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Formulations and Antibacterial Activity of Shallot (*Allium cepa* L.) Peel Extract Patch against *Streptococcus pyogenes*

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Abstract

Background: *Bacterial pharyngitis is an inflammatory condition in the back of the throat caused by Streptococcus pyogenes. Patients are often prescribed antibiotics and antiinflammatories to alleviate pain and discomfort while reducing bacterial growth in the throat. However improper and prolonged use of antibacterial and antiinflammatory agents increases the risk of bacterial resistance and side effects. An often discarded Shallot (Allium cepa L.) peel rich in flavonoids with great antibacterial and anti-inflammatory properties is potentially used as an alternative treatment for bacterial pharyngitis.* ***Objective:*** *This study aimed to develop shallot peel extract as an antibacterial against Streptococcus pyogenes.* ***Methods:*** *Patch was formulated with variations in extract concentration of 5% (F1), 10% (F2), and 15% (F3) to observe their influence on weight uniformity, thickness, folding endurance, surface pH, moisture content, and antibacterial activity using disc diffusion.* ***Results:*** *All formulations produce slightly heavy and thicker but uniform patches (CV<5%), surface pH suitable for application in the skin (4.6-4.9), flexible and durable patches with high folding endurance (> 300 folds), good moisture content (<10%) and moderate to strong antibacterial activity (inhibition zone diameter ranging from 9 to 13.67 mm). Variations in extract concentration in the formula significantly influenced the thickness, weight, folding endurance, and also the antibacterial activity of the patches. Higher concentrations of extract produce thicker and heavier patches but stronger antibacterial activity against pharyngitis pathogens.* ***Conclusion:*** *Therefore, antibacterial patches containing up to 15% shallot peel extract are potentially used as an alternative treatment for pharyngitis.*

Keywords: *antibacterial, patch, pharyngitis, shallot peels, streptococcus pyogenes*

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INTRODUCTION

Bacterial pharyngitis is an inflammatory condition caused by a bacterial infection in the pharynx, nasopharynx, and tonsils. *Streptococcus pyogenes* or group A beta-haemolytic streptococcus is the predominant cause of bacterial pharyngitis in children and adults accounting for up to 30% of all cases (Tadesse et al., 2023). In Yogyakarta, pharyngitis remains among the top 10 infectious diseases with the highest incidence affecting 10,269 patients in 2023 (Badan Pusat Statistik Yogyakarta, 2023). Pharyngitis features persistent symptoms such as burning sensation, pain, itching, dryness, recurrent cough, and irritation that worsen during swallowing which affect the patient's daily life (Ding et al., 2020). Bacterial pharyngitis treatment management aims to alleviate pain and discomfort while reducing bacterial growth in the throat. Therefore, patients are often prescribed antibiotics and antiinflammatory/analgesic agents to shorten the course of the disease and improve symptoms. However, misidentification of the pathogenic bacteria and irrational use of antibiotics can cause bacterial resistance, leading to severe complications, especially in children (Cots et al., 2015). Moreover, long-term usage of oral anti-inflammatory and analgesic drugs potentially leads to cellular toxicity, allergic reactions, and side effects. This clarifies the need to find safer alternatives for pharyngitis therapy.

