

Physical characteristics and antibacterial properties of pandanus peel-off gel mask against *Propionibacterium acnes*

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Abstract

Acne is a skin inflammation disease occurs due to physiological and hormonal changes, as well as infection with *Propionibacterium acnes* bacteria. This study aims to see the potential of pandan leaf extract (*Pandanus amaryllifolius* Roxb.) to be used as a peel-off gel mask based on its physical characteristics and antibacterial properties on *Propionibacterium acnes* bacteria. Pandan leaves were extracted using maceration technique with ethanol as solvent. Peel-off gel mask was made using hydroxypropyl methylcellulose, polyvinyl alcohol, propylene glycol, methylparaben, propylparaben, distilled water, and pandan leaf extract. Antibacterial test was conducted by determining bacterial inhibition zone after being given negative control, positive control (clindamycin 1%), gel mask with extract concentration of 10%, 15%, and 20% respectively. As a result, pandan leaf extract contains flavonoids, alkaloids, polyphenols, tannins, and terpenoids. Physical characteristics of gel mask including organoleptic, homogeneity, pH, drying time, spreadability, and viscosity meet Indonesian national standards. Peel-off gel mask of pandan leaf extract has antibacterial properties at all concentration levels where at concentrations of 15% and 20% the inhibition zone is not statistically different from standard antibiotics. Thus, it can be concluded that pandan leaf extract has the potential to be used as a peel-off gel mask as an anti-acne medication.

Keyword: Peel-Off Gel Mask; Pandan; Pandanus Amaryllifolius; Acne; Anti-Acne; Propionibacterium Acnes

1. Introduction

Acne is a skin inflammation that is common in postpubescent teens and estimated to affect 9.4% of the global population so that make it the eighth most prevalent disease worldwide. This skin disease occurs due to physiological and hormonal changes characterized by lesions on the face and other areas of the body. In addition to physiological factors and hormonal changes, another factor that causes acne is infection with *Propionibacterium acnes* bacteria. These bacteria are able to break down free fatty acids in sebum, which then clogs the skin pores and triggers inflammation [1-2]

There were various topical and oral drugs are available for acne in the market like Salicylic acid, Isotretinoin, Erythromycin, Clindamycin, Triclosan, Minocycline, Tetracycline, and Metronidazole. However, there are many findings showed that these drugs have limitations with respect to toxicity and side effects such as skin drying, headache, and nausea. In addition, an excessive use of these drugs over a long time can lead to the rising resistance of bacteria. That's why researchers continue to look for medicinal ingredients that are effective, safe and low-cost anti-acne drugs, among others by exploring herbal resources [3].

Herbal resources known to have anti-acne properties are plants that contain phytochemicals that have anti-oxidant, anti-inflammatory, immunomodulatory, antibacterial antiandrogenic, reducing sebum production, and lipogenesis inhibitory activities. The phytochemicals including polyphenols such as resveratrol, myricitrin, schisandrin, terchebulin,

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alpha-mangotin, curcumin, ellagic acid and epigallocatechin 3-gallate. Additionally, alkaloids and terpenoids such as berberine, ursolic acid, lupeol also known to have anti-acne properties [4].

Among medicinal plants known to contain phytochemicals that have anti-bacteria, anti-inflammatory, and antioxidant is *Pandanus amaryllifolius* [5-6]. Phytochemicals screening showed that pandanus plant extract containing alkaloid, tannin, anthraquinone, flavonoid, terpenoid, coumarin, saponin and reducing sugar [7]. In addition, pandanus leaf extract is rich in essential oil consisting of pyranone, coumaran, 1,4-di-tert-butylphenol, pinane, ethyl palmitate, phytol, purpurogallin, squalene, decamethyltetrasiloxane, and vitamin E [8].

In order to evaluate whether phytochemical compounds in pandanus plant, the ethanol leaf extract of the plant is formulated in a peel-off gel mask. The gel mask then characterized and tested for its anti-bacterial properties against *Propionibacterium acnes* bacteria.

