

Evaluation and Antibacterial Activity Test of Peel-off Mask Preparation from Combination of *Pegagan* Leaves (*Centella asiatica* (L) Urb.) and Charcoal Powder

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Abstract

Pegagan leaf (*Centella asiatica* (L) Urb.) is a plant that contains flavonoids, tannins, triterpenoids, and saponins that function as antibacterials. *Pegagan* leaf is combined with activated charcoal powder, which has the job of absorbing facial oil, so that it is formulated in the form of a peel off mask to produce a synergistic effect. This study aims to formulate, evaluate, and test the antibacterial effectiveness of the peel-off mask preparation of *pegagan* leaf extract at concentrations of 1.5%, 2%, and 2.5%. This study uses an experimental method by testing the parameters of the physical evaluation requirements and testing antibacterial activity with the disc method on the peel off mask preparation. The results were evaluated for 28 days, and the results of the organoleptic test were stable, homogeneity stable, pH (5.6–6.2), dry time (16–25 minutes), dispersion (6.1–6.4 cm), and irritation (2 respondents). The effectiveness test against the bacteria *Propionibacterium acnes* has inhibitory power of 12.3 mm in the strong category. So, it can be concluded that the peel off mask produced has stable physical characteristics and can be used as an antibacterial mask.

Keywords: activated charcoal; antibacterial; *pegagan* leaf; peel off mask.

INTRODUCTION

Unhealthy environmental conditions due to the impact of air pollution such as incomplete combustion from motor vehicles, cigarette smoke, solar radiation, and other pollutants can trigger free radicals. This will result in the destruction of body cells. The effect on the skin is in the form of slowly decreasing skin elasticity, which results in the appearance of hyperpigmentation or tanned spots and also wrinkles (Ningsih *et al.*, 2016).

The skin is not the only organ that has a vital function. Having smooth and healthy skin is a matter of pride, especially for teenagers and women. But like other parts of the body, the skin can also experience disorders or diseases, one of which is acne (Albastomi, 2019).

Acne is a common thing experienced by millions of people in various parts of the world, involving disorders of the pilosebaceous unit. This disorder occurs due to clogging of the skin pores, resulting in inflammation of pus sacs. Acne sufferers in Indonesia continue to increase every year. Acne is also triggered by the development of *Propionibacterium acnes* bacteria. This bacterium is one of the gram-positive rod-shaped bacteria and is a normal part of the skin flora that is involved in the production of acne (Ervina *et al.*, 2020). In overcoming skin problems

such as acne, Indonesians have always used plants as medicine, and not a few are used as skin care ingredients and cosmetics (Ramadhania, 2018).

One of the medicinal plants in Indonesia that has antibacterial activity that can fight acne-triggering bacteria is *Pegagan* (*Centella asiatica* (L.) Urb.). This is supported by the bioactives contained in it that have antibacterial activity, such as saponins, tannins, flavonoids, and so on (Soebagio *et al.*, 2020). *Pegagan* also contains madecassic acid, asiatic acid, and asiaticoside, which can help heal wounds (Budi & Rahmawati, 2020). *Pegagan* is a type of plant that can be used in the treatment of scaly and dry facial skin. By synthesizing collagen, it can regenerate tissue levels and can be used as a raw material for skin care (Sumiati *et al.*, 2019). Cosmetics have been known since ancient times, with natural raw materials used to beautify themselves.

There are various cosmetics, including face masks. The use of a face mask can unclog pores, soften the skin, and remove cosmetic residue that is difficult to remove with regular cleansers. Regular use of face masks can also reduce the appearance of fine lines and wrinkles and prevent premature aging (Widyarti *et al.*, 2019). Peel-off face masks are a type of face mask that is superior in its

use because they can be easily removed like an elastic membrane (Sulastrri & Chaerunisaa, 2018). Peel-off masks can be used to cleanse and moisturize the skin. Most facial peel off masks use polymers that can thin the layers on the face. Peel-off masks come in dry and gel forms. Applying a face mask in the process takes 15-30 minutes to dry by applying a layer on the face (Oktavia *et al.*, 2021). Based on research (Lestari *et al.*, 2020) the combination of palm kernel shell activated charcoal peel-off mask and PVA will result in the skin's surface structure becoming cleaner and smoother after use.