Recently, plant-derived herbal medication has gained substantial attention from researchers for its lower toxicity. In Indonesia, shallot *(Allium cepa* L.*)* plants have been used orally or topically as folk remedies to treat various diseases such as colds, fever, ulcers, stomachaches, and asthma (Henri & Hakim, 2020). Shallot is the most produced horticultural crop in Indonesia accounting for nearly 2 million tons annually (Badan Pusat Statistik, 2024). However, the bulbs and leaves are the most utilized part of shallot plants in the medicinal field. Whereas, shallot peels which are often discarded as waste in shallot cultivation are also proven as an abundant source of phytochemicals such as phenolics and flavonoids. Metabolomic profiles of shallot peels extracted by ultrasound-assisted extraction display rich bioactive such as flavonoids, stilbenes, terpenoids, phenolic acids, and sulphur compounds. These bioactives are responsible for their strong antioxidant activity and inhibition of several enzymes involved in metabolism, memory, and pigmentation (Moldovan et al., 2024). According to Fredotović et al. (2021), 100 g of shallot peel extract contains ± 600 mg of quercetin glucosides, ± 70 mg of quercetin aglycone, and ± 4 mg of anthocyanins. Shallot peel extract has proven to show a strong antibacterial activity against pharyngitis major pathogen, *Streptococcus pyogenes* with MIC of 500 µg/mL (Fredotović et al., 2021). A similar study also reported concentrations of 5%, 10%, and 15% of shallot peel extract had an average inhibition zone diameter of 0.683 cm, 0.830 cm, and 1.137 cm respectively (Wirdia et al. 2017). Shallot peel extract also has high antioxidant activity with an IC_{50} value of 86 μg/mL which contributes to its antiinflammatory activity. Juliadi & Agustini (2019), developed a cream formulation containing 0.32% shallot peel extract that can reduce up to 50% swelling in carrageenan-induced paw edema which shows that the extract has great anti-inflammatory activity. A compress made from shallot is also able to reduce children's body temperature during fever, highlighting the antipyretic ability of the plants via promoting vasoconstriction in response to the cold sensation of shallot exposure to the skin (Mardhiah, 2022). Therefore, shallot peel extract is potentially used as an alternative medicine for pharyngitis treatment.

Despite its medicinal properties, shallot peel extract has an extremely unpalatable bitter taste and strong sulphuric odor, which may decrease patient adherence, especially in pediatrics. A study reported that medicines with poor palatability lower the patient's adherence, whereas those with good palatability have a positive influence (Alkilani et al., 2023). Therefore in this study, shallot peel extract was formulated in the patch and intended to be applied on the skin preferably around the throat region. Skin application via transdermal routes has several advantages including bypassing first-pass metabolism, preventing therapeutic discomfort, and increasing patient compliance while ensuring precise dosing (Wong et al., 2023). In this study, three patch formulations containing different concentrations of shallot peel extract were developed and evaluated for their influence on the physical characteristics and antibacterial activity of the patchs.

MATERIALS AND METHODS

Materials

Shallot peels were collected from the local market (Bantul, Yogyakarta), 70% ethanol (Brataco), excipients used for patch formulation were distilled water, hydroxypropyl methylcellulose 4000 (HPMC) (Sigma), propylene glycol (Bratachem), methylparaben (Bratachem), menthol (Bratachem), and phosphate buffer (Merck). Reagents and bacteria used for antibacterial activity assay were H_2SO_4 (Merck), *Streptococcus pyogenes* ATCC 19615 (Yogyakarta

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Health and Calibration Laboratory Center), Mueller Hinton Agar (MHA) (Merck), Nutrient Broth (Merck), $BaCl_2$ 1% (Merck), and amoxicillin Disc Oxoid CT0061B (Oxoid).

Equipments

Rotary evaporator (Heidolph Hei-Vap Platinum 1), oven (Binder), pH meter (Ohaous Aquasearcher AB33M1-F), digital caliper (Mitutoyo 500-196-30) and biological safety cabinet (Monmouth Guardian Class II).

Methods

Preparation of shallot peels extract

The freshly collected shallot peels were sorted and washed with water to remove impurities. Then, the peels were dried at a temperature of 60^{o}C. Subsequently, dry sorting was carried out and dried shallot peels were pulverized with a blender (Setiani et al., 2017). A total of 100 g powdered shallot peels was macerated using 1 L of 70% ethanol with gentle stirring every 1 hour for 10 hours, and the mixture was left at room temperature away from the light for one day. The mixture was then strained and the solid residue was remacerated twice using 1 L of 70% ethanol. All the filtrates were combined and concentrated using a rotary evaporator at 60°C and 80 rpm (Setiani et al., 2017). The concentrated extract was then further tested to determine the presence of flavonoids qualitatively. A shallot peel extract sample (2 mL) was taken in test tubes and was added 5 drops of concentrated H_2SO_4. A red color formation in the test tube indicates the presence of flavonoid in the extract (Atika, 2021).