2. Materials and Methods

2.1. Plant sample and extraction

Plant samples of *Pandanus amaryllifolius* were collected from a farmer in suburb of Bandar Lampung City, Lampung Province, Indonesia. The taxonomic identification of the plant was done by taxonomist in the Botanical Laboratory, University of Lampung. The Fresh leaves of the plant were washed and rinsed using running tap water. After air-dried the leaves were grinded into small particles and macerated in 96% ethanol for 24 hours. After being macerated for four times, the extract evaporated using rotary evaporator under low pressure at 50°C until viscous extract formed.

2.2. Qualitative phytochemical test

Phytochemical tests were conducted to determine the content of secondary metabolites in ethanol extract of *Pandanus* leaves. Qualitative phytochemical tests were conducted as follows.

2.2.1. Tannin test

0.5 g sample + distilled water + 2 drops of 10% FeCl₃ solution. Positive results are indicated by a change in the colour of the sample to blackish blue.

2.2.2. Saponin test

1 mL sample + warm water, then shaken for 30 seconds. Positive results are indicated by the presence of foam.

2.2.3. Flavonoid test

Extract sample + ethanol + n-hexane. Then residue + 10 ml 80% ethanol + 0.5 g Mg + 0.5 M HCl. Positive results are indicated by a change in the colour of the sample to pink or purple.

2.2.4. Alkaloid test

0.5 mL sample + 5 drops of chloroform liquid + 5 drops of Mayer's reagent (1 g of KI dissolved in 20 mL of distilled water, added 0.271 g of HgCl₂ until dissolved). Positive results are indicated by a change in the color of the sample to brownish white.

2.2.5. Polyphenol test

Folin-Ciocalteu reagent (F-C) (phosphotungstic acid and phosphomolybdic acid) with phenolic compounds. Positive results produce molybdenum-tungsten blue.

2.2.6. Steroid test

0.5 mL sample + 0.5 mL glacial acetic acid + 0.5 mL H₂SO₄. Positive results are indicated by a change in the colour of the sample to blue or purple.

2.2.7. Terpenoid test

0.5 mL sample + 0.5 mL glacial acetic acid + 0.5 mL H₂SO₄. Positive results are indicated by a change in the colour of the sample to red or yellow.

2.3. Preparation of peel-off gel masks

The peel-off gel mask formulation was made using ingredients with concentrations as presented in Table 1.

Table 1 Formulation of peel-off gel mask

No.	Material	Concentration	Utility
1	Pandanus leaf extract	10%, 15%, 20%	Active substance
2	HPMC	2%	Gelling agent
3	PVA	10%	Thin film
4	Propylene glycol	10%	Humectant
5	Methylparaben	0.2%	Preservative
6	Propylparaben	0.02%	Preservative
7	Distilled water	up to 100ml	solvent

HPMC (hydroxypropyl methylcellulose) is put into a beaker glass, dissolved in distilled water, then heated at 80 C while stirring until it expands (M1). PVA (polyvinyl alcohol) is developed in hot distilled water on a hotplate at 80 C, stirred constantly until completely homogeneous (M2). Pandanus leaf extract, methyl paraben, propyl paraben are dissolved in propylene glycol on a water bath (M3). M1 and M2 are put into a mortar and ground until homogeneous, then M3 is added to the mixture and stirred until homogeneous. After that, distilled water is added until the weight reaches 100 g.

2.4. Characterization of peel-off gel masks

- Organoleptic test. The organoleptic properties of the masks were assessed based on parameters such as colour, odour, texture and consistency through visual inspection.
- Homogeneity test. The preparation is smeared on a glass object or petri dish, then visually observed for coarse grains or lumps.
- pH test. The pH of the masks was tested by dissolving 1 g of gel mask preparation in 10 ml of distilled water that were stirred to uniform. The pH meter then dipped into the dissolved gel and the pH values were recorded.
- Spreadability test. The spreadability properties of the masks were carried out by placing 1 gram of the peel-off gel preparation between two horizontal glass plates (10 cm× 20 cm). Then, a standard weight of 25 grams is applied to the upper plate, and the spreading diameter is measured after one minute.
- Drying time test. One gram of each formula was weighed and evenly spread using a mask brush over a 7 x 7 cm area on the forearm skin. The gel was then allowed to dry until the film could be peeled off, and the drying time was recorded using stopwatch.
- Viscosity test. The viscosity measurement of the peel-off gel mask was performed with a Brookfield-Viscometer. The gel was rotated at 10, 20, 30, 40, 50, and 60 rpm. At each speed, the corresponding dial reading was noted.