The purpose of this study was to evaluate and test the antibacterial activity of peel-off mask preparations made from a combination of *Centella asiatica* (L.) Urb. leaves and activated charcoal powder.

MATERIALS AND METHODS

Study area

The tools used in this study were a rotary evaporator (Dlab®), laminar air flow, oven (Mettler®), autoclave (Wiseclave®), analytical balance (Kern®), Erlenmeyer (Pyrex®), pH meter (Atc®), measuring cup (Pyrex®), petri dish (Normax®), hot plate (Kern®), stirring rod, glass slide, 20x20cm glass, stopwatch, dropper, spatel, filter paper, maceration vessel, spirit burner, ose needle, disc paper (Whatman®), parchment paper, spatula, cotton swab, tissue, and tube mask.

The materials used in this study included *Pegagan* leaves (*Centella asiatica* (L.) Urb), activated charcoal powder (Bumi agung®), 70% ethanol, 96% ethanol, PVA, HPMC, glycerin, propylenglycol, methyl paraben (Golden era®), propyl paraben (Golden era®), aquadest, nutrient agar media (Merck®), Mediolin® gel, and *Propionibacterium acne* bacteria.

Procedures

Sampling

Pegagan leaves were obtained from Kenagarian Sungai Tanang, Agam Regency, West Sumatra. Plant identification was carried out by Dr. Nurainas at the Herbarium Andalas, Department of Biology, FMIPA, Andalas University, Padang, West Sumatra.

Sample Preparation

Pegagan leaves totaling 3.5 kg were washed thoroughly using running water and dried at 60 °C using an oven for 5 hours to obtain *Pegagan* leaf simplisia (Handayani & Qamariah, 2019).

Extraction

Pegagan leaf simplisia powder (600 g) was macerated using 70% ethanol until submerged within 4x24 hours. Then filtering was done to separate the filtrate from the pulp. The filtrate obtained was then concentrated using a

rotary evaporator until a thick extract was obtained (Purdiyanti, 2017).

Phytochemical Screening (Soebagio *et al.*, 2020)

Flavonoid Identification

A total of 0.1 g of extract was added to 2 ml of 70% ethanol as well as 50 ml of hot water that had been brought to a boil. Then a few drops of concentrated HCl and 0.1 g of magnesium powder were added. Flavonoids are characterized by the formation of red, yellow, and orange colors.

Identification of Tannins

A total of 0.1 g of extract was added to as much as 2 ml of 70% ethanol and FeCl₃ to as much as 3 drops. The presence of tannin compounds is indicated by the appearance of blue, green, or precipitate.

Identification of Triterpenoids

A total of 0.1 g of extract was added to as much as 2 ml of 70% ethanol, then put into a test tube, followed by the addition of concentrated H₂SO₄ 1 drop and acetic anhydride 3 drops. If a purple, red, or brown color is formed, the sample is positive for triterpenoid.

Raw Material Inspection

The ethanol extract of *pegagan* leaves was examined for organoleptic, solubility, and phytochemical screening; glycerin, PVA, HPMC, propylenglycol, propyl paraben, methyl paraben were examined according to the requirements listed in the handbook Pharmaceutical excipients, 6th edition, Indonesian Pharmacopoeia III edition.

