

Antibacterial & Antioxidant Properties of Leave & Stembark Extract of *Artocarpus Heterophyllus* as the Component of Peel-Off Mask

Ahmad Budiman Siregar

Department of Chemistry

Graduate School-Department of Chemistry, Universitas Sumatera Utara

Rumondang Bulan

Department of Chemistry

Universitas Sumatera Utara. PUI Minyak Atsiri Eucaliptus

Yuniarti Yusak

Department of Chemistry

Universitas Sumatera Utara

Abstract

Antibacterial and antioxidant activities have been conducted using methanol extract of leave and stem bark of *Artocarpus* for its application as peel-off mask. The raw material was macerated using methanol and the obtained filtrate was evaporated until the concentrated crude extract was obtained. The crude extract is tested using several tests, such as phytochemistry screening, antibacterial test to *Propionibacterium acnes* and *Staphylococcus aureus* with the concentration of extract of 50; 100; 150 and 200 ppm, and antioxidant test using DPPH method. The additional test was conducted for evaluating the characteristic of peel-off mask, such as homogeneity, pH, organoleptic and irritation test. The result of phytochemistry screening showed those extracts were dominated with tannin and saponin. The antibacterial test to *P. acnes* showed the leave extract with concentration of 50, 100, 150 and 200 ppm showed the inhibition zone 9,6; 9,9; 10,4 and 11,3 mm. The other extract, the stem bark extract, provided the inhibition zone 8,4; 8,8; 9,5 and 10,5 mm at the similar concentration of extract. For *Staphylococcus aureus*, the leaves extract showed the inhibition zone 7,5; 7,8; 8,2 and 8,8 mm, while the stem bark extract 6,2; 6,4; 7,7 dan 9,2 mm. Those result revealed that the leave extract is more powerful to inhibit *P. acnes* than stem bark extract. But the different result was obtained when discussed about the inhibition of *S. aureus*, the stem bark extract showed the higher activity than leave extract. The measurement of antioxidant of leave extract at concentration 50; 100; 150 and 200 ppm showed the following activity data 22,91; 31,90; 32,76 and 40,47%, then the stem bark extract has the following activity data 43,89; 64,66; 70,02; 70,44%. These data showed the antioxidant activity of stem bark extract is higher than stem bark extract. The clinical testing of peel-off mask formulation showed that the F1, F2 and F3 has a good homogeneity and the pH was 6,7; 6,7 and 5,8. The irritation test showed a negative result for all of formulation.

Keywords: Antibacterial, Antioxidant, *P. Acnes*, *S. Aureus*, Jackfruit

I. INTRODUCTION

The problem that being faced in the field of medicine today is the bacterial resistance against antibiotics in the developing and developed countries. Therefore, a lot of research has been conducted that focused in the production of antibiotics from the synthetic process and secondary metabolite from natural product to deal with the bacterial resistance [1].

Bacteria are microorganisms that cannot be seen with the naked eye, but can only be seen with a microscope. Among them are *Staphylococcus aureus* and *Propionibacterium acnes* bacteria. *Staphylococcus aureus* is a coccus-shaped bacterium with a diameter of about 1 μm and it arranged in irregular groups [2]. In human, *Staphylococcus aureus* is found in the nose, skin, throat, and others. These bacteria can cause various infections, such as pneumonia, meningitis, empyema, endocarditis, zits, pyoderma, or impetigo [3] [4].

Propionibacterium acnes is a rod-positive gram-bacteria and it is a normal skin flora that plays a role in the formation of acne [5]. *Propionibacterium acnes* secretes hydrolytic enzymes which cause damage to the polysebaceous follicles and produce lipase, hyaluronidase, protease, lecithinase and neuramidase, which play an important role in the inflammatory process [6].