Preparation of shallot peels extract containing patch

Three patches with varied concentrations of shallot peel extract were prepared using the solvent evaporation method with a formula modified from Wardani & Saryanti (2021) as listed in Table 1. Initially, HPMC 4000 was dispersed in distilled water and let swell properly until a clear and viscous solution was formed. The shallot peel extract was solubilized in 15 mL of water: ethanol 70% mixture (1:2). The dissolved extract was then mixed with HPMC 4000 and stirred until homogeneous. Futhermore, methylparaben was dissolved in a separate container with propylene glycol and added to the HPMC 4000 and extract mixture. Lastly, menthol and the remaining water and ethanol 70% were added to the mixture and stirred until homogeneous. The resulting mixture was allowed to stand for 20 hours to remove bubbles and then poured into square mold of 3.5x3.5 cm per patch. The mixture was then dried in the oven at 50°C until a dried patch was formed. The patches were placed in the desiccator for 20 hours and peeled from the mold. Patches were stored in a closed container (Wardani & Saryanti, 2021).

Physical characterization of shallot peels extract patches

Physical appearance evaluation

Physical appearance of patches were observed for its color, transparency, roughness of the surface texture, and odor (Mariadi & Bernardi, 2023).

Weight uniformity test

Three patches from each formula were weighed in an analytical balance. Then the average weight, standard deviation (SD), and % coefficient of variation (CV) of each formula were calculated. A patch was considered to have a uniform weight if the CV value ≤ 5% (Setiawan & Setiawan, 2024).

Patch thickness measurements

Patch's thickness for each formula was measured using a digital caliper having an accuracy of 0.01 mm at three different points. The thickness of each patch was determined as the average of measurements on three points (Surpiadi & Sherlyke, 2023).

Folding endurance

The patch was folded repeatedly in the same position until it broke. A patch was considered to have good folding endurance if it resists>200 folds without breaking (Setiawan & Setiawan, 2024).

Table 1. Formula of shallot peel extract patches

Composition	Function of Material	Concentration (%)		
		F1	F2	F3
Shallot peels extract	Active ingredient	5	10	15
HPMC 4000	Polimer	7	7	7
Propylene glycol	Plasticizer	10	10	10
Methylparaben	Preservative	0,3	0,3	0,3
Menthol	Perfume	3	3	3
Ethanol 70%	Solvent	40	40	40
Aquadest	Solvent	to 100 mL	to 100 mL	to 100 mL

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pH measurements

To measure the surface pH of the patch, the patch needs to be soaked in 10 mL phosphate buffer pH 6.8. After 2 hours, a pH meter was used to measure the surface pH. Reading on the pH meter was determined as surface pH. The measurement was replicated three times for each formula (Maddeppungeng et al., 2023).

Moisture content analysis

The initial weight (w1) of each patch was independently measured using an analytical balance. Then patch was transferred into an oven at 40ºC for 24 hours and dried until constant final weight (w2) was achieved. Moisture content was calculated as a percentage of [w1-w2]/w2. Three readings were recorded and the average was calculated as a percentage of moisture content (Hikma et al., 2024).

Antibacterial activity of shallot peel extract patch

A disk diffusion method was chosen to determine the antibacterial activity of the three patches. Bacterial suspensions were adjusted to a bacterial cell density of 1.0×10^8 CFU/mL (or 0.5 McFarland turbidity units). A sterile swab soaked in the bacterial suspension was used to inoculate the entire surface of blood agar medium. Patches from each formula were cut into 6 mm in diameter using a paper cutter and placed with slight pressed onto the agar. A 6 mm in diameter placebo patch was used as the negative control and amoxicillin Disc Oxoid 25 µg as a positive control. The petri dish was placed in incubator at 37°C for 24 hours, then the inhibition zone was observed by measuring the clear zone diameter using a caliper (Sfeir et al., 2013). The assay was done in triplicates.

