The Physicochemical Characteristic and Inhibition Zone of Eschericia coli in Ketepeng (Cassia alata L.) Leaf Extract Transparent Soap

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Keywords: Inhibition Zone of Eschericia coli, Ketepeng Leaf Extract, Physicochemical Characteristic, Transparent Soap.

Abstract: Ketepeng *(Cassia alata)* is a wild plant that is traditionally recognized as a medication for skin diseases. The leaf of Ketepeng has a phytochemical content that plays a role to inhibit the growth of Eschericia coli. A transparent soap with an addition of leaf extract is one of the innovations to increase the economic value of the Ketepeng plant. This research aims to identify the effect of adding leaf extract on the quality of transparent soap based on the Indonesian National Standards (SNI). The experiment design that used is Completely Randomized Design (RAL) with a three-time repetition. It comprised three different treatments consist of the addition of leaf extract as much as 2, 4, and 6 grams in 50-gram coconut oil as the basic material of soap. The result of this research indicated that increasing the concentration of leaf extract might increase saponification value, hardness, pH, inhibition zone of Eschericia coli ATCC 25922. It was also reported to increase the attribute texture of the transparent soap, as well as to decrease water content, foam stability, free alkali, and soap transparency. The best treatment in this study was the transparent soap which was added by 6 grams of ketepeng leaf extract.

1 INTRODUCTION

The COVID-19 (coronavirus) pandemic requires people to live clean. Washing hands with antiseptic soap is one way to prevent COVID-19. Transparent soap with the addition of ketepeng left extract is an alternative to antiseptic soap. According Widiarto, Janiarta, Intan and Hajiriah (2018) antiseptic soap is a soap that contains chemical compounds that are used to kill or inhibit the growth of microorganisms on living tissues such as the surface of the skin and mucous membranes. The use of antiseptics is highly recommended when there is an epidermal disease because it can slow the spread of the disease the spread of the disease.

Ketepeng leaf extract has secondary metabolite compounds for example alkaloid, flavonoid, tannin, anthraquinone, and saponin have antibacterial activities against Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa (Ekwenye and Okorie, 2010; Lumbessya, Abidjulua, and Paendonga 2013). Ecoli is a gram-negative bacteria that can cause skin diseases. However, it can be prevented by the use of antibacterial soap (Stevens, Nicholas, and David 2003). Generally, secondary metabolite compounds have antibacterial. Thus, they can be used as active materials in a soap-making process. Meanwhile, saponin contains several properties like the foaming agent, easily soluble, surfactant compounds, and antibacterial (Tebogo, 2004).

Indonesia is home of tremendous natural resources. One of the natural resources is Ketepeng plant which is known as a cure and anti-bacteria for skin problems such as ringworm and itching (Kusmardi, Kumala, and Triana, 2007). Traditionally, Ketepeng plant is applied by rubbing or sticking its leaves to the affected skin. Esimone, Nworu, Ekong, and Okereke (2007) discuss that this plant has an excellent wound-healing characteristic. The results of herbal soap which adding ketepeng leaves extract with ethanol extract (95%) indicate has antibacteria activities covering S. aureus, B. subtilis, E. coli, P.

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aeruginosa, and C. Albicans. The soap with adding of ketepeng extract on the market.

Ecoli is microorganism which can serve as an indicator towards the possible existence of pathogen. Pathogen is gram negative bacteria that can cause skin diseases. However, it can be prevented by the use of antibacterial soap (Stevens et al. 2003). antibacterial properties such as saponin, tanin, terpenoid (Ugbabe, Ezeunala, Edmond, Apev and Salawu., 2010), alkaloid (Jasim, Hussein, Hameed and Kareen 2015), flavonoid, tripenoid (Cushnie and Lamb, 2005). Thus, they can be used as active materials in a soap-making process. Meanwhile, saponin contains several properties like foaming agent, easily soluble, surfactant compounds, and antibacterial (Widyasanti and Hasna, 2017 ; Tebogo, 2004).