2.5. Anti-bacterial test and experimental design

The *Propionibacterium acnes* Roxb. bacterial isolate was obtained from AGAVI Laboratory (PT. Agritama Sinergi Inovasi, Bandung, West Java, Indonesia). The bacteria then rejuvenized using nutrient agar (NA) incubated at temperature of 37°C for 24 h. Next, the bacterial suspension is made by inserting 1 ose loop of bacteria into a test tube containing 10 ml of 0.9% physiological NaCl solution. Then the test tube is shaken until homogeneous, then the turbidity is equalized with the McFarland standard.

To find out the anti-bacterial properties of the peel-off gel mask containing pandan leaf extract, 5 types of treatments were prepared as follows.

- Negative control (C-)—gel mask without pandanus leaf extract;
- Positive control (C+)—gel mask containing 2% clindamycin;
- Pandanus extract 1 (PE-1)—gel mask with 10% pandanus leaf extract;
- Pandanus extract 2 (PE-2)—gel mask with 15% pandanus leaf extract;

- Pandanus extract 3 (PE-3)—gel mask with 20% pandanus leaf extract.

Antibacterial test was conducted using disc diffusion method. Sterile cotton swab was dipped into bacterial suspension then rubbed on the surface of agar media and left for 1-5 minutes so that the suspension blends with agar. Furthermore, disc paper that has been soaked in gel with various concentrations of active ingredients is placed on the media using tweezers. The media was then incubated at 37°C for 1x24 hours. The inhibition power of gel mask was determined by measuring the diameter of the clear zone around the disc using callipers.

2.6. Statistical analysis

Statistical analyses for the antibacterial activity were performed using ANOVA and Least square difference (LSD) at significance level of 5%. The ANOVA analysis and LSD post hoc test used are in SPSS version 26 software.

3. Results and Discussion

3.1. Phytochemical content

The qualitative phytochemical test of pandan leaf extract conducted in this study revealed compounds as shown in Table 1. The screening results showed that the ethanol extract of pandan leaves contains flavonoids, terpenoids, polyphenols, tannins and alkaloids.

Table 1 Results of phytochemical test of ethanol extract of cherry leaves

No.	Secondary metabolite	Test observation	Results
1	Flavonoid	The colour of the solution changes to yellow	+
2	Terpenoid	No colour change	+
3	Polyphenol	Colour changes to brownish yellow	+
4	Tanin	The colour changes to blackish blue	+
5	Saponin	No foam occurred	-
6	Alkaloid	The colour changes to brownish white	+
7	Steroid	No changes in colour	-

Notes: (+): Secondary metabolite compounds present in the extract ; (-): No secondary metabolite present

These results are similar to screening conducted by other previous researchers that *Pandanus amaryllifolius* plant leaves contain flavonoids, polyphenols, tannins, and alkaloids [9,10]. However, the screening conducted in this study failed to find steroids which according to Hidayani *et al.* (2021) are contained in pandan leaf extract [11]. In addition, this study also failed to find saponins as reported by Burhana *et al.* (2023) [12]. The difference in extraction results is likely caused by differences in extraction methods whether liquid-liquid extraction, solid phase extraction, solid liquid extraction and supercritical extraction. The extraction results are also determined by the type of solvent and the extraction procedure [13, 14].

3.2. Characterization of peel-off gel masks

The results of the peel-off gel mask characterization test containing pandan leaves at different concentrations are presented in Table 2.

The characteristics of the gel mask obtained were all within the limits recommended in the Indonesian National Standard SNI 16-4399-1996. The homogeneity test results show that the gel mask is homogeneous, the pH is in the range of 4.5-8, the drying time is in the range of 15-30 minutes, the spreadability is 5-7 cm, the viscosity is in the range of 2000-5000 pcs. Based on these physical characteristics, the pandan leaf extract gel mask preparation can be developed as a standard peel-off gel mask [15,16].

