Effect of betel type (*Piper* sp) and concentration of betel leaf extract on quality and antibacterial activities of glycerine bar soap

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**Abstract.** Betel is an Asian medical plant that has antibacterial properties. Addition of betel extract to soap during its production steps may affect the quality characteristics, organoleptic and antibacterial activity of glycerine bar soap. This research aims to identify the effect of red and green betel by applying its ethanol extract on soap. This study used a Factorial Completely Randomized Design (FCRD) using the factor of betel types, red betel (S1) and green betel (S2) and concentrations of betel extract, which are 2.5%, 5%, 7.5%, and 10%. The collected data were analysed using the Analysis of Variance test (ANOVA) and continued with the Duncan test Multiple Range Test (DMRT). Test results showed that the soap has moisture contents between 1.24% – 5.64%, free alkalis between 0.03% – 0.44%, and pH with range 10.72 – 11.31. Red betel soap has a higher moisture content and free alkali levels as the added betel concentration increases. For its aroma, colours, and transparency, panellists hedonically tended to like the red betel soap more than the green. The transparent soap with addition 2.5% red betel extract has the best quality, as well as organoleptic and antibacterial activity characteristics when compared to other glycerine bar soaps.

1. **Introduction**

Soap is a product of hydrolysis of fatty acid with sodium or potassium. Currently, soap is a daily necessity that functions as a cleanser and beauty product [1]. Soap consumption is increasing every year in Indonesia and unfortunately national production is unable to make a supply that meets this demand. This has caused the amount of soap imported into Indonesia to reach up to 110,203,081 tons in June 2019 [2]. Generally, soap is available in two forms bar and liquid. There are also different types of soap using sodium (NaOH) and liquid soap using potassium (KOH). Soap can be divided into three types based on the clarity: opaque, translucent, and transparent or commonly known as glycerine soap bar [3].

Transparent soap has advantages over other soaps. Transparent or glycerine soap bar has a clear appearance and produces a soft foam because it contains a lot of glycerine which functions as a moisturizer [4]. Therefore, when consumers