This research uses coconut oil as a basic ingredient in making soap. Coconut oil contains saturated fatty acids which play a role in the foaming properties of the soap. In particular, Lauric acid (C12:0) in coconut oil produces soap with high solubility and soft foam characteristics (Anggraini, Ismanto, and Dahlia, 2015; Habib, Kumar, Sorowar, Karmoker, Khatun and Al-Reza, 2016). This becomes the basis of the research because it is assumed that the addition of ketepeng leaf extract with different concentrations can affect the physicochemical characteristics of the resulted soap and increase the inhibition zone of Escherichia coli. This research could be antiseptic soap alternative in the pandemic situation.

2 MATERIAL AND METHODS

2.1 Raw Material

This research was conducted in two phases. It started by preparing sorting ketepeng leaves, extraction, and a transparent soap making process. The ketepeng leaf were taken into 5 parts from left to right of the stem base of the Ketepeng. Prepare 50 g coconut oil. After that, the soap involved chemical materials by using NaOH (merck) 5 g, NaCl (merck) 0.2 g, citric acid 0.2 g, glycerin (brataco) 6.5 g, aquadest 5 ml, sugar (brand: Gulaku) 7.5 g and ethanol (merck) 70% 7.5 g. Pure inoculant of ATCC 25922 Escherichia coli.

2.1.1 Ketepeng Leaf Extraction

The process of ketepeng leaf extraction was performed by refining 20 g ketepeng leaves which had been sorted and dissolved with a 200 ml alcohol solvent. The ratio of ingredients and solvents was 1:10. Having filtered the basic ingredients, then the filtrate was evaporated by using a rotary evaporator (RV-10, IKA-WERKE, Germany) at a 70ºC temperature, a speed of 160 rpm, and a pressure of 2.5. The process took place for 9 hours in order to obtain thick extracts (Hernani, Bunasor and Fitriarti, 2010).

2.1.2 Making of Transparent Soap

The transparent soap was made by heating 50 g coconut oil at 70ºC temperature. After that, 5 g NaOH was dissolved to 5 ml of water and stirred until dissolved. Put the solution in the oil and then add 7.5 g of ethanol and 7.5 g of sugar. Prior to that, the ethanol and sugar should be dissolved. Finally, add glycerin by 6.5 g. All materials were stirred until the stock of soap was formed. It was followed by adding 0.2 g of NaCl, 0.2 g of citric acid, and extract of ketepeng leaf by 2, 4, 6 g until all the mixture was immunized. After that, the mixture was poured into a mold and it was cooled until the soap hardens. The resulted soap was then examined in several tests like free alkali test, water content test, hardness, pH test, saponification value, foam stability, inhibition zone test, and organoleptic test of color, texture, transparency.

2.2 Statistical Analysis

This research used a completely randomized design using 3 (three) repititions. After gathering the data, if F table was smaller than F count, further test was conducted namely the least significant different (LSD) with 0.01 rate. The observation parameter covered:

2.3 Parameter Analysis

2.3.1 Water Content

This research counted 5 gram of transparent soap in a petri dish and put in the oven at 105ºC temperature for 4 hours. Then, the soap was cooled, desiccated, weighted, and heated for 1 hour to obtain a constant weight. After that, the water content was calculated by using the following formula:

2.3.2 pH

The soap was weighted 1 g and dissolved in 10 ml aquadest. When necessary, the mixture could be heated to accelerate the dissolution process. After that, a pH meter was dipped in the solution. Acidity degree (pH) was observed to obtain and take note the results.

2.3.3 Hardness

To measure the level of hardness towards the resulted soap, the research employed a penetrometer tool.