Manufacture of peel-off mask

PVA was developed using hot water until fully expanded, then homogenized (mass 1). HPMC was developed on top of distilled water ad inflated (mass 2), then mass 2 was put into mass 1. Gotu kola extract was dissolved with 96% ethanol until dissolved, then propyl paraben and methyl paraben were dissolved with 96% ethanol until dissolved (mass 3). Mass 3 is included in mass 1 while still stirring in the beakerglass. Next, add little by little propylenglycol, glycerin, and activated charcoal powder while still stirring. Finally, add distilled water ad 100 ml until a homogeneous gel mass is formed (Handayani & Qamariah, 2019).

Physical Evaluation of peel-off mask

Organoleptical Test

The organoleptic test was carried out by observing changes in the shape, smell, and color of the mask preparation.

Homogeneity Test

The homogeneity test was carried out by placing 0.1 gram of gel between two glass objects and observing

whether the surface was evenly smooth and the base was homogeneous. The preparation is said to be homogeneous if there are no coarse particles that can be palpated (Pramiastuti *et al.*, 2019).

pH Test

The pH measurement was carried out using a pH meter that had previously been calibrated using standard dapar (pH 7 and pH 4) (Pramiastuti *et al.*, 2019). A total of 1 gram of gel was weighed and dissolved with 10 mL of distilled water and stirred homogeneously, then the pH meter was dipped and the results obtained were recorded (Arman & Mansauda, 2021).

Drying Time Test

A total of 1 gram of Peel-off mask gel was applied to 7 cm of arm skin. Then calculate the speed of drying the gel to form a film layer using a stopwatch (Handayani & Qamariah, 2019).

Spreadability Test

A total of 0.5 grams of gel was placed on a glass with a size of 20x20 cm. Then it was covered with another glass, a load of 125 grams was added, and after 1 minute, the diameter of the spreading gel was measured (Istiqamah & Anindhita, 2018).

Irritation Test

The open patch test was used for the irritation test on the skin of 10 volunteers, the peel-off mask gel preparation was applied in an area of 2.5 cm² on the forearm, then allowed to stand for 15 minutes, and the reaction that appeared was observed (Rinaldi *et al.*, 2021).

Antibacterial Activity Test

Sterilization of Equipment

All equipment, including media, should be sterilized before use by autoclaving at 121 °C for 15 minutes. Ose tweezers and needles were sterilized by direct burning over flame (Oktavia *et al.*, 2021).

Preparation of Nutrient Agar Medium

5 grams of nutrient agar were dissolved in 250 ml of sterile hot water, then stirred using a stirring rod until dissolved, and sterilized in an autoclave at 121 °C for 15 minutes (Husnani & Rizki, 2018).

Preparation of a Pure Culture Suspension of Test Bacteria

Stock cultures of test bacteria were taken using a sterile ose needle and then suspended in 10 ml of physiological NaCl (Husnani & Rizki, 2018).

Preparation of the sample solution

The peel off mask of *Centella asiatica* (L.) Urb leaf extract was made with concentrations of 1.5%, 2%, and 2.5%, respectively. The sample solution was made by weighing each preparation as much as 10 grams and then

dissolved with aqua p.i. in 10 ml for a positive control and dissolved with aqua p.i. in a ratio of mediclin: aqua p.i.(1:1). F0 as a negative control (Husnani & Rizki, 2018).

Disc preparation

Paper discs with a diameter of 0.6 cm were prepared, soaked for 15 minutes in each sample solution aseptically, and then placed on the media used (Husnani & Rizki, 2018).

Inhibition Testing

Inhibition testing of a peel-off mask of *Centella asiatica* (L.) Urb leaf extract was carried out by diffusion method using disc paper. The test bacterial suspension was poured as much as 1 ml into a Petri dish, then 10 ml of sterile NA medium that had been cooled to a temperature of 40-45 °C were added. Next, the Petri dish was closed, shaken until it was homogeneously mixed, and allowed to freeze. The disc paper that has been soaked in the sample solution is placed on the surface of the Nutrient Agar (NA) medium, which contains the test bacteria, and then incubated in an incubator at 37 °C with an inverted position for 2x24 hours. The zone of inhibition formed was measured using a caliper (Husnani & Rizki, 2018; Afriani & Rahmayulis, 2022).