Data analysis

The results of the physical evaluation and antibacterial activity of the patch were analyzed using Statistical Products and Services Solutions (SPSS) software. The testing process started with determining normality using Kolmogorov-Smirnov method and assessing homogeneity through the Levene test. The analysis continued using One-way Analysis of Variance (ANOVA) method to observe differences between formulas since the data were normal and uniform ($p>0.05$). The data were reported to be significant when p-value <0.05.

Yield and flavonoid identification of shallot peels extraction

The maceration of 100-gram shallot peels with 70% ethanol as a solvent yielded 10% w/w concentrated extract. The maceration method used in this study was more effective compared to previous studies with the same solvent which obtained a lower yield of 5.92% w/w due to the short maceration time (1 day). In a study conducted by (Handoyo, 2020) it was stated that the length of extraction time used was one of the factors that influenced the yield value obtained. In addition, the type of solvent polarity, the ratio or concentration of the solvent used, and the particle size of the simplicia could also be factors that influenced the yield value. As seen in Figure 1, shallot peel extract obtained in this study tested positive for flavonoid as signified by a red color change after the sample was treated with concentrated H_2SO_4. The red color. Indicates a complexation between sulfuric acid and flavonoids presence in the extract via an oxidation-reduction reaction (Atika, 2021).

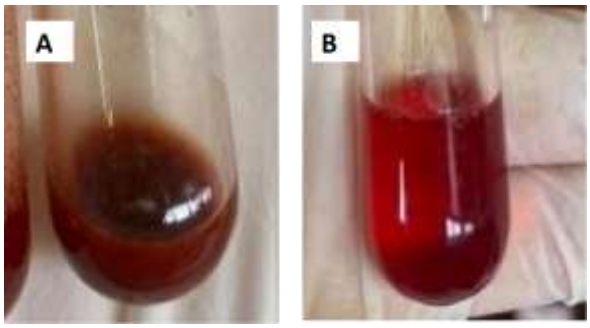

Figure 1. Flavonoid identification in shallot peels extract. (A) shallot peels extract and (B) shallot peels extract and concentrated H_2SO_4 which resulted in a bright red color solution

RESULTS AND DISCUSSION

Results

Extracts yield

Based on the data in Table 1, the 96% ethanol extract of *Ipomoea pes-caprae* leaves yields 20,63%, while the stem extract only reaches 10,09%.This yield is calculated from the ratio of the weight of the extract to the weight of the initial dry plant.The leaf extract is produced from 300 g of dry plant into 61.91 g of extract, while the stem with an initial weight of 213 g of dry plant yields 21.491 g of extract.

Physical characteristics evaluation of shallot peels extract patch

Physical appearance of all patches

The variation of extract concentration (5-15%) in the patch formulation using HPMC as matrix polymer resulted in patches with different colors and transparency. Higher extract concentration intensifies the reddish-brown color and lowers the transparency of the patch. As seen in Figure 2 and Table 2, all patches have a smooth surface, and the addition of menthol to the formulation successfully masks the sulphuric smell

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of shallot peel extract. Thus increasing patient acceptability.

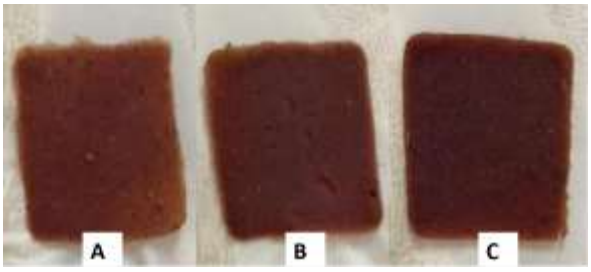

Figure 2. Physical appearance of shallot peel extract patches (A) F1 with 5% extract, (B) F2 with 10% extract, (C) F3 with 15% extract