2.3.4 Foam Stability

1 g of sample was dissolved in 9 ml water and then poured in a reaction tube. Then, the mixture was shaken by using vortex for 30 seconds. This process resulted foam and the height of the foam was measured. The sample foam was left for 1 hour and the foam was measured again. If the sample number was more than one, the dimensions of all tubes must be similar. To measure, the foam stability, the following formula could be performed (1).

$$\frac{\text{Final foam height (mm)}}{\text{Initial foam height (mm)}} \text{x } 100\ \% \quad (1)$$

2.3.5 Saponification Value

The soap was weighted 1.5 g in 250 ml of *Erlenmeyer*. Add 50 ml NaOH solution in alcohol. After that, the solution was covered by a condenser and boiled for 30 seconds. Then, add several drops of phenolphthalein indicators and titrate with 0.5 N HCl until its color changed from pink to transparent. Saponification measure following formula (2).

$$\frac{(V2-V1)\ x\ N\ x\ 0{,}04}{0{,}258\ x\ W}\ x\ 100\% \quad (2)$$

Description:
V_2 = The volume of the sample titration
V_1 = The volume Blanco titration
N = Normality 0,5 HCl
0,04 = Atom Weight NaOH
0,258 = The average saponification value of coconut oil
W = Sample weight

2.3.6 The Number of Free Alkali

The transparent soap was weighted 10 g and dissolved in 50 ml of hot alcohol. After that, drop indicators pp 2-3 drops, refluxed for about 30 minutes. Then the soap was cooled and titrated by using KOH 0.1% until it turned to pink. Take note the titration volume and calculate the free alkali (3).

$$\frac{VxBMx\ N}{W\ x\ 1000}\ x\ 100\% \quad (3)$$

Description:
V = Titration volume
BM = molecule weight
W = Sample weight

2.3.7 Bacteria Inhibition Zone Test

1) Microbe Rejuvenation Test

NA Media was weighted 5 g and dissolved by aquadest by 250 ml. Sterilization was performed until the temperature reached 121ºC. Keep it still for 15 minutes then the media was put into reaction tubes respectively to form titled NA. After the solid titled NA was obtained from the Eschericia coli ATCC 25922 breed as much as one inoculating loop. Then, they were inoculated in the titled NA media. The tested bacteria were incubated for 24 hours at 37ºC and it could be used as tested bacteria.

2) Making test bacterial suspension

Test bacteria resulted from rejuvenation process went through suspension process by using NaCl 0.9% solution which was put into respective reaction tubes. After that, it was mixed with a sterilized NA media and then immunized.

3) Bacteria inhibition zone test

NA media was turned to a solid form in a petri dish. After being solid, prepare a bacteria suspension test. Dip a stick with cotton at the end on the bacteria suspension test than swab vertically and horizontally on the NA surface which had been solidified until all surfaces were covered. Leave it until all surfaces dried. Meanwhile, each transparent soap sample was melted on a hot plate. Soak disc paper for 1 hour in the soap sample which was melted and then dry the disc paper.

After all NA surfaces dried, the disc paper which was soaked in the transparent soap was put on the NA surface by using pin set. It was then incubated at 37ºC for 24-48 hours. The petri dish which was incubated for 24-48 hours then was observed and measured to obtain its inhibition diameter.

2.3.8 Organoleptic Test

Organoleptic test was performed to score the attribute of transparency and texture. Panelists in this research were 25 semi-trained panelists. The assessment score was based on the level of quality where 1 (one) indicated the lowest score and 7 (seven) meant the highest.

3 RESULT AND DISCUSSION

3.1 Water Content

High water content in the soap may cause a hydraulic reaction between water and fat which is not sapofinied to form free fat acid and glycerol. This may cause the decrease of quality during storage (Vivian, Nathan, Osano, Mesopirr and Omwoyo, 2014). The high water content may lead the soap to easily dissolve in water, shrink, and have a soft texture (Hambali, Bunasor, Suryani and Kusumah 2005). In addition, it may cause a short span of the soap storage (Habib, et.al., 2016). Table 1 shows that the water content kept decreasing as the addition of ketepeng leaf extract. The water content of soap which was added by ketepeng leaf extract by 2, 4, and 6 gram decreased by 18.15, 12.09, and 10.78%. According to Indonesian National Standard (SNI), the maximum water content of soap is 15%.

Table 1: Water content of Transparent Soap.