Table 2 Characterization result of peel-off gel mask of Pandanus leaf extract

Parameters	Characteristics of peel-off gel by concentration of extract		
	Extract 10%	Extract 15%	Extract 20%
Organoleptic	Brownish green in colour, semi-solid in shape, has a distinctive leafy odour.	Brownish green in colour, semi-solid in shape, has a distinctive leafy odour.	Brownish green in colour, semi-solid in shape, has a distinctive leafy odour.
Homogeneity	Homogeneous, no coarse particles	Homogeneous, no coarse particles	Homogeneous, no coarse particles
pH	4.69	4.73	4.80
Spreadability	6.8 cm	6.9 cm	7 cm
Drying time	18 minutes and 10 seconds	18 minutes and 33 seconds	20 minutes and 38 seconds
Viscosity	3.108 cps	4.080 cps	5.784 cps

3.3. Anti-bacterial properties

The results of the pandan leaf extract test on the growth of *Propionibacterium acnes* bacteria based on the inhibition zone are presented in Table 3.

Table 3 Growth inhibition zone of *Propionibacterium acnes* bacteria by pandanus peel-off gel mask

Treatment	Inhibition zone (mm)			
	1	2	3	Mean
Control -	0	0	0	0 ^a
Control + (Clindamycin 1%)	13.53	15.5	18.13	15.72 ^c
Extract (10%)	9.95	9.35	7.85	9.05 ^b
Extract (15%)	11.53	14.85	13.83	13.4 ^c
Extract (20%)	13.58	15.2	18.15	15.64 ^c

Mean values followed the same superscript is not difference statistically by LSD 5%

Based on the data in Table 3, it is revealed that pandanus peel-off gel has antibacterial properties against *P. acnes* bacteria. Gel containing extract concentrations of 10%, 15%, and 20% all cause inhibition zones in bacterial growth compared to negative controls. Even at concentrations of 15% and 20%, the efficacy is statistically the same as the standard antibiotic clindamycin.

The antimicrobial properties of pandan leaf extract (*P. amaryllifolius*) have indeed been previously revealed by other researchers. Kusmawati & Hikmah (2022) found that pandan leaf extract can inhibit the growth of *Streptococcus mutans* bacteria [17]. Next, Wijayanti et al. (2025) found that pandan leaf extract has antibacterial properties on *Staphylococcus aureus*, *Escherichia coli*, and antifungal on *Pityrosporum ovale* [18]. The antifungal properties of pandan leaf extract were also found by Sinaga et al. (2021), so pandan leaf extract is also recommended as an anti-dandruff drug [19].

Bioactive compounds in pandan leaf extract that are suspected to have antimicrobial properties (antibacterial and fungal) are flavonoids, alkaloids, and polyphenols. Flavonoids are known to be anti-Gram-positive and Gram-negative bacteria. While alkaloids are antimicrobial against *Proteus vulgaris*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Vibrio cholera*, *Mycobacterium Tuberculosis*, against *Aspergillus niger* and *Candida albicans* fungi. Phenolic acid compounds are known to be anti-bacterial against *Bacillus subtilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* [20].

To better understand the real efficacy of bioactive compounds in pandan leaf extract, a more detailed and quantitative screening of the chemical compounds contained is needed. In this study, the bioactives detected were still limited to a group of compounds. In fact, each group of compounds consists of many varieties. Flavonoids, for example, consist of

apigenin, baicalein, catechin, chrysoeriol, coumestrol, cyanidin, delphinidin, daidzein, diosmetin, datiscetin, eriodictyol, epicatechin, gardenin, genistein, hesperetin, isorhamnetin, isocytoside, kaempferol, luteolin, morin, myricetintaxifolin, naringenin, pelargonidin, peonidin, quercetin, quercetagenin, sinensetin, tamarixetin, tangeretin, and vitexin [21-22]. Furthermore, alkaloids consist of various types such as alkaloidal amines, glyoxaline, indole, isoquinoline, imidazoline, lupinane, piperine, piperidine, pyrrolidine, phenanthridine, quinoline, quinazoline, tropane, and terpenoid [23].