One kind of natural product that mostly used by people for treating any kind of health problem, including infection that caused by bacteria is leave and stem bark jackfruit (*Artocarpus heterophyllus* Lam.) The leave of jackfruit contains flavonoid, saponin and tannin that can act as antimicrobial and induce the production of new skin cell [7]. Also, the stem bark of jackfruit has some secondary metabolites, such as morin, sianomaklurin, flavonoid and tannin. Especially for the flavonoid that found in stem bark of jackfruit, there are some new compounds that has been discovered, such as morusin, artonin E, artokarpin, cycloartobiloxanton,

and artonol B. The bioactivity of those flavonoid compounds can be used as anticancer, antiinflammation, diuretic, and antihypertension [8].

II. MATERIALS & METHODS

The leave and stem bark of jackfruit (*Artocarpus heterophyllus* Lam) were obtained from Sigambal, Sumatera Utara. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{CeSO}_4 \cdot 4\text{H}_2\text{O}$, H_2SO_4 96%, DPPH were obtained from Sigma Aldrich. Ethanol and methanol were obtained from Merck. All chemicals were used without further treatment.

A. Phytochemical Screening Test

1) Alkaloid Test

The methanol extract of jackfruit leaves and stem bark were placed in several test tubes. The tube I was dropped with Wagner reagent, formation of brown/reddish precipitate indicates the presence of alkaloids. The tube II was dropped with Mayer reagent, formation of a yellow colored precipitate indicates the presence of alkaloids. Tube III was penetrated by Boucahardat reagent, formation of a chocolate colored precipitate indicates the presence of alkaloids and IV tube was dropped with Dragendorf reagent, formation of red precipitate indicates the presence of alkaloids.

2) Test for flavonoids

Jackfruit leaf methanol extract and bark were inserted into 2 test tubes. The I tube was dropped with 10% NaOH, if a violet blue solution was formed, it is positive for flavonoids. Tube II was added with concentrated HCl and Mg powder, if an orange solution was formed, it is positive for flavonoids.

3) Terpenoid Test

The methanol extract of jackfruit leaves and stem bark were inserted in a test tube then 1% CeSO_4 in 10% H_2SO_4 was added. If a brownish red precipitate was formed, it positively contains terpenoids.

4) Tanin Test

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

5) Saponin test

0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes, it indicates the presence of saponins.

B. Antioxidant Activity Test

1) Activity Test of Blanket Solution Antioxidants

A total of 2.5 ml of ethanol p.a and 1 ml of 0.3 mM DPPH solution was added into a test tube, homogenized and left for 30 minutes in a dark room. Then the absorbance is measured with a wavelength of 515 nm.

2) Test of Sample Antioxidant Activity

A total of 2.5 ml of methanol extract of jackfruit leaves and 5 ppm jackfruit bark also with 1 ml of 0.3 mM DPPH solution were added into a test tube, homogenized and left in a dark room for 30 minutes. Then the absorbance was measured with a maximum wavelength of 515 nm. The same treatment was carried out for concentrations of 50, 100, 150 and 200 ppm of extracts.

3) Antibacterial Activity Test of Jackfruit Leaf and Bark of Jackfruit Extract

Disc paper that has been soaked in the extract was placed in the petri dish that have contained specific bacteria, then incubated in an incubator at 35°C for 18-24 hours. The diameters of the inhibition zone around the disc paper were measured.

4) Making Masks

a) Mask Formulation from Jackfruit Leaf Methanol Extract

The peel-off mask was prepared by following the formulation in the Table 1 below:

Table – 1

Mask Formulation from Jackfruit Leaf Methanol Extract

Ingredient	Concentration (g)		
	F ₁	F ₂	F ₃
PVA	14	-	-
HPMC	-	2	-
Gelatin	-	-	30
Glycerin	5	5	5
Nipagin	0,2	0,2	0,2
Alcohol 96%	12,5	12,5	12,5
Aquadest	100	100	100
Extract	0,5	0,5	0,5

F = Formulation

C. Evaluation of Mask of Jackfruit Leaf Methanol Extract

1) Organoleptic

This observation included the observation of color, odor and clarity.

2) Homogeneity

The prepared gel was applied on the object glass, then the glass was clamped with another object glass. The homogeneity was observed with naked eyes.

