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Antibacterial Activity Peel-off Mask Ethanol Extract of Pomegranate Peel (*Punica granatum* L.) against *Staphylococcus epidermidis* and *Staphylococcus aureus*

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ABSTRACT

Background: Pomegranate peel (*Punica granatum* L.) is a part of the pomegranate which is used as a traditional medicine for acne. The ethanol extract of pomegranate peel contains alkaloid, phenolic, flavonoid, and saponin compounds which have potential as antibacterial agents.

Aim: This study aims to make peel-off masks of the ethanol extract of pomegranate peels and to determine the antibacterial activity against acne-causing bacteria *Staphylococcus epidermidis* and *Staphylococcus aureus*.

Method: The maceration method was used to obtain pomegranate peel extract with 96% ethanol solvent. Phytochemical screening is used to determine the content of the extract. Preliminary test of antibacterial activity was carried out using the disk diffusion method to determine the MIC value. The extract then made into a peel-off mask preparation based on HPMC gel and PVA as gelling agent then evaluated physically and tested for its antibacterial activity by agar well diffusion.

Result: Pomegranate peel extracted contains alkaloids, flavonoids, saponins, and phenolics which show antibacterial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus* with a MIC value of 1.9 mg/ml. The physical properties of peel-off masks showed that formula F(1) and F(2) met the requirements with extract concentrations 1.56% and 3.12%. The antibacterial activity test of the peel-off mask of the ethanol extract of pomegranate peel had an inhibition zone of 9-15mm in the medium to strong category.

Conclusion: Based on physical evaluation and antibacterial activity test, F(2) was the most optimum formula with inhibition against *Staphylococcus epidermidis* and *Staphylococcus aureus* of 13 mm and 14.5 mm, respectively.

Keywords: Pomegranate peel, antibacterial, *Staphylococcus epidermidis, Staphylococcus aureus*, peel-off mask

BACKGROUND

Acne is a skin disease that is often found in Indonesian society (Sahala et al., 2016). *Acne vulgaris* is a chronic inflammatory disease of the pilosebaceous follicles which is characterized by the presence of comedones, papules, nodules, and pustules (Amalia & Sulistiyowati, 2019). Indonesian Cosmetic Dermatology reports that the percentage of people suffering from acne is increasing every year, 60% in 2006, 80% in 2007 and 90% in 2009. Treatment to treat acne both physically and chemically requires a relatively high cost because it must be done repeatedly by professionals. Physical treatments include facial and laser therapy, then chemical treatments such as the use of pharmaceutical preparations containing antibiotics. The topical use of antibiotis can

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cause side effects including irritation and allergies. In addition, long-term use of antibiotics can cause resistance and trigger hypersensitivity reactions (Sibero et al., 2019).

The pomegranate plant (*Punica granatum* L.) has been used naturally to treat sore throats, coughs, urinary tract infections, digestive disorders, skin disorders, arthritis, and to treat tapeworms (Kumari et al., 2012). Even though it is believed to have many health benefits, processed pomegranate peels are still quite rare in society. Several phytochemical compounds from pomegranate peels are known to inhibit the growth of pathogenic bacteria. Previous research stated that pomegranate peel contains bioactive compounds that have potential as antibacterial in the form of alkaloids, phenolics, flavonoids, ellagitannins, and proanthocyanidins (Benslimane et al., 2023; Prihantoro et al., 2006). Based on the research results of Nozohour et al., (2018) pomegranate peel extract was able to inhibit the growth of *Staphylococcus aureus* bacteria, with MIC and MBC values of 25 mg/ml and 50 mg/ml, respectively. This is in line with research conducted by Benslimane et al., (2023) which showed that pomegranate peel extract had antibacterial activity against *Enterococcus faecalis, Staphylococcus epidermidis, Klebsiella oxytoca*, *Enterobacter bugandensis*.

A peel-off face mask is a type of face mask that can be easily removed like an elastic membrane, so it is considered more practical because it makes it easier for users to clean products from facial skin (Fauziah et al., 2020). At the time of removing the peel-off mask from the face, dead skin cells and dirt/sebum on the face can also be removed to reduce the potential for pore blockages which can cause acne (Velasco et al., 2014). In addition, the use of gel-based peel-off facial masks is also beneficial in hydrating the skin because most of its composition is water (Grace et al., 2015). Therefore, this study aims to look at the antibacterial activity of peel off mask preparations of pomegranate peel extract (*Punica granatum* L.) extracted with 96% ethanol against *Staphylococcus epidermidis* and *Staphylococcus aureus*. This is intended to optimize the utilization of pomegranate peels into a useful and valuable product.

METHODS

Extraction

Fresh pomegranate fruits were purchased from a local market. The peels of pomegranate were removed manually and dried using an oven at 50°C, and ground into powder by a blender. To prepare the extract, 668 g of each powder was soaked in 96% ethanol solution (1:5 ratio) in a closed container and was shaken for 24 hours at dark room. The extracts were filtered through Whatman No. 41 filter paper and concentrated under vacuum at 50°C using a rotary machine, and the obtained extract was stored at 8°C and later used.

Phytochemical Screening

The Phytochemical test is an initial testing method that provides an overview of the class of compounds in plants as indicated by the colour testing reaction using a colour reagent.

a) Alkaloid test. The extract was dissolved using 2N HCl and divided into four tubes. The first tube was added Dragendorff, the second tube was added by Mayer, the third tube was added by Wagner, and the fourth tube was used as a control.

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b) Flavonoid test. 2 mL of the sample is dissolved in 2 mL of water, added a little Mg powder, 1 drop of concentrated HCl, and 5 mL amyl alcohol. The yellow or orange colour formation indicates the presence of flavonoid compounds.
c) Saponin test. Put 0.5 gram extract in a test tube and give it 10 ml of warm distilled water. Shaken for 1 minute.
d) Phenolic test. 0.5 gram extract was given 1 mL of 10% $FeCl_3$. Positive result is marked with a change in colour to dark blue, blue black or greenish black.

Isolation and identification of Bacteria

The assayed microorganisms used in this study were as follows: Local clinical isolates *S. epidermidis* and *S. aureus* obtained from microbiological laboratories of health centre of Rumah Sakit Nasional Diponegoro. Strains were identified using gram staining profiles and bacterial structures.

Assay for Antibacterial Activity of Pomegranate extract

The antibacterial activities of extracts were evaluated using disk diffusion agar method. The minimum inhibitory concentration (MIC) was determined. Bacterial suspensions equivalent to a 0.5 McFarland turbidity were prepared in sterile normal saline solution from clinical and reference isolates. A sterile swab dipped into the inoculum tube containing bacterial suspensions and then was cultured on the Nutrient agar. Sterile filter paper disc (6 mm in diameter) was impregnated with pomegranate peels extracts (30µL) for 10–15 minutes and allowed to dry completely for 20–25 minutes, then evenly placed on the surface of previously inoculated cultures. Clindamycin antibiotic discs were positive control and DMSO was negative control for comparison of inhibition zone with sample. Plates were incubated at 37°C for 24 hours, until visible growth of bacteria was evident in control plates. Clearly visible inhibition zones around discs were measured in 2 directions and averaged. The antibacterial activity was expressed according to the diameter of inhibition zone produced by extract against test bacteria.

Formulation of Peel-Off Gel Mask Pomegranate Peel Extract

The peel-off gel masks of Pomegranate peel extract formulation consist of Polyvinyl Alcohol (PVA), Hydroxypropyl Methylcellulose (HPMC), Glycerine, Nipagin, Nipasol, Alcohol, and Water. PVA is a good film forming and water-soluble PVA concentration of 10-16%. HPMC as a gelling agent (also used to thicken and provide texture through gel formation). Nipagin and Nipasol as a preservative, and the recommended concentration for nipagin are 0.02-0.3%, and for nipasol are 0.01-0.6%. Glycerine as a softener, Oleum citric as a fragrance and Alcohol 96% as a solvent for nipagin and nipasol.

Table 1. Formulation of Peel-Off gel mask Pomegranate peel extract

Materials	Concentration (% w/w)			
	F0	F1	F2	F3
Extract	-	1,56%	3,12%	6,25%
Polyvinyl alcohol (PVA)	13%	13%	13%	13%
HPMC	3%	3%	3%	3%
Glycerine	10%	10%	10%	10%
Methyl paraben	0,18%	0,18%	0,18%	0,18%

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Materials	Concentration (% w/w)			
	F0	F1	F2	F3
Propyl paraben	0,02%	0,02%	0,02%	0,02%
Oleum citric	qs	qs	qs	qs
Aquadest	Ad 100%	Ad 100%	Ad 100%	Ad 100%

Prepare a water bath with a temperature of 80°C. Add PVA which has been added with aquadest into a water bath, stirring until homogeneous forms a gel base. In another container, HPMC is added with aquadest and stirred until it swelled. Put HPMC that has blown into the PVA gel base and stir until homogeneous. Dissolve nipagin and nipasol with 96% alcohol. Add glycerine, solution nipagin nipasol, and oleum citric stir until homogeneous. Add extract and distilled water until it reaches 30 g of peel-off gel mask.

The Quality Analysis of The Peel-Off gel Mask Formulation

The quality test of mask formulation includes the organoleptic test, pH, Homogeneity, Spread ability, and Dry time. Organoleptic tests were carried out by looking at the gel peel-off mask preparation's colour, shape, and smell. Homogeneity test 1 gram of the gel sample is smeared on the glass slide to form a thin layer. Covered with a prep glass. Homogeneous if there are no coarse grains and even texture. The pH test was carried out using a pH meter. The pH meter that has been calibrated is then dipped into the preparation which has been dissolved with distilled water up to the mark, then the pH value of the peel-off mask preparation will be read.

Spread ability test by taking a 0,5 gram of gel is placed in the middle of a round glass scale, covered with another glass then left for 1 minute. Then given a load of 50 gram, 100-gram, 150 gram and 200 gram. Let stand for 1 minute each load. The spread ability test determines the distribution diameter of the peel-off gel mask preparation when applied to the skin. The adhesion test was carried out by placing 0.25 gram of the preparation on two glasses in the test equipment, then given a load of 1 kg for 5 minutes. After that, an 80-gram load was placed on the test equipment, and the time of releasing the two glass objects was recorded. The drying test was carried out by weighing 1 gram of gel applied to the back of the hand with an area of 3x3 cm and calculating the time required for the gelled stock to dry.

Assay for Antibacterial Activity of The Peel-Off gel Mask

The antibacterial activities of extracts were evaluated using the well diffusion agar method. Bacterial suspensions equivalent to a 0.5 McFarland turbidity were prepared in sterile normal saline solution from clinical and reference isolates. A sterile swab dipped into the inoculum tube containing bacterial suspensions and then was cultured on the Nutrient agar. The wells were made using a cork borer (6 mm in diameter). Put 0.1 gram of peel off mask into the well. Clindamycin gel was positive control and F0 gel was negative control for comparison of inhibition zone with sample. Plates were incubated at 37°C for 24 hours, until visible growth of bacteria was evident in control plates. Clearly visible inhibition zones around discs were measured in 2 directions and averaged. The antibacterial activity was expressed according to the diameter of the inhibition zone produced by extract against test bacteria.

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RESULT AND DISCUSSION

Extraction

The maceration method is used because it is relatively easy to do using simple equipment and relatively low cost. The maceration method can also prevent damage to the active substance at too high a temperature (Marjoni, 2016). The immersion process of simplicia causes damage to the cell walls and membranes caused by the difference in pressure between inside and outside the cell, so that secondary metabolites in the cytoplasm can dissolve in organic solvents (Wendersteyt et al., 2021). The 96% ethanol is used as a solvent because it is universal so it can dissolve polar, semi-polar and non-polar compounds, is easily available, non-toxic, and the temperature required for thickening is not too high. This is supported by research conducted by Benslimane et al., (2023) which stated that the ethanol extract of pomegranate peels had higher levels of total flavonoids and total phenols than methanol and acetone extracts. The results of the ethanol extract of pomegranate peel can be seen in Figure 1.

Figure 1. Pomegranate peel extract

The liquid extract resulting from the maceration process was then thickened using a rotary evaporator at 50°C. The extraction result of pomegranate peel is in the form of a viscous liquid with a purplish colour and a distinctive smell of pomegranate skin with a viscous extract weight of 235.92 g with an extract yield calculation of 35%. Yield determination is carried out to determine the approximate amount of simplicia needed to manufacture a certain amount of viscous extract.

Phytochemical Screening

Phytochemical screening are carried out qualitatively using reagents to determine the chemical compound content in the extract. The phytochemical screening of the extract can be seen in Table 2.

Table 2. Phytochemical Screening of Pomegranate Peel extracted

Test	Reagent	Result	
		Parameters	Result
Alkaloid	HCl + Dragendorff	Orange precipitate	(+)
	HCl + Wagner	Brown precipitate	(+)
	HCl + Mayer	White precipitate	(+)
Flavonoid	Mg + HCl + amyl alcohol	Yellow colour in the amyl alcohol layer	(+)
Saponin	Warm aquadest	Foam 1-10 cm	(+)
Phenolic	$FeCl_3$ 5%	Deep Green or Blue colour	(+)

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Alkaloid

In the alkaloid test, HCl 2N is used to withdraw the alkaloid compounds in the extract by forming alkaloid salts. This is because the alkaloids are alkaline, so when an acid is added, an alkaloid salt will form (Harborne, 1987). A positive alkaloid result is indicated by the presence of potassium alkaloid precipitate with dragendorff reagent containing potassium tetraiodo bismutat which will form an orange precipitate. Mayer's reagent containing potassium tetraiodomercurate (II) will form a white precipitate and Wagner's reagent containing potassium iodide will produce a brown precipitate (Ergina et al., 2014).

Flavonoid

Qualitative identification of flavonoids was carried out by adding HCl, Mg metal and amyl alcohol (Maslahat et al., 2017). According to Harborne (1987), the yellow color in the reaction results is because the flavonoid compounds contained in the extract will be reduced with Mg and HCl to produce flavylium salts in the amyl alcohol layer.

Saponin

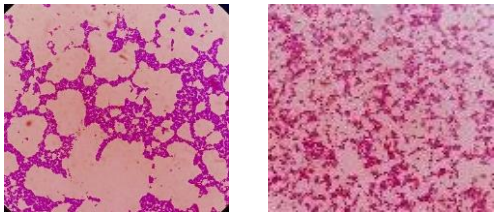
Identification of saponins was carried out by foam formation test. Positive saponin results were shown by the presence of 1 cm high foam after shaking, and constant foam for 10 minutes (Dewi et al., 2021). Formation of foam occurs due to the presence of hydrophilic and hydrophobic groups in saponins which can bind water and air during shaking to produce foam (La et al., 2021).

Phenolic

A qualitative test for phenol can be carried out by adding $FeCl_3$ reagent which will react with the hydroxyl groups on phenol and form a blue or black colour (Dewi et al., 2021). Based on the results of the phytochemical tests conducted, it can be concluded that the ethanol extract of pomegranate peels positively contains alkaloids, flavonoids, saponins and phenols. This is in line with the research by Benslimane et al., (2023) and Prihantoro et al., (2006) which stated that pomegranate peel contains alkaloids, flavonoids, and phenols (ellagitannins).

Isolation and identification of Bacteria

The bacteria to be used are two types of acne-causing bacteria, namely *Staphylococcus epidermidis* and *Staphylococcus aureus*. Before being used for the test, gram staining of bacteria is carried out which aims to identify microbes. The results of the gram staining of the two bacteria are shown in Figure 2.



(a) **(b)**

Figure 2. Gram staining profiles and bacterial structures of *Staphylococcus epidermidis* (a) and *Staphylococcus aureus* (b)

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Figure 2(a) shows that the cultured bacteria are *S.epidermidis* which is cocci/spherical in shape (Büttner et al., 2015). Figure 2(b) shows that the cultured bacteria are *S.aureus* with grape-like cocci (Tong et al., 2015). Both bacteria are gram-positive bacteria which are marked with a purple colour on the results of gram staining due to the high amount of peptidoglycan in the cell wall of gram-positive bacteria, so that they can maintain the colour of crystal violet-iodine even when given an alcoholic acid solution (Retnowati et al., 2011).

Antibacterial Activity of Pomegranate Peel extract Assay

The antibacterial activity test of pomegranate peel extract aims to determine the extract's ability to inhibit the growth of the test bacteria which is shown in the form of an inhibition zone on the test medium. The results of the extract inhibition zone on bacterial growth can be seen in Table 3.

Table 3. Diameter of zone inhibition (mm) of Pomegranate Peel extract against *Staphylococcus epidermidis* and *Staphylococcus aureus*.

Concentration (%)	Diameter of zone inhibition (mm)	
	Staphylococcus epidermidis	***Staphylococcus aureus***
Control (+)	38 mm	32 mm
50	22 mm	19 mm
25	19 mm	17 mm
12,5	18 mm	16 mm
6,25	16 mm	15 mm
3,125	14 mm	14 mm
1,56	11,5 mm	11 mm
0,781	8 mm	8 mm
0,390	6 mm	6 mm
0.195	3 mm	4 mm
0,097	0 mm	0 mm
0,048	0 mm	0 mm
0,024	0 mm	0 mm
Control (-)	0 mm	0 mm

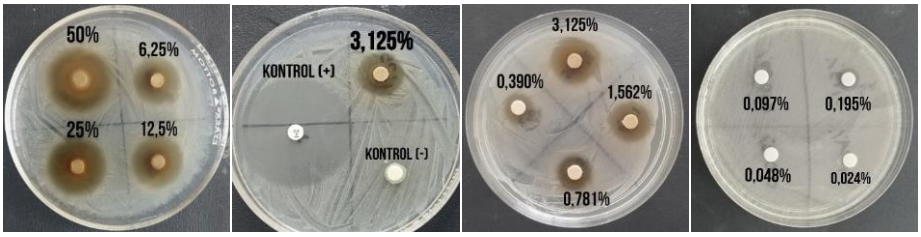

Figure 3. Antibacterial activity of pomegranate peels extracts against *Staphylococcus epidermidis* determined by agar disk-diffusion method.

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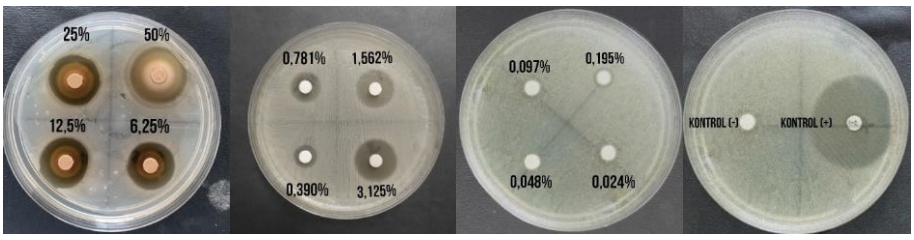

Figure 4. Antibacterial activity of pomegranate peels extracts against *Staphylococcus aureus* determined by agar disk-diffusion method.

Antibacterial activity test of pomegranate peel extract was carried out by agar diffusion method with Nutrient Agar (NA) media for *S.epidermidis* and *S.aureus* bacteria. The use of 10% DMSO as a solvent in the dilution series is due to the nature of the viscous extract which is difficult to dissolve in water. DMSO is a solvent that can dissolve compounds that are polar and nonpolar and does not provide inhibition in bacterial growth so it will not interfere with the results of observations in the antibacterial test of the ethanol extract of pomegranate peel (Huda et al., 2019). The Minimum Inhibitory Concentration (MIC) value was obtained based on the measurement of the Diameter of Inhibitory Area (DDH) of bacterial growth formed around the disc paper. The smallest concentration that produces a clear zone is concluded as the MIC value.

The results of the antibacterial activity test of pomegranate peel extract showed that the greater the concentration of the test, the higher the ability of the test material to inhibit the growth of acne-causing bacteria. Based on the results of the antibacterial activity test of pomegranate peel extract, it was found that pomegranate peel extract had an inhibitory effect on *S.epidermidis* and *S.aureus* bacteria at a concentration of 0.195% with an inhibition value for both types of bacteria of 5 mm and 4 mm.

Based on the results of the agar diffusion disk test, the MIC value of the ethanol extract of pomegranate rind against *S.epidermidis* and *S.aureus* was 0.195% or equivalent to 1.95 mg/ml. When compared with research conducted by Nozohour et al., (2018), stated that the MIC value of the ethanol extract of pomegranate peel was 25 mg/ml against *S.aureus* bacteria, whereas according to Benslimane et al., (2023) stated the MIC value of the extract ethanol of pomegranate peel against *S.epidermidis* of 0.05 mg/ml. The difference in MIC values can be caused by differences in genotypes, climatic conditions, and physiological growth stages of the pomegranate samples used as samples. These differences can affect the concentration of pomegranate metabolite content so that it will also produce different antibacterial activities (Farsi et al., 2023).

The Quality Analysis of The Peel-Off gel Mask Formulation

Organoleptic

Organoleptic testing aims to determine the aesthetic value of the appearance of peel-off masks in the form of shape, colour, and smell of peel-off masks. This is also related to comfort when using the resulting peel-off mask gel (Afianti & Murrukmihadi, 2017). The organoleptic results of the peel-off mask can be seen in Table 4 and Figure 5.

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Table 4. Organoleptic peel-off mask formulation

Test type	F(0)	F(1)	F(2)	F(3)
Gel form	Viscous gel	Viscous gel	Viscous gel	Viscous gel
Colour	Transparent	Transparent yellow	Yellow	Brownish yellow
Scent	Citrus scent	Citrus scent	Citrus scent	Citrus scent
Feel	Sticky	Sticky	Sticky	Sticky

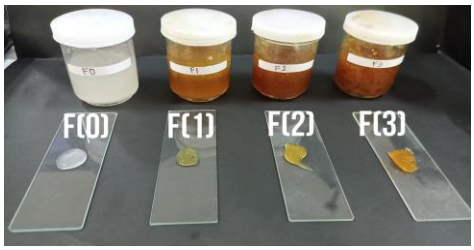

Figure 5. Organoleptic peel-off mask formulation

Organoleptically, the peel-off mask preparations produced in F(1), F(2) and F(3) containing pomegranate peel extract had a brownish yellow colour, whereas in F(0), which is the basic formula for peel-off masks, off looks transparent (colourless). The colour consistency produced by F(3) has a darker brown colour than F(1) and F(2). This was due to the higher concentration of the extract contained in F(3) compared to F(2) and F(1). The higher the concentration of the extract contained in the formula, the higher the colour consistency produced. The four peel-off masks produced are in gel form, thick with an orange smell resulting from the addition of citric oleum as a fragrance to the formula.

Homogeneity

The purpose of the homogeneity test on peel-off mask preparations is to see the homogeneity of the ingredients in the mask formula which is characterized by the absence of lumps or coarse particles in the peel-off mask (Sulastri & Chaerunisaa, 2016). The results of the peel off mask homogeneity test can be seen in Table 5 and Figure 6.

Table 5. Homogeneity peel-off mask formulation

Formula	Parameters	Result
F(0)	No coarse particles	Homogeneous
F(1)	No coarse particles	Homogeneous
F(2)	No coarse particles	Homogeneous
F(3)	No coarse particles	Homogeneous

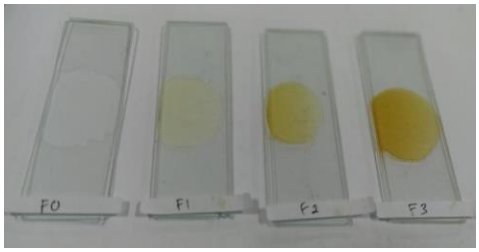

Figure 6. Homogeneity peel-off mask formulation

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Of the four formulas, F1, F2, F3, and F0 had good homogeneity with no lumps or coarse particles visible in the peel-off mask preparation. Only air bubbles were visible caused by too fast stirring during the manufacturing process.

pH test

The pH test aims to ensure that the peel-off mask has a pH that matches the skin's pH, thereby preventing irritation when using the peel-off mask. Topical preparations should have a pH that matches the pH of the skin, namely 4.5-6.5 (Rejeki et al., 2021). The results of the peel off mask pH test can be seen in Table 6.

Table 6. pH of peel-off mask formulation

Formula	pH
F(0)	6,23
F(1)	5,25
F(2)	4,76
F(3)	4,57

Based on the table above, the four formulas have a pH ranging from 4.57 to 6.23 so it can be concluded that the four formulas have met the pH requirements so as to minimize the possibility of irritation when used. Topical preparations that do not match the pH of the skin can cause skin problems. An alkaline pH will cause dry and scaly skin, while an acidic pH can cause skin irritation (Fauziah et al., 2020).

Spreadability test

The purpose of doing a spreadability test is to see the ability of the gel to spread over the surface of the skin during application, the greater the spreadability, the easier the preparation is to be smeared (Andini, 2017). However, if the spreading power is too great, the preparation will flow easily on the skin surface, which results in less optimal absorption of the active substance (Rejeki et al., 2021). The results of the peel-off mask spreadability test can be seen in Table 7.

Table 7. Spreadability of the peel-off mask formulation

Weight	Formulas			
	F(0)	F(1)	F(2)	F(3)
0 g	4,8 cm	4,0 cm	4,0 cm	3,6 cm
50 g	5,5 cm	4,6 cm	4,2 cm	3,8 cm
100 g	5,5 cm	5,0 cm	4,4 cm	4,0 cm
150 g	5,7 cm	5,3 cm	5,0 cm	4,2 cm
200 g	5,7 cm	5,3 cm	5,2 cm	4,5 cm

Based on table 7, F(0) has the greatest spreading power and F(3) has the smallest spreading power. Based on the results of the gel spreadability test obtained, it can be concluded that the use of extract concentrations in the formula will affect the spreadability of the gel. This happens because the higher the concentration of the extract used, the higher the viscosity of the preparation. The spreading power is affected by the viscosity (Priawanto, 2017). The higher the viscosity of the preparation, the smaller the resulting spreading power. Based on the table above, the peel-off mask gel formulas F(0), F(1), F(2) have met the requirements for good dispersion

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and F(3) did not meet the requirements for good dispersion around 5-7 cm (Sulastri & Chaerunisaa, 2016).

Adhesion test

The adhesion of pomegranate extract peel-off masks was tested to measure the mask's ability to adhere well to the skin so that it can properly release active substances during the drying process (Wahyuni et al., 2022). Peel off mask adhesion test results can be seen in Table 8.

Table 8. Adhesion of the peel-off mask formulation

Formulas	Time (s)
F(0)	58
F(1)	114
F(2)	189
F(3)	605

The adhesive ability of peel-off masks is affected by the presence of a bond between the preparation and the skin which allows absorption of the active substance into the skin. If the bond between the gel and the skin is not optimal, the drug will be easily removed by the skin (Syam et al., 2021). Based on table 8, the four formulas have an adhesive power of 58-605 seconds. This shows that the resulting pomegranate peel extract peel-off mask meets the requirements, namely not less than 4 seconds (Wahyuni et al., 2022).

Dry time test

The purpose of the dry time test is to find out how long it takes for a peel-off mask to dry on the surface of the skin and form a film that can be peeled off (Andini, 2017). The dry times of the four formulas can be seen in table 9.

Table 9. Dry times of the peel-off mask formulation

Formulas	Time (m:s)
F(0)	26:16
F(1)	27:02
F(2)	28:48
F(3)	29:47

Based on table 9, the four formulas have dry times ranging from 26-29 minutes. From the observations, it is known that the higher the extract content, the longer it takes for the preparation to dry. Based on the results obtained, it can be concluded that the four formulas have met the requirements for a good peel-off mask drying time, which is between 15-30 minutes (Syam et al., 2021).

Based on the results of the evaluation of the peel-off mask preparation of pomegranate peel extract, it was found that the formulas F(0), F(1), and F(2) had fulfilled all the physical evaluation requirements. Meanwhile, F(3) with an extract concentration of 6.25% did not meet the spreadability test requirements. This is due to the higher concentration of the extract in the preparation which affects the viscosity of the preparation thereby reducing the spreadability of the preparation.