Weight uniformity and thickness of shallot peel extract patch

A weight uniformity test was done to confirm the consistency of the patch preparation. A low % coefficient of variation (CV) value indicates that each patch produced with the same formula and production process has uniform weight (Maddeppungeng et al., 2023; Andriani et al., 2024). The data presented in Table 3 showed that the CV value of all formulations ranging from 2.29-2.8% meet the weight uniformity requirements. According to Wardani & Saryanti (2021), patch weight with a CV value of ≤ 5% is considered uniform. The weight uniformity of the patch correlates with the drug content uniformity, consistent weight ensures that each patch delivers the intended dose, preventing under or over-dosing, which could compromise treatment efficacy or safety (Lall & Rathore, 2024). As observed in Table 3, each formula resulted in a patch with a different weight, and heavier patches were obtained in the formula using higher concentrations of the extract. Statistical analysis was used to determine the effect of extract concentrations on the weight of patches. The result found that all three formulations have significantly different weights ($p < 0.05$), which indicates that variation in extract concentration in the formula affects the weight of the resulting patch. A similar pattern was observed in the thickness measurements of the patch. Formula made of higher extract concentration of extract resulted in thicker patches, with the formula with the highest extract concentration (F3) showing maximum thickness. The average thickness of all formulations was significantly different ($p < 0.05$), which indicated that extract concentration influenced patch thickness. However, all formulas form thick patches with a thickness larger than 1 mm which is slightly larger than the requirements (≤ 1 mm in thickness) (Fuziyanti et al. 2022). Thin and light patches were preferred by the patient for their wearability and comfort leading to patient compliance. Thicker patches tend to detach more easily from the skin. However, this problem can be resolved by adding an adhesive backing membrane to the matrix patch. The thickness of the patches also affects the drug release rate from the patch matrix, which in this case will influence the patch performance (Gunarti et al., 2024). Thinner patches offer a faster drug release rate due to shorter diffusion pathways for drug molecules to penetrate through the skin (Lall & Rathore, 2024). The thickness of the patch matrix produced by the solvent casting method is not only influenced by matrix composition but also by the mold dimension and volume of the matrix poured onto each mold. In this study, the use of small mold of 3.5x3.5 cm per patch for 30 mL of matrix mass resulted in thicker patches.

Table 2. The physical appearance of shallot peel extract patches

Test Parameters	F1	F2	F3
Color	Light reddish brown	Reddish brown	Dark reddish brown
Smell	Mint	Mint	Mint
Texture	Smooth	Smooth	Smooth
Transparency	Low	No	No

Table 3. Physical properties test results of shallot peel extract patch

Test Parameters	Formula		
	F1	F2	F3
Weight (gram)	0.784±0.02[a]	0.855±0.02[b]	0.972±0.03[c]
Weight uniformity/CV (%)	2.29	2.80	2.67
Thickness (mm)	1.10±0.03[a]	1.15±0.01[b]	1.20±0.04[c]
Folding endurance (folds)	>750	586.33±6.66[b]	367±19.86[c]
Surface pH	4.80±0.04	4.78±0.06	4.90±0.00
Moisture content (%)	8.65±0.68	7.65±0.95	8.39±1.72

*a, b,c (significant difference $p<0.05$)

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Folding endurance

Folding endurance is a parameter to evaluate the durability and flexibility of the patch. Good quality patches should maintain their integrity with general skin folding when applied or during storage (Mo et al., 2022). The flexibility of the patches is greatly affected by the concentration of plasticizer used in the formula. However, based on the result tabulated in Table 3. extract concentration also influenced the elasticity of the patch, with lower extract concentration generating more tear resistance patches due to its thinness. Higher extract concentrations produce a thicker patch which is difficult to fold and tear easily. The folding resistance of the three patch formulations exceeds the requirements of ≥ 200 times recommended by Nisa et al. (2016). This is influenced by the presence of the appropriate amount of plasticizer (propylene glycol) in the formula to regulate the patch flexibility. The plasticizer will bind to the polymer matrix and increase the volume of the cavity between the polymer chains, which reduces the crystallinity of the polymer chains. Enhanced polymer movement can increase the flexibility and elasticity of the patch. However, if the excess amount of extract was introduced, it disrupted the crosslinking between plasticizer and polymer, creating more cavities between polymer chains causing it to break more easily (Maddeppungeng et al., 2023).