3) Spread Ability Test

The prepared gel was weighed 1 gram then placed on a glass with size of 20x20 cm, then it was covered by mica and given a ballast, after 1 minute the diameter was measured.

4) pH test

A total of 1 g of prepared gel was dissolved with 10 mL of aquadest. The electrode of pH meter was dipped to the gel solution, the obtained pH was recorded.

5) Drying Speed Test

The prepared gel mask was applied to the skin of the back of the hand and then the dry speed to form a peel-off layer from the gel phase was measured using a stopwatch.

6) Irritation Test

The irritation test was carried out on 10 panelists, on the left hand of the panelist was tested with the prepared gel (F₁, F₂, and F₃).

III. RESULTS & DISCUSSION

Table – 2
Result of Phytochemical Constituent Screening Test of Leave and Bark of Jackfruit

No	Test	Reagent	Leaves	Stem bark
1	Flavonoid	FeCl ₃ 5%	-	-
		NaOH 10%	-	-
		H ₂ SO ₄	-	-
2	Alkaloid	Bouchardat	-	+
		Dragendorff	-	-
		Meyer	-	-
3	Terpenoid	CeSO ₄ 1%	-	-
		Salkowsky	-	+
4	Steroid	CeSO ₄ 1%	-	-
		Salkowsky	-	-
5	Tanin	FeCl ₃ 5%	+	+
6	Saponin	Aquadest	+	+

(+) positive (-) negative

The results of phytochemical screening tests of jackfruit leaves and bark that has been carried out (Table 2) with Bouchardat, Meyer and Dragondrof reagents did not give a color change indicating that it was negative for alkaloid. The results of terpenoid testing with 1% CeSO₄ did not form a brownish red precipitate then the results were negative. Jackfruit leaves and bark were positive to contain saponins in the presence of foam when the extract is shaken. When the extract was tested with FeCl₃ 5%, a blackish blue solution was obtained it indicated a positive result of tannin compounds.

A. Test Activity of Leaf Antioxidants & Jackfruit Bark

Table – 3
Antioxidant Activity Test Results of Jackfruit Leaf Methanol Extract

Concentration	Absorbance		%Inhibition	
	Leaves	Stem bark	Leaves	Stem bark
Blank	0,467	0,467		
50 ppm	0,360	0,262	22,91	43,89
100 ppm	0,318	0,165	31,90	64,66
150 ppm	0,314	0,140	32,76	70,02
200 ppm	0,278	0,138	40,47	70,44

Based on Table 3 shows that there is a decrease in the absorbance of DPPH with the addition of jackfruit leaf extract and jackfruit bark in DPPH solution compared to blank solution without the addition of jackfruit leaf extract and jackfruit bark. The greater decrease in absorbance shows greater antioxidant activity. This positive result is indicated with the color of the solution changes from dark purple to bright yellow and absorbance at its maximum wavelength will disappear. The decrease in absorbance value occurred due to the electron transfer of the antioxidant hydrogen atom to DPPH. DPPH is a free radical molecule that is stabilized by resonance forms.

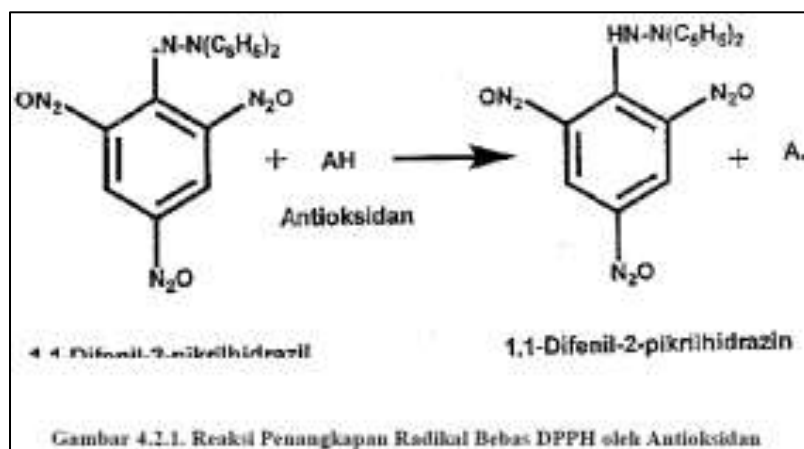


Fig. 1: Reaction of Free Radical Dpph by Antioxidan

From the Table 3, the IC₅₀ of each extract can be calculated by plotting the concentration of the test solution and the percent of the inhibition of DPPH as a parameter of antioxidant activity. The IC₅₀ for jackfruit leaf extract was 52.08 mg / ml and for jackfruit bark extract was 33.93 mg / ml.

