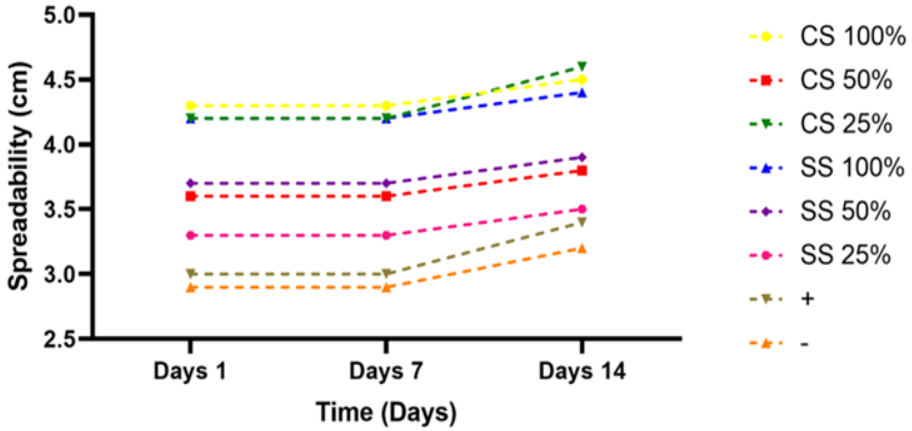

FIGURE 2. Spreadability of gel preparation of *S. epidermidis* extract

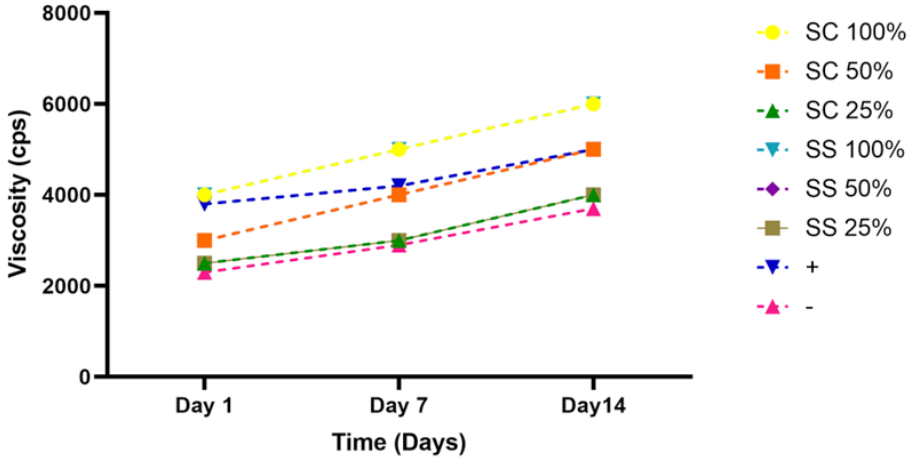

FIGURE 3. Viscosity of the topical gel of commensal *S. epidermidis* extract.