Surface pH measurements

Safety is an important quality in developing a dosage form. Patch that is intended to be used on the skin must not irritate the skin upon usage. A Surface pH of the patch should be inside 4.5-6.5 to be tolerated by the skin (Gunarti et al., 2024). pH of the patch is also essential to maintain the stability of the active ingredients. Quercetin a compound belonging to the flavonoid family is the major bioactive in shallot peel extract. Quercetin has molecular stability in the pH range of 1-6 and undergoes autooxidation in pH 7-10 (Momić et al., 2007). Data tabulated in Table 3 showed that the surface pH of all three patch formulas was around 4.7-4.9 which was acceptable for use while also maintaining the stability of the active ingredients. Although the formulas made from different extract concentrations resulted in a patch with varied surface pH, the effect was observed to be not significant ($p>0.05$).

Moisture content analysis

Moisture content can affect the microbial stability and physical integrity of the patch during storage (Andriani et al., 2024). High moisture content patches are prone to microbial contamination which increases skin irritation and compromises the patient's health. High moisture content can also reduce patch adhesion to the skin, leading to early detachment of the patch from the skin (Lall & Rathore, 2024). However, completely dried patches with low moisture content create brittleness, therefore a good quality patch is required to have moisture content in the range of 1-10% % (Fuziyanti et al., 2022). The results in Table 3 showed that all formulations produced patches with good moisture content (≤10%) which ensured the patch stability and tolerability to the skin during application. Although the extract concentration did not significantly affect the moisture content of the patch, the hygroscopic sugar-derivate bioactive that is present in the shallot peels extract works with propylene glycol as a humectant and plasticizer in the formula to help retain the moisture inside the patch (Nurhamidah & Nurrochman, 2022).

Antibacterial activity assay

In the disc diffusion method, the inhibition zone is often measured to evaluate the antibacterial effect of the material, and a larger zone area means stronger antibacterial activity. Table 4 and Figure 3 showed that shallot peel extract formulated in the patch still had great antibacterial activity against *Streptococcus pyogenes* with inhibition zones ranging from 9-11.67 mm, and that the excipients in the patch did not influence the antibacterial activity of the extract (the placebo patch had zero inhibition zone). Although the inhibition zone were smaller than the standard antibiotic, the result still indicated promosing potential of shallot peel extract as a natural antibacterial agent. The inhibition zone diameter increased as the concentration of the shallot peel extract increased ($p<0.05$). The result in this study was correlated to the study conducted by Wirdia et al. (2017), that the inhibition zone of 5-15% extract was 6.83 mm, 8.30 mm, and 11.37 mm respectively, showing that increasing extract concentration will increase the inhibition zone. According to Safitri et al. (2017), the antibacterial activity of a compound based on the inhibition zone is divided into 4 categories, very strong (>20 mm), strong (10-20 mm), moderate (5-10 mm), and weak (<5 mm). Therefore, from the result presented in Table 3, we can summarize that F1 with the lowest extract concentration has moderate antibacterial activity. Meanwhile, F2 and F3 with higher extract concentrations have strong antibacterial activity though slightly lower than the positive control (amoxicillin disc 25 µg).