B. Antibacterial Activity Test of Leaves and Jackfruit Bark

Table – 4
Antibacterial Activity Test of Leaves & Jackfruit Bark

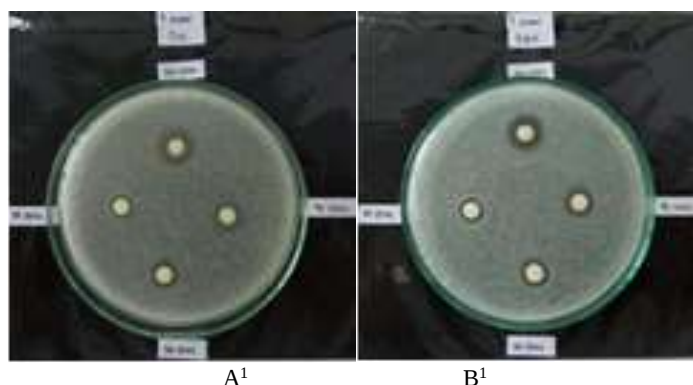
No.	Bacteria	Sample	Diameter of Inhibition Zone (mm)			
			200 ppm	150 ppm	100 ppm	50 ppm
1	<i>Staphylococcus aureus</i>	Stem bark	9,2	7,7	6,4	6,2
2	<i>Staphylococcus aureus</i>	Leaves	8,8	8,2	7,8	7,5
3	<i>Propionibacterium acnes</i>	Stem bark	10,5	9,5	8,8	8,4
4	<i>Propionibacterium acnes</i>	Leaves	11,3	10,4	9,9	9,6

The results of the research can be seen in Table 4. Jackfruit leaf extract can effectively inhibit bacterial growth at a concentration of 50 ppm. This shows that jackfruit leaf extract more effectively inhibits the growth of *Propionibacterium Acnes* and *Staphylococcus aureus* bacterial colonies compared to jackfruit bark extract with *Propionibacterium Acnes* and *Staphylococcus aureus* bacteria.

In this study, antibacterial activity test of jackfruit leaves and jackfruit bark was carried out. Jackfruit leaves and bark were tested against *Propionibacterium Acnes* and *Staphylococcus aureus*. From the phytochemical screening of jackfruit leaves and bark, tannins and saponins were detected, among the detected compounds, one of them was active as antibacterial [9][10]. Tannin that contained in the leave and stem bark of jackfruit is a compound that active as antibacterial that can inhibit the function of the cytoplasmic membrane in bacteria. At low concentrations these compounds can also damage the cytoplasmic membrane which causes the leaking of important metabolites that activate the bacterial enzyme system, while at high concentrations can precipitate cell proteins. [11].

Microorganisms have different response to the materials that used to turn them off. There are differences between species depending on the water content and pH of the environment and from the age of the cell or spore and so on.

The exponential destruction or destruction depends not only on the type of organism but on the various environmental conditions [12].



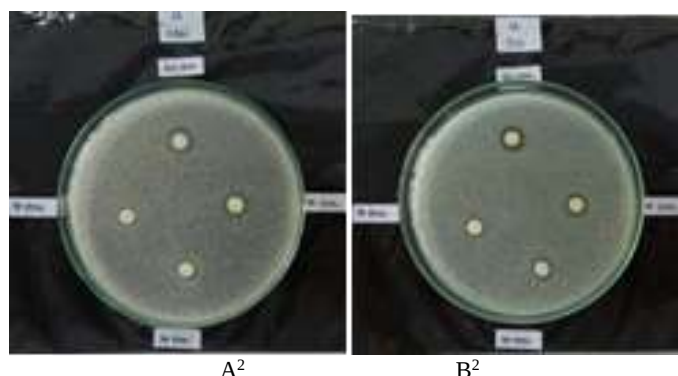


Fig. 2: Inhibitory Zones of Jackfruit Leaf Methanol Extract (A1B1) and Stem Jackfruit Skin (A2B2) Against *P. Agnes* (A1A2), *S. aureus* (B1B2) Bacteria, with extract concentrations of 50, 100, 150 and 200 ppm.