Ketepeng Leaf Extract Treatment	Water Content (%)
2 gram	18.15^{a} ±0.23
4 gram	12.09^{b} ±0.41
6 gram	10.78^{c} ±0.28

The decrease of water content in the soap which was added by ketepeng leaf extract was resulted from active compound of saponin contained in the plant. This compound possessed a characteristic to absorb water which could decrease the percentage of water in the soap. Widyasanti and Hasna (2017) in their study on transparent soap with white tea extract claim that the more white tea extract added to the soap, the more water will be absorbed by sugar so that the water in the soap decreases. Saponin is a glycoside compound which will produce glycone (sugar) and glycone (non-sugar) if hydrolyzed. Sugar has hygroscopic properties and causes the soap to solidify.

3.2 Saponification Value

The saponification value gives information about the solubility in water and soap formation (Ohimain, Izah, and Fawari, 2013). The degree of saponification depends on the type of ion, its ionic strength, the temperature of the solution, and above all on the pH of the solution (Briscoe, Evans, and Tabor, 1976). Saponification value indicates the neutralization process between fatty acid and base in the process of soap stock. If the saponification is not perfect, it may increase the percentage of free alkali or unsaponified fat acid. As a result, it can decline the quality of the soap.

The addition of leaf extract increased significantly the value of saponification (Tabel 2). The increase of this value is a result of alkaloid content in the leaf extract. Alkaloid constitutes a hydrogen base so that the addition of leaf extract will affect the solution's basicity. Cotte, Checroun, Susini, Dumas, Tchoreloff, Besnard and Walter (2006) explain that the process of triolein saponification with various types of lead salts on pH base can increase the percentage of soap amount. Sears and Schulman (1964) argue that pH 13 saponification is essentially complete. At a lower pH 8.5 the degree of association between the cation and the fatty acid could be less than complete.

Table 2: Saponification Value of Transparent Soap.

Ketepeng Leaf Extract Treatment	Saponification Value (Mg/NaOH)
2 gram	155.5^{c} ± 0.81
4 gram	164.8^{b} ± 0.18
6 gram	178.2^{a} ± 0.46

3.3 Hardness

Level of hardness test aims at understanding the efficiency of the soap when used. Hard soap is considered higher resistance towards damage or form changing as a result of physical disturbances.

Table 3: Hardness of Transparent Soap.

Ketepeng Leaf Extract Treatment	Hardness
2 gram	0.5^{c} ±0.04
4 gram	1.1^{b} ±0.05
6 gram	1.4^{a} ±0.02

Level of hardness on the soap correlated with the percentage of water content and saponification value. The water content decreases when the leaf extract is added and the level of hardness increases too. Similarly, the increase also occurs to saponification value that can affect the hardness of the soap. Saponifaction value provides information about the soap forming process (Ohimain et al. 2013). A high value of saponifation that has a lot of greased fat indicates a high quality of the soap (Ketaren, 1986 cited in Kusumaningsih and Hastuti, 2014). Soap texture can be affected by the length of hydrocarbon chain and oil doubled bond.

3.4 Foam Stability

Foam stability is measured by esthetic value of a soap product. Customers perceive that good soap is those which produce much foam. In fact, the amount of foam is not necessarily correlated positively with its ability to clean dirt. There is no requirement regarding the minimum or maximum of foam height for soap.

The results of this research revealed that the addition of leaf extract in the soap would make the foam stability low. Based on LSD (0.01) soap that was added 4 and 6 grams of the leaf extract was significantly different from a 2-gram addition. The concentration of leaf extract increases significantly decrease the stability of the foam.

Table 4: Foam Stability of Transparent Soap.

Ketepeng Leaf Extract Treatment	Foam Stability
2 gram	$55.5^a \pm 1.08$
4 gram	$38.4^b \pm 0.79$
6 gram	$35.7^b \pm 0.92$

Ketepeng's leaf which is extracted by using ethanol contains alkaloid, saponin, flavonoid, tannin, and antraquinon compounds (Lumbessya et al. 2013). Jin-Young Park et al. (2005) argue that saponin has several properties such as easy to dissolve in water, surfactant, and the ability of form stable foam in solution. For these reasons, saponin is often used as an agent of foaming, emulsifier, and detergent in meal and non-meal products. However, as the concentration of leaf extract increases, the stability of the foam decreases. This is in line with a study by Widyasanti and Hasna (2017) in transparent soap with white tea extract. Adding white tea extract would make the foam stability low. This occurs because the foam stability is influenced by ethanol content in the white extract. Putri (2017) supports that methanol and ethanol in the extracts play a role as antifoaming agent in the soap.