Data Analysis

The data obtained were analyzed using a one-way ANOVA statistically through the SPSS 25 program with a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Fresh samples of *pegagan* leaves were obtained from Kenagarian Sungai Tanang Agam Regency, West Sumatra. Identification was carried out at the Herbarium Andalas, Faculty of Biology, Andalas University, the results obtained were the type of plant studied in the form of (*Centella asiatica* (L.) Urb.). The extraction process was carried out by maceration technique using 70% ethanol solvent and obtained a thick extract of 345.75 g with a yield of 57.6%.

Phytochemical Profile of Pegagan Leaves

Based on the results of phytochemical tests on the ethanol extract of *pegagan* leaves, it is positive for flavonoids, tannins, and triterpenoids from the reagent reactions used. Secondary metabolite compounds such as saponins, tannins, and flavonoids contained in *pegagan* leaves are claimed to have antibacterial activity. The results of phytochemical screening are shown in Table 2.

There are various chemical contents in *pegagan* that have many benefits for humans. *Pegagan* contains compound components, including triterpenoids, which include brachnid acid, madasiatic acid, steroids, tannins, glycosides, alkaloids, asiatic acid, madecoside, and

asiaticoside. Triterpenoids from pegagan are claimed to have potential as antioxidants, antifungals, and antibacterials (Sulastris *et al.*, 2017). Besides using pegagan leaves as the main active substance that functions as an antibacterial, activated charcoal powder is also used to absorb dirt and oil on the face. These two ingredients have a synergistic effect on inhibiting acne growth. Therefore, pegagan leaf extract and activated charcoal powder were chosen to be the active substances of the peel-off mask preparation in this study. The peel-off mask preparation was chosen because it is easier, more efficient, and more effective to wear on the face until it dries and produces a thin, elastic, transparent film layer, and is also easy to clean without having to be washed (Rismayanti *et al.*, 2021).

The ingredients used in making peel-off masks include polyvinyl alcohol (PVA), which functions as a gelling agent or film-forming base; glycerin and propylenglycol as humectants that can moisturize the skin in high humidity conditions and can bind water from the air; propylenglycol can maintain the water contained in the preparation so that during storage, the stability and physical properties of the preparation can be maintained (Frida *et al.*, 2018), hydroxypropyl methylcellulose (HPMC) as a viscosity enhancer; methyl paraben and propyl paraben as preservatives to anticipate microbial contamination due to the high water content in the preparation (Rowe & Q.E., 2015). The peel-off mask was made in 4 variations of extract concentration with the aim of comparing the antibacterial effectiveness and evaluating the physical quality of good peel-off masks from each concentration with positive control and negative control (F0) with preparation evaluation parameters in the form of spreadability test, organoleptic, homogeneity, pH, dry time, irritation test, and antibacterial inhibition.

Organoleptical tests are carried out as quality control for a preparation. Observations were made visually and

using the five senses. The results of observations for 28 days showed that the peel-off mask remained stable and no changes occurred during storage. This preparation is said to have met the requirements of the stability test because there is no change in odor, color, or shape (Ambari *et al.*, 2020). The results of the evaluation of peel-off mask preparations are shown in Table 3.

Observation of the homogeneity of the preparation was carried out within 28 days. From the results of observations on days 0 7 14 21 and 28, all formulas remained homogeneous, characterized by the absence of coarse particles in the preparation (Fauziah *et al.*, 2020). The pH test was carried out using a calibrated pH meter.

Table 1. Peel-off Mask Formula Design.