In Figure 2 it can also be seen that the differences in concentration also influence extracts in inhibit bacterial activity. There is a linear correlation between the diameter of inhibition and the concentration of leaf extracts and jackfruit bark. The greater the concentration of the jackfruit leaf and bark extract given the greater the clear zone formed. Whereas if we use methanol and water solvents without the provision of leaf extracts and clear zone jackfruit stems which form a bit this proves that the substances contained in the extract of the leaves and bark of jackfruit can affect anti-bacterial activity.

C. Mask Test

1) Organoleptic Test

The peel off gel mask of methanol extract was prepared with certain conditions.

Table – 5
Organoleptic Test Results

Sample name	Consistency	Colour	Fragrance
<i>F</i> ₁	thick rather liquid	black brown	Sedikit asam
<i>F</i> ₂	thick rather liquid	black brown	Sedikit asam
<i>F</i> ₃	thick rather liquid	black brown	Sedikit asam

Remarks: F = Formulation

2) Homogeneity Test

The homogeneity test of the three gel masks showed good homogeneity. The test results must show a homogeneous. The ingredients that used in making mask of methanol extract of jackfruit leaves must be well dispersed [13].

Table – 6
Homogeneity Test Results

Konsentrasi Gel	Homogeneity
<i>F</i> ₁	+
<i>F</i> ₂	+
<i>F</i> ₃	+

(+) homogeneity

3) pH stability test

The result of pH stability test can be seen in Table 7. The prepared masks, all of them meet the requirements of the pH, it around 4.5-6.5 [14]. The pH value should not be too acidic because it can cause skin irritation and also should not too alkaline will cause scaly skin [15].

Table – 7
pH Test Results

Parameter			
pH	<i>F</i> ₁	<i>F</i> ₂	<i>F</i> ₃
	6,7	6,7	5,8

4) Spread ability Test

The good gel dispersion is 5-7 cm. In that range, the gel mask will have a good property especially a comfortable during the use of the mask [16]. From the observations on the diameter of the spread of the prepared gel masks, the diameter of the spread of each gel was not met the requirement because the prepared gel mask has a slightly liquid consistency so that the ability to spread is wider.

Table – 8
Results of Spread Power Test

Sample	Diameter (cm)
<i>F</i> ₁	7,76
<i>F</i> ₂	7,82
<i>F</i> ₃	7,79

5) Drying Speed Test

From the observations, the prepared mask from jackfruit leaf extract has a drying time in the range of 18-19 minutes. This drying speed test shows how long it takes for the mask to dry out in its application. The good drying time for mask is in their range of 15-30 minutes after application [17].

Table – 9
Drying Test Results

Sample	Drying time (minute)
F ₁	21
F ₂	25
F ₃	23

6) Irritation Test

From the observations, there was no visible change in the skin after test. It means there was no irritation reaction from 10 panelists. That result indicated the gel mask can be used safely and does not cause irritation to the skin.

Table – 10
Irritation Test Results

Sample	Eritema	Edema
F ₁	-	-
F ₂	-	-
F ₃	-	-

IV. CONCLUSION

From the results of the research can be concluded:

- 1) Based on phytochemical screening test, jackfruit leaf and bark extract contained tannin and saponin compounds.
- 2) The test results of antioxidant activity of methanol extract of jackfruit leaves and bark bark showed a strong antioxidants activity, 52.08 mg / ml and 33.93 mg / ml, respectively.
- 3) The higher the concentration of methanol extract of leaves and jackfruit bark, the higher the inhibitory effect on the growth of *Propionibacterium Acnes* bacteria with inhibitory zones of 9.6 to 11.3 mm at the concentration of 50-200 ppm and *Staphylococcus aureus* in the concentrations of 50-200 ppm with zones. inhibition of 6.2–9.2 mm
- 4) Jackfruit leaf methanol extract better inhibitory effect on *Propionibacterium Acnes* than methanol extract of jackfruit bark, and jackfruit leaf methanol extract better inhibitory effect on *Staphylococcus Aureus* than methanol extract of jackfruit bark.