Beside saponin, alkaloid also affects the stability of foam in soap. Saunders (1935) widely claims that the tension of soap surface is affected by pH. pH itself is influenced by the amount of organic base like alkaloid. Widyasanti and Hasna (2017) posit that alkaloid has base properties causing it to increase the degree of acidity in soap. According to Gwi-Taek Jeong, Hwa-Won Ryu, Yung-Il Joe, Don-Hee Park and Tanner (2002) base pH can potentially decrease the foam stability. Hence, it is assumed that the addition of leaf extract will increase alkaloid content in the soap that it can decrease the foam stability.

Ketepeng extract contains pigment chlorophyll, the pigment can arise in the solution can increase surface tension, thereby reducing foam stability. The presence of dissolved substances in the liquid will increase viscosity which in turn will increase surface tension and result in reduced foaming ability.

3.5 pH

Incomplete hydrolysis from saponification process produced high pH values so that acidity degree (soap pH) should be measured. Normal soap pH is around 9.0-10.8 (Gusviputri, Meliana, Aylianawati and Indraswati 2013). pH 10 has base properties and is good for skin. If the pH is <9, it might cause the skin dry. Meanwhile, pH values which are >10.8 may cause skin irritations. Table shows that the addition of leaf extract by 2, 4, and 6 gram has acidity degree or pH around 9.33-9.67. It indicates that pH of transparent soap which is added by the leaf extract has met SNI 06 3532 1994.

Table 5: pH of Transparent Soap.

Ketepeng Leaf Extract Treatment	pH
2 gram	$9.33^a \pm 0.94$
4 gram	$9.67^a \pm 0.47$
6 gram	$9.67^a \pm 0.47$

3.6 Free Alkali

Free Alkali in soap is caused by the presence of alkali which does not react with fat acid during a saponification process (Zulkifli and Estiasih, 2014). The increase of concentration of leaf extract significantly raises the saponification values. It leads to the decline of free alkali because of the lack of alkali which reacts with fatty acid. Based on SNI 06 3532 1994 regulations, the value of free alkali in soap should be a maximum of 0.1%. In this research, the LSD (0.01) test of free alkali towards the three treatments was not significantly different. It shows us that the tested soap is safe. High free alkali (>0.1%) will lead to skin irritations (Hernani et al. 2010) and dry skin (Widyasanti and Hasna, 2017).

Table 6: Free Alkali of Transparent Soap.

Ketepeng Leaf Extract Treatment	Free Alkali (%)
2 gram	$0.0060^a \pm 0.006$
4 gram	$0.0056^a \pm 0.006$
6 gram	$0.0026^a \pm 0.003$

3.7 Inhibition Zone

Escherichia coli is bacteria that cause diarrhea, urinary tract infection, meningitis, peritonitis, mastitis, septicemia, pneumonia, and food poisoning (Brooks, Butel and Morse 2008). Inhibition zone is an active substance's or compound's ability to inhibit the growth of microbe. It is marked by the existence of transparent zone on agar media. Based on LSD (1%) test, the inhibition zone of transparent soap towards the three treatments was significantly different. The more the addition of leaf extract on the soap would be able to inhibit the growth of Escherchia Coli ATCC 25922. The difference of the inhibition zone size from each concentration stemmed from the difference of active compound contents. It was shown by the size of the inhibition zone which increasingly widespread.

Juliansyah and Paotonan (2017) support that the higher the concentration of the extract, the more active compounds which potentially serve as antibacterial. The ketepeng leaf extract contains secondary metabolite compounds like alkaloid, flavonoid, anthraquinone, and saponin. These compounds constitute as phenolic compounds which has a phenol group. Hernani et al. (2010) argue that the phenol group has bacteriacide properties because it may cause protein coagulation and bacteria cell membranes may become lysis.