4. Conclusion

Pandanus amarrilifolius leaf extract contains bioactive compounds that are antibacterial such as polyphenols, flavonoids, and alkaloids. Gel masks containing pandan leaf extract have characteristics that comply with the Indonesian National Standard (SNI 16-4399-1996). Furthermore, pandan leaf extract has been proven to have antibacterial properties against *Propionibacterium acnes*. Thus, it can be concluded that pandan leaf extract has the potential to be used as a peel-off gel mask as an anti-acne medication.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

References

- [1] Tan JK, Bhate K. A global perspective on the epidemiology of acne. *Br J Dermatol*. 2015 Jul;172 Suppl 1:3-12. doi: 10.1111/bjd.13462. PMID: 25597339.
- [2] Bhatia A, Maisonneuve JF, Persing DH. *Propionibacterium Acnes* and Chronic Diseases. In: Institute of Medicine (US) Forum on Microbial Threats; Knobler SL, O'Connor S, Lemon SM, et al., editors. *The Infectious Etiology of Chronic Diseases: Defining the Relationship, Enhancing the Research, and Mitigating the Effects: Workshop Summary*. Washington (DC): National Academies Press (US); 2004. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK83685/>
- [3] Vora J, Srivastava A and Modi H. Antibacterial and antioxidant strategies for acne treatment through plant extracts. *Informatics in Medicine Unlocked*, Volume 16, 2019, Pages 100229
- [4] Soleymani S, Farzaei MH, Zargarani A, Niknam S, Rahimi R. Promising plant-derived secondary metabolites for treatment of acne vulgaris: a mechanistic review. *Arch Dermatol Res*. 2020 Jan;312(1):5-23. doi: 10.1007/s00403-019-01968-z. Epub 2019 Aug 26. PMID: 31448393.
- [5] Lomthong T, Chorum M, Samaimai S, Thongpoem P. Antioxidant and antibacterial activities of *Pandanus amaryllifolius* Roxb. (Pandanaceae) prop roots and its application for a novel bacterial cellulose (Nata) fermentation by enzymatic hydrolysis. *J App Biol Biotech*. 2022;10(4):147-152. DOI: 10.7324/JABB.2022.100420
- [6] Nurunnahar, Mosaddik A, Zahan R, Maniruzzaman M, Uddin MH, Rahman MA and Khatun S. Analgesic, anti-inflammatory and CNS depressant activities of *Pandanus foetidus* Roxb. leave. *International Journal of Pharmaceutical Research and Applications* Volume 8, Issue 1 Jan-Feb 2023, pp: 2035-2041.
- [7] Quyen NTC, Quyen NTN, Nhan LTH and Toan TQ. Antioxidant activity, total phenolics and flavonoids contents of *Pandanus amayllifolius* Roxb. IOP Conf. Series: Materials Science and Engineering 001 (2020) 012019 doi: 10.1088/1757-899X/991/1/012019
- [8] Zakaria MM, Zaidan UH, Shamsi S and Gani SSA. Chemical Composition of Essential Oils from Leaf Extract of Pandan, *Pandanus amaryllifolius* ROXB. *Malaysian Journal of Analytical Sciences*, Vol 24 No 1 (2020): 87 – 96
- [9] Aini R & Mardiyarningsih A. Pandan leaves extract (*Pandanus amaryllifolius* Roxb) as a food preservative. *Jurnal Kedokteran dan Kesehatan Indonesia (JKKI)* 2016;7(4):166-173