Material	% Concentration			
	F0	F1	F2	F3
Pegagan leaf extract	-	1,5	2	2,5
Activated charcoal powder	-	1	1	1
PVA	10	10	10	10
HPMC	1	1	1	1
Glycerin	10	10	10	10
Propylenglycol	10	10	10	10
Methyl Paraben	0,15	0,15	0,15	0,15
Propyl Paraben	0,15	0,15	0,15	0,15
Ethanol 96%	15	15	15	15
Aquadest ad	100	100	100	100

Table 2. Phytochemical Screening Results of an Ethanol Extract of Pegagan Leaves.

Secondary metabolite compounds	Ethanol extract of pegagan leaves	
	Results	Color
Flavonoids	+	Yellow red
Tannins	+	Blue green
Triterpenoids	+	Red brown

Table 3. Evaluation Results of peel-off mask preparation of pegagan leaf extract combined with activated charcoal powder.

Formula	Day to	Smell	Color	Shape	Homogeneous	pH	Dry time
F0	0	Typical	Clear white	Semi-solid	Homogeneous	6,3	16:35
	7	Typical	Clear white	Semi-solid	Homogeneous	6,3	16:16
	14	Typical	Clear white	Semi-solid	Homogeneous	6,2	17:18
	21	Typical	Clear white	Semi-solid	Homogeneous	6	16:09
	28	Typical	Clear white	Semi-solid	Homogeneous	6,2	16:16
F1	0	Typical	Black	Semi-solid	Homogeneous	6,2	21:03
	7	Typical	Black	Semi-solid	Homogeneous	6,3	21:23
	14	Typical	Black	Semi-solid	Homogeneous	6,1	21:31
	21	Typical	Black	Semi-solid	Homogeneous	6	20:19
	28	Typical	Black	Semi-solid	Homogeneous	6,1	22:53
F2	0	Typical	Black	Semi-solid	Homogeneous	6,3	21:20
	7	Typical	Black	Semi-solid	Homogeneous	6	22:05
	14	Typical	Black	Semi-solid	Homogeneous	5,8	22:31
	21	Typical	Black	Semi-solid	Homogeneous	6	22:06
	28	Typical	Black	Semi-solid	Homogeneous	6,1	22:46

Table 3. Cont.

Formula	Day to	Smell	Color	Shape	Homogeneous	pH	Dry time
F3	0	Typical	Black	Semi-solid	Homogeneous	5,9	21:56
	7	Typical	Black	Semi-solid	Homogeneous	5,5	22:45
	14	Typical	Black	Semi-solid	Homogeneous	5,3	24:16
	21	Typical	Black	Semi-solid	Homogeneous	5,8	25:25
	28	Typical	Black	Semi-solid	Homogeneous	5,8	25:27

Table 4. Inhibition Test Results of peel-off mask against *Propionibacterium acnes* bacteria.

Formula	Inhibition zone diameter (mm)			Mean diameter of inhibition zone (mm)
	I	II	III	
F0	0	0	0	0
F1	10,25	10,35	10,55	10,38
F2	11,2	11,4	11,7	11,43
F3	12,0	12,25	12,65	12,3
Mediklin®	16,1	21,7	25,1	20,93

Testing was carried out every week for four weeks. The evaluation results show that all formulas have a pH range of 5.6-6.2, which means that all preparations meet the pH test requirements and are in accordance with the normal skin pH range of 4.5-6.5 (Utami *et al.*, 2020). The rise and fall of the pH of the preparation during storage shows a preparation that is less stable. The changing pH value can be influenced by the decomposition of the media at high temperatures during storage or manufacture to produce bases or acids. Changes in pH can also be triggered by environmental factors, including poor storage, temperature, and unstable combinations of extracts in the preparation because they are oxidized (Putra, 2017). This shows that if the pH is below 4.5, it can cause skin irritation and scaly skin if the pH exceeds 6.5 (Noviyanty, 2020).