Table 7: Inhibition Zone of Transparent Soap.

Ketepeng Leaf Extract Treatment	Inhibition Zone (mm)
2 gram	$1.8^{c} \pm 0.24$
4 gram	$4.0^{b} \pm 0.47$
6 gram	$6.0^{a} \pm 0$

In this research, the inhibition zone of transparent soap which was added leaf extract by 2 gram (1.8 mm) was categorized weak. The addition of the extract by 4 gram (4 mm) and 6 gram (6 mm) was grouped medium. Magdalena and Kusnadi (2015) explain the level of resistance of negative Gram bacteria (Escherichia coli ATCC 25922) was high One of the reason was because the structure of bacteria cell walls of the negative gram bacteria was complex. It had three layers, outside layers lipoprotein, middle layer lipopolysaccharide which served to block antibacterial bioactive materials, and inside layer peptidoglycan which had high lipid contents (11-12%). This made the active compound of leaf extract difficult to penetrate the nonpolar lipid layer on Escherichia coli ATCC 25922.

3.8 Organoleptic Test

An organoleptic test for transparent soap with leaf extract covered attribute of transparency and texture ranging from 1 – 7. Soap texture assessment is performed by pressing samples using fingers. In the other side, transparency test is done by using the senses of vision by observing samples with the help of light (Meilgaard, civele and Carr, 1999).

Table 8 show that the texture attributes of the three treatments were not significantly different. It can be seen from the results of the panelist's assessment which pointed out that the three treatments had the same texture (from neutral to rather hard). Meanwhile, the attributes of transparency in the three different treatments were very significant (neutral to transparent).

Table 8: Organoleptic Test of Transparent Soap.

Ketepeng Leaf Extract Treatment	Organoleptic Test	
	Texture	Transparency
2 gram	4,32	$5{,}48^{a}$
4 gram	4,40	$5{,}36^{a}$
6 gram	4,72	$4{,}20^{b}$

The increased concentration of leaf extract could improve the texture of the soap. This result is correlated with the decrease of the soap water content. Low water content in soap increases the hardness of the soap (Tabel 1). Based on the organoleptic test, The texture attributes of the three treatments were not significantly different. It indicates that the addition of leaf extract does not affect consumer perceptions towards texture attributes.

Meanwhile, adding 2 and 4 grams of ketepeng leaf extracts was significantly different from 6 grams on the attribute of transparency (Tabel 8). It shows that the addition of ketepeng extract affects consumer perceptions of the attributes of transparency. The more addition of leaf extract to transparent soap, the browner and less transparent soap is. According to Anggraini et al. (2015) a heating process causes chlorophyll (green) to become a compound of pheophytin (olive green). Therefore, if the soap is added by more leaf extract, the soap will turn to dominantly brown. The flavonoid content contained in the extract also affects transparency. According to Ahmad, Hasan, Muhamad, Bilal, Yusof and Idris (2018) the brightness level of transparency decreases with the increased content of flavonoids and phenol compounds.

Based on the Indonesian National Standards (SNI) for solid bath soap, the treatment of the addition of 6-gram ketepeng leaf extract had a better quality soap than the two treatments. The treatment also had the largest ATCC 25922 Escherichia coli inhibition zone as much as 6 mm despite having low foam stability by 35.7. Finally, the organoleptic score of texture attribute was 4.72 (rather hard) and the transparency value was 4.20 (from neutral to rather transparent).

4 CONCLUSION

In conclusion, increasing the concentration of leaf extract could increase saponification, hardness, pH, Escherichia coli inhibition zone ATCC 25922, and the texture attributes of transparent soap. In addition, it is capable of reducing water content, foam stability, free alkali and soap transparency.

The best treatment in this study was transparent soap which was added by leaf extract as much as 6 grams, although it produced soap which its transparency attributes ranged from neutral to rather transparent.

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