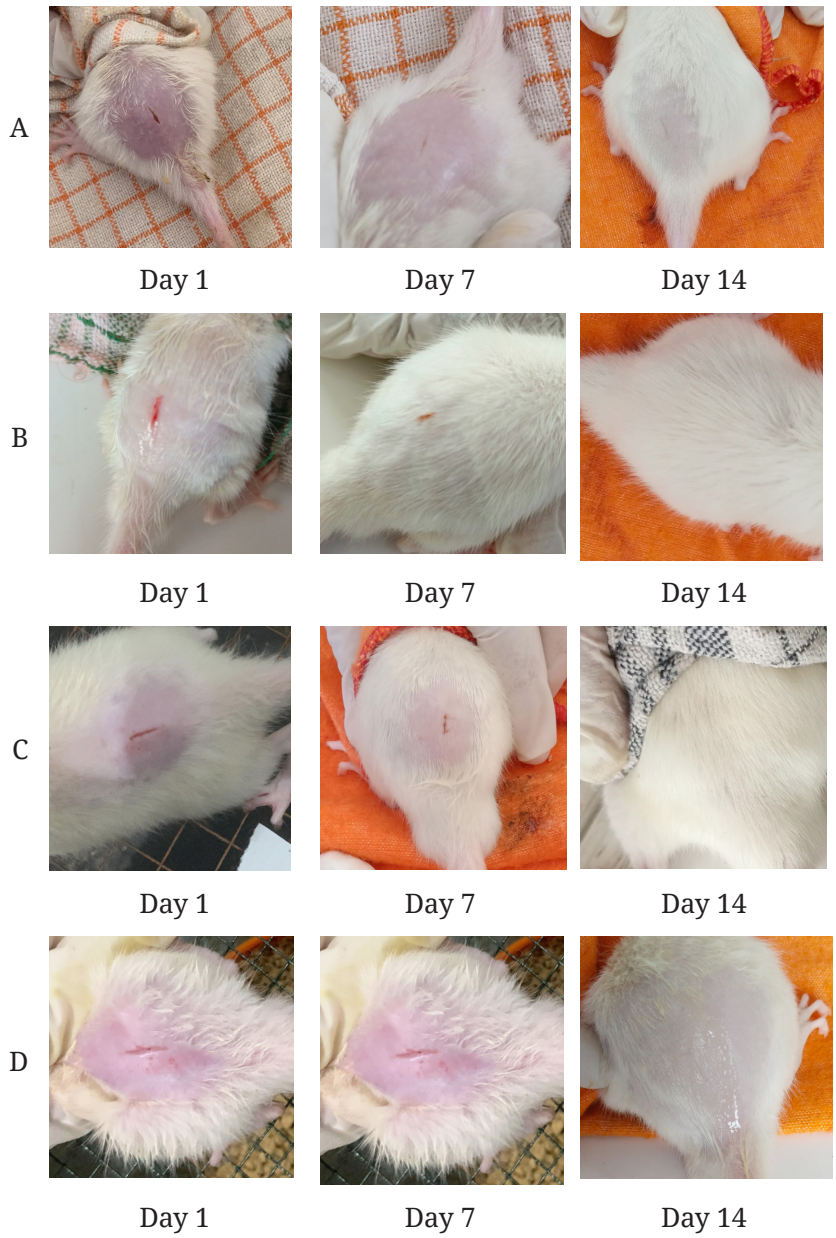

FIGURE 4. Macroscopic observation of wound healing process after topical gel application in all groups at day 1, 7, and 4. A) commensal *S. epidermidis* 100%; B) standard *S. epidermidis* 100%; C) negative control; and D) positive control.

Wound healing activity

Macroscopic wound healing process after topical application of the gel preparation of S. epidermidis extracts at dose of 100% on day 1, 7 and 14 (FIGURE 4). The results of the wound healing process observation after topically administration of the gel preparation of *S. epidermidis* extracts on incision wounds of rats are presented in FIGURE 5. Overall, a significant increase in wound healing after the administration of the gel preparation of *S. epidermidis* extracts both from commensal and standard bacteria compared to negative control was observed ($p < 0.01$ and $p < 0.0001$). However, there was no significant difference in accelerated wound healing for 25% CS treatment on day 11 and day 14 compared to negative controls ($p > 0.05$). Administration of the gel preparation less than 14 days has provided wound closure that looks the same as before incision wound treatment.