REFERENCES

- [1] Wang Y., Lu Z.X., Wu H., Lv F.X. (2009): Study on the antibiotic activity of microcapsule curcumin against foodborne pathogens. *International Journal of Food Microbiology*, 136: 71–74.
- [2] Chung Y.C., Chen C.Y. (2008): Antibacterial characteristics and activity of acid-soluble chitosan. *Bioresource Technology*, 99: 2806–2814.
- [3] Rios, J. L. & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*, 100, 80–84.
- [4] Satish, S., Raghavendra, M. P. & Raveesha, K. A. (2008). Evaluation of the antimicrobial potential of some plants against human pathogenic bacteria. *Advances in Biological Research*, 2, 44.
- [5] Kadler BK, Mehta SS, Funk L. *Propionibacterium acnes* infection after shoulder surgery. *Int J Shoulder Surg* 2015;9:139e44.
- [6] Hudek R, Sommer F, Kerwat M, Abdelkawi AF, Loos F, Gohlke F. *Propionibacterium acnes* in shoulder surgery: true infection, contamination, or commensal of the deep tissue? *J Shoulder Elbow Surg* 2014;23:1763e71.
- [7] Ibrionke AA, Comparative study of the chemical composition and mineral element content of *Artocarpus heterophyllus* and *Treculia africana* seeds and seed oils. *Bioresour Technol*, 99: 5125-5129, (2008).
- [8] Chrips, NR, Balasingh GR and Kingston C (2008). Nutrient constituents of neglected varieties of *Artocarpus heterophyllus* Lam. from Kanyakumari district, South India. *Journal of Basic and Applied Biology* 2(3&4) 36-37.
- [9] Jagtap UB, Bapat VA (2010). *Artocarpus*: A review of traditional uses, phytochemistry and pharmacology. *J. Ethnopharmacol.* 129:142-166.
- [10] Khan MR, Omoloso AD, Kihara M (2003). Antibacterial activity of *Artocarpus heterophyllus*. *Fitoter* 74:501–505.
- [11] Rios JL, Recio MC (2005). Medicinal plants and antimicrobial activity. *J. Ethnopharmacol.* 100:80-84.
- [12] Velazquez MME (2005). Emergence and spread of methicillin-resistant *Staphylococcus aureus*. *Pub. Health Mex.* 5:1-17.
- [13] Reveny, J., Surjanto, J.T., & Lois, C. Formulation of Aloe Juice (Aloe vera (L) Burm. f.) Sheet Mask as Anti- Aging. 2016., *International Journal of PharmTech Research*, 9(7), 105-111
- [14] Minh, N.G., Investigation of Pickled Water Spinach (*Ipomoea aquatic*) Fermentation by *Lactobacillus* sp. *International Journal of Multidisciplinary Research and Development*, 2014, 1(5): 71-80.
- [15] Paithanker, V. V. 2010. Formulation and evaluation of herbal cosmetic preparation using safed musli. *International Journal of PharmTech Research*, 2(4), 2261-2264
- [16] Jadoon, S., Karim, S., Asad, M. H. H. B., Akram, M. R., Kalsoom Khan, A., Malik, A., & Murtaza, G. 2015. Anti-aging potential of phytoextract loaded-pharmaceutical creams for human skin cell longevity. *Oxidative medicine and cellular longevity*.
- [17] Shai, A., Maibach, H. I., and Baran, R., *Handbook of cosmetic Skin Care*, Second Edition, UK: Informa Healthcare, 2009, 6.