- [10] Sa'adah SM, Putri FR, Ibtisam RA, Arrohmah RS & Fitriyah. Phytochemical analysis of secondary metabolite compounds of Pandanwangi leaf extract (*Pandanus amaryllifolius*). J. Nat. Scien. & Math. Res. Vol. 9 No. 2 (2023) 135-142
- [11] Hidayani CE, Ginting CN & Chiuman L. Analysis of Anti-Bacterial Activity of Ethanol Extract Fragrant Pandan Leaves (*Pandanus amaryllifolius* Roxb) Against the Growth of Disease Cause Pathogen Bacteria Using the Agar Diffusion Method. Budapest International Research in Exact Sciences (BirEx)Journal Volume 3, No 3, July 2021, Page: 213-228
- [12] Burhana NA, Akbar MF, Putri AS, Agustina E, Lusiana N & Purnamasari R. Comparison of Pandan Leaf Extract (*Pandanus amaryllifolius*) Using Ethanol and N-Hexane to The Content of Bioactive Compounds. The 3rd International Conference on Sustainable Health Promotion (ICOSHPRO), Surabaya, 30-31 August 2023: 48-60
- [13] Siddiqui SA, Ali Redha A, Salauddin M, Harahap IA, & Rupasinghe HPV. (2023). Factors Affecting the Extraction of (Poly)Phenols from Natural Resources Using Deep Eutectic Solvents Combined with Ultrasound-Assisted Extraction. Critical Reviews in Analytical Chemistry, 55(1), 139–160. <https://doi.org/10.1080/10408347.2023.2266846>
- [14] Patel K, Panchal N & Ingle P. Review of Extraction Techniques Extraction Methods: Microwave, Ultrasonic, Pressurized Fluid, Soxhlet Extraction, Etc . International Journal of Advanced Research in Chemical Science (IJARCS) Volume 6, Issue 3, 2019, PP 6-21
- [15] Anugrah N, Permadi A, Rahayu A, Maryudi, Suharto TE & Nazzal S. Comparative Analysis of Anti-aging Serum based SNI 16-4399-1996 using Aloe Vera, Binahong Leaves (*Anredera cordifolia*), and Mangosteen Peel (*Garcinia mangostana* L) Extracts. CHEMICA : Jurnal Teknik Kimia Vol. 11, No. 1, April 2024, pp. 17-25
- [16] Uce Lestari, Muhaimin Muhaimin, Yuhana Yuhana, Yuliawati Yuliawati. Physical Properties of Peel-Off Gel Mask Ethanol Extract of Surian Leaves (*Toona sinensis*) as an Antioxidant. Indonesian Journal of Pharmaceutical Science and Technology (IJPST) - SUPP 1(1), 2023; 90-99
- [17] Wijayanti R, Susmayanti W, MelianaDF, Fauziyah AH, Anjeline AM, Putri DY & Ulya FT. Antibacterial and Antifungal Activities of *Pandanus amaryllifolius* Leaf Extract, Fractions, and Isolate and Their Role in Anti-Dandruff Shampoo Optimization. Sciences of Pharmacy. 2025; 4(2):51-65.
- [18] Kusmawati FN & Hikmah NA. "Effect of Pandan Leaf Extract as Antibacterial on the Growth of *Streptococcus mutans* on Heat Cured Acrylic Resin Plate". EC Dental Science 21.3 (2022): 42-48
- [19] Sinaga A, Siregar S, Rizky VA & Topia R. Antifungal Effectiveness Test Fragrant Leaf Ethanol Extract (*Pandanus amaryllifolium* Robx) Against Fungus *Pityrosporum ovale* in Vitro. Journal of Engineering and Technology for Industrial Applications, 2021, pp. 42-46, doi:10.5935/jetia.v7i31.773.
- [20] Allemailem KS. Antimicrobial Potential of Naturally Occurring Bioactive Secondary Metabolites. J Pharm Bioallied Sci. 2021 Apr-Jun;13(2):155-162. doi: 10.4103/jpbs.JPBS_753_20. Epub 2021 May 26. PMID: 34349474; PMCID: PMC8291113.
- [21] Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. Scientific World J. 2013;2013:162750. doi: 10.1155/2013/162750.
- [22] Sarbu LG, Bahrin LG, Babii C, Stefan M, Birsa ML. Synthetic flavonoids with antimicrobial activity: A review. J Appl Microbiol. 2019;127:1282–90. doi: 10.1111/jam.14271.
- [23] Othman L, Sleiman A, Abdel-Massih RM. Antimicrobial activity of polyphenols and alkaloids in Middle Eastern plants. Front Microbiol. 2019;10:911. doi: 10.3389/fmicb.2019.00911.