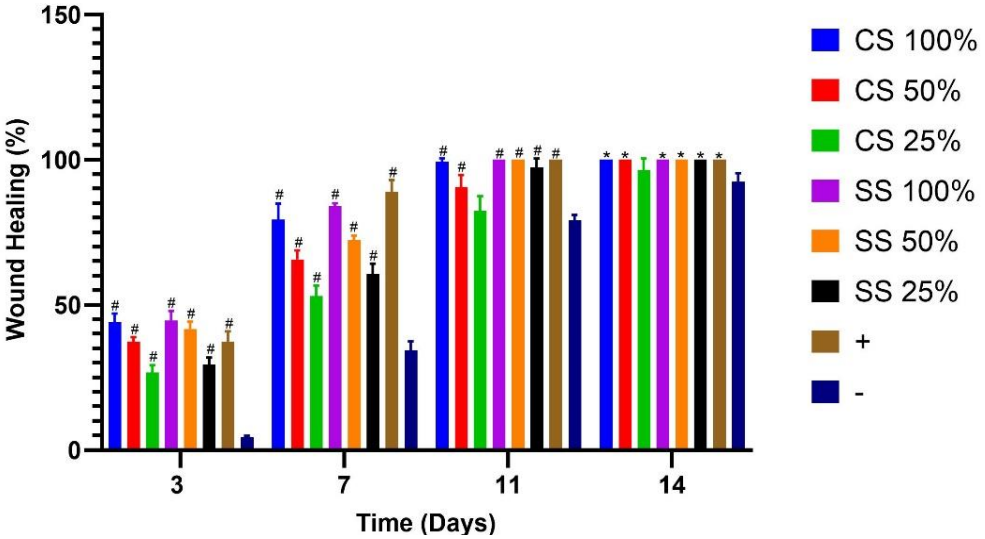

FIGURE 5. Wound healing activity of the topical gel preparation of S. epidermidis extract. CS= commensal S. epidermidis extract; SS= standar S. epidermidis extract. Data are expressed as mean ± SD for three rats in each group. *P < 0.01, #P < 0.0001 compared to the negative control group (-) on respective days. Statistical analysis that has been carried out using ANOVA followed by Bonferroni's post hoc test.

DISCUSSION

In this study, the topical gel preparation of *S.epidermidis* extract demonstrated good physical stability based on the observations of organoleptic properties, pH value, dispersion, homogeneity, and viscosity. No significant change in organoleptic properties during the storage was observed. The pH value did not also significantly change during storage and still met the requirement of a gel preparation.[20]

The freshness of a topical gel preparation is considered good if it has a dispersion value of 3-5 cm.[17] The good dispersion of a gel preparation is characterized by easily applied without excessive pressure. Therefore, it can increase the surface area of the gel preparation in contact with the skin and cause the active constituents well distributed at the site of application.[21]

The homogeneous properties of all of the topical gel preparations of *S. epidermidis* extract were still considered good quality during storage. The viscosity still meets the requirement of viscosity values of a gel preparation that is in the range of 2000 – 4000 Cps.[15] On day 7 and day 14, the viscosity values increased in all of the topical preparations in this study. The NaCMC properties as a base gel that can absorb water may cause an increase of the topical gel preparation.[13]

This study proved that topical administration of the gel preparation of *S. epidermidis* extracts can accelerate wound healing time in the incision wound rats model. The effect of *S. epidermidis* extracts on wound healing have been reported in previous studies. Linehan *et al.*[8] reported that *S. epidermidis*-induced CD8+ T cells promoted improved re-epithelization of the affected skin and accelerated wound repair. Furthermore, Leonel *et al.*[6] reported that *S. epidermidis* promote

wound healing via the accumulation of CD8+ T lymphocytes in the skin, and suppress cutaneous tumour formation via 6-N-hydroxyaminopurine secretion. Other compounds for wound healing secreted by *S. epidermidis* were also reported by some authors such as LTA and lipopeptide 78. The LTA was proven to inhibit both inflammatory cytokine release keratinocytes and inflammation triggered by injury through a TLR2-dependent mechanism.[9] Whereas, the lipopeptide 78 activates β-catenin to inhibit skin inflammation.[10]

CONCLUSION

This present study demonstrated the topical gel preparation containing *S. epidermidis* extracts can accelerate wound healing process compared with negative control. This results support further study of this preparation in the treatment and management of wounds.

ACKNOWLEDGMENT

This study was funded by the Directorate General of Higher Education, Research and Technology, Ministry of Education, Culture, Research and Technology of the Republic of Indonesia (Contract number: 187/E5/PG.02.00. PL/2023). The authors would like to thank the Indonesian Biofilm Research Collaboration Center BRIN-UGM-Unsoed-UMKT for suporting the bacterial isolate and some materials.

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