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Table 4. Antibacterial activity test results of shallot peel extract patch

Replication	Inhibition Zone Diameter (mm)				
	Concentration of Shallot Skin Extract			Amoxicilin Disc 25 µg	Placebo Patch
	5%	10%	15%		
1	9	12	13	30	0
2	9	11	14	31	0
3	9	12	14	30	0
Average	9.00	11.67	13.67	30.33	0
SD	0	0.58	0.58	0.58	-

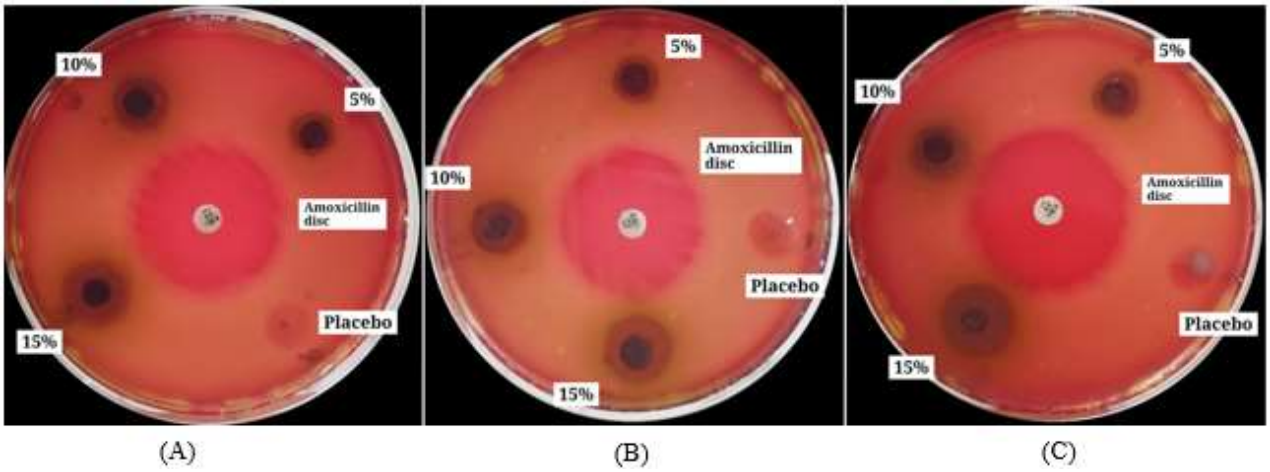

Figure 3. Inhibition zone of shallot peels extract against agar containing *Streptococcus pyogenes* culture , replication 1(A), replication 2(B), replication 3(C)

CONCLUSION

Good physical properties and antibacterial activity of the patch formulation intended to be used in the skin for pharyngitis treatment are important to ensure the safety and effectiveness of the treatment, along with increasing patient acceptability and compliance. In this study, all formulations produced slightly heavy and thicker but uniform patches, surface pH suitable for application in the skin, flexible and durable patches with high folding endurance, and good moisture content which ensure microbial stability and physical integrity during storage. Variations in shallot peel extract concentration in the formula significantly influenced the thickness, weight, folding endurance, and also the antibacterial activity of the patches. Higher concentrations of the extract produced thicker and heavier patches but stronger antibacterial activity (the average inhibition zone is 13.67 mm) against pharyngitis pathogen. Therefore, antibacterial patches containing up to 15% shallot peel extract were potentially used as an alternative treatment for pharyngitis. However, more assays such as in vitro release and permeation studies are needed to confirm that the matrix patch is capable of delivering the active ingredients through the skin. Skin irritation and hedonic tests with panelists are also needed to ensure the safety and comfortability of the antibacterial patch made of shallot peel.

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AUTHOR CONTRIBUTIONS

Conceptualization, S.N.A., L.B.S., C.A.E.; Methodology, L.B.S., C.A.E.; Software, N.A.Z.; Validation, S.N.N., D.A.A., C.A.E.; Formal Analysis, K.S.M., N.A.Z.; Investigation, D.A.A., C.A.E.; Resources, S.N.A., L.B.S., K.S.M., N.A.Z., S.N.N.; Data Curation, K.S.M., N.A.Z., D.A.A., C.A.E.; Writing - Original Draft, S.N.A., L.B.S., K.S.M., N.A.Z., S.N.N., D.A.A., C.A.E.; Writing - Review & Editing,

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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