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Antibacterial Activity of The Peel-Off gel Mask Assay

The antibacterial activity test of the peel-off mask aims to determine the peel-off mask's ability to inhibit the growth of the test bacteria which is shown in the form of an inhibition zone on the test medium. The results of the extract inhibition zone on bacterial growth can be seen in Table 10.

Table 10. Diameter of zone inhibition (mm) of the peel-off gel mask against *Staphylococcus epidermidis* and *Staphylococcus aureus*

Consentration (%)	Diameter of zone inhibition (mm)			
	Staphylococcus epidermidis	Category	*Staphylococcus aureus*	Category
F(0)	0 mm	Weak	0 mm	Weak
F(1)	9 mm	Medium	10 mm	Medium
F(2)	13 mm	Strong	14,5 mm	Strong
F(3)	15 mm	Strong	15 mm	Strong
Control (+)	39 mm	Very strong	29 mm	Very strong

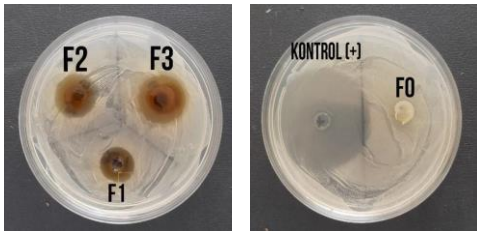

Figure 7. Antibacterial activity of the peel-off gel mask against *Staphylococcus epidermidis* determined by agar well-diffusion method.

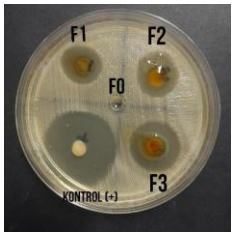

Figure 8. Antibacterial activity of the peel-off gel mask against *Staphylococcus aureus* determined by agar well-diffusion method.

The results of the observation of the antibacterial inhibition test of the peel-off gel mask preparation of the ethanol extract of pomegranate peel showed that the peel-off mask gel containing only base (F0) did not have an inhibition zone so it would not interfere with the observations, while the peel-off mask gel that was contains extracts and clindamycin gel preparations provide an inhibition zone against *S.epidermidis* and *S.aureus* bacteria. This is based on the results of measuring the average diameter of the inhibition around the wells of the gel ethanol extract of pomegranate peels during the 24-hour incubation period.

The diameter of the inhibition zone that has been measured is interpreted as the inhibition zone is in four categories, namely the weak category (<5 mm), medium (5-10 mm), strong (10-

20 mm), and very strong (20 mm or more) (Sa'adah et al., 2022). Formulation with a concentration of 1.56% (F1) with a diameter of inhibition zone against *S.epidermidis* and *S.aureus* bacteria 9 mm and 10 mm respectively in the medium category, then formulation with a concentration of 3.125% (F2) with a diameter of inhibition of bacteria *S.epidermidis* and *S.aureus* respectively 13 mm and 14.5 mm in the strong category, then the formulation with a concentration of 6.25% (F3) with a diameter of inhibition zone against both *S.epidermidis* and *S.aureus* bacteria of 15 mm with strong category for *S.epidermidis* and *S.aureus*. The positive control clindamycin gel had an inhibition zone diameter for *S.epidermidis* and *S.aureus,* respectively 45 mm and 35 mm indicating a very strong inhibition zone. Meanwhile, for the negative control, the peel-off mask base (F0) did not produce a clear zone, which means that the peel-off mask base did not inhibit the growth of *S.epidermidis* and *S.aureus* bacteria.

CONCLUSION

The ethanol extract of pomegranate peel (*Punica granatum* L.) has antibacterial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus* which was tested in vitro with a MIC value of 0.19% or equivalent to 1.9 mg/ml. Pomegranate peel extract (*Punica granatum* L.) peel-off mask formulations F(1) and F(2) fulfilled all the requirements in the physical evaluation of preparations, while F(3) did not meet the spreadability requirements. The peel-off mask made can properly release the active substance of pomegranate peel extract so that it can provide antibacterial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus* with F(1) providing moderate category antibacterial effect, F(2) and F(3) providing antibacterial effect strong and positive control clindamycin gel provides very strong antibacterial effect.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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