The dry time test aims to determine the time it takes for the preparation to dry on the skin surface and produce a film layer. Ideally, the dry time of the preparation ranges from 15-30 minutes starting from the application of the mask until the mask dries on the surface of the skin. The range of dry times for all formulas was 16-25 minutes. These results show that all formulas meet the dry time test requirements that have been set. The dry time range of F0 was 16-17 minutes, F1 was 20-22 minutes, F2 was 21-22 minutes, and F3 was 21-25 minutes. The dry time of F0 was faster than the three formulas containing pegagan extract and activated charcoal powder. The increase in drying time was due to the evaporation of ethanol when the packaging was opened for too long during the test. The function of ethanol in the peel-off mask formula is to shorten the drying time of the mask, therefore if the ethanol has evaporated, the drying time of the preparation becomes longer (Sutriningsih & Astuti, 2017).

The spreadability test aims to see the potential distribution of the preparation when applied to the skin. The easier the application, the greater the surface area of contact of the preparation with the skin, thus the more optimal the absorption of substances in the skin. Face mask preparations are declared good if they have a spreadability of 5.0-7.0 cm. The results of the evaluation of the average diameter of the spreadability of each formula for 28 days were 6.1 cm, 6.2 cm, 6.4 cm, and 6.4 cm, respectively. The higher the concentration of the extract used, the faster the spreadability, and vice versa; the lower the concentration of the extract, the longer the spreadability of the preparation (Noviyanty, 2020). The evaluation results showed that all formulas met the specified spreadability requirements.

The irritation test was carried out using an open patch test (patch test) on 10 volunteers by applying F0-F3 preparations covering an area of 2.5 cm² on the back of the hand. This test was carried out for 3 consecutive days, 3 times a day, for 15 minutes, with the parameters of red, itchy, and swollen reactions (Putri *et al.*, 2021). Symptoms that arise are observed, and irritation will generally occur with the onset of skin reactions shortly after contact or attachment to the skin, or this condition is called primary irritation (Anonim, 1985). The test results showed mild irritation in 2 out of 10 respondents to the use of F0-F3 in the form of itching and redness, but the irritation disappeared after a few minutes. From the observations that have been made on the standardization of pegagan extract, the pH is 4.7, which meets the requirements, and when applied to the back of the hand, it does not experience irritation. The possibility of this reaction is due to the sensitivity and nature of the skin of different respondents. However, the onset of irritation is not long-only a few minutes after the mask is applied (Numberi, 2020).

The inhibition test on mask preparations is carried out using the disc diffusion method. The advantages of this method are that it is cheap, easy, and fast because the process does not require special tools. This test aims to determine the ability of pegagan leaf extract combined with activated charcoal powder to inhibit the growth of *Propionibacterium acnes* bacteria. Nutrient agar is used as a growth medium because it is a common medium used in bacterial growth (Retnaningsih *et al.*, 2019). The test results are seen in the clear zone formed on the media. Descriptively, all formulas showed antibacterial

activity with different inhibition zone diameters. F3 is the formula with the largest inhibition zone diameter of 12.3 mm with a strong category, while the Mediklin® comparison is 20.93 mm, but to determine the best formula requires statistical testing using SPSS 25. The test results show that the greater the concentration of the extract, the greater the diameter of the clear zone formed. Inhibition test results are shown in Table 4.

ANOVA Test

The prerequisite for conducting the Anova test is that the data must be normally distributed and homogeneous. This test is a multivariate analysis technique that functions to distinguish more than two groups of data by comparing their variances. Based on the results of the ANOVA test, it can be seen whether there is a significant or real difference from all formulas on inhibitory power. If the significance value <0.05 , it can be concluded that there is a significant difference in all formulas. The results of the anova test showed a significance value <0.05 on the inhibitory power, so H_0 was rejected, so it can be concluded that there is a significant difference in the inhibitory power between all formulas.

CONCLUSIONS

Based on the research that has been done, it can be concluded that the peel-off mask of *pegagan* leaf extract and activated charcoal powder has stable physical characteristics and can be used as an antibacterial mask for *Propionibacterium acnes*.

Competing Interest: The Authors have no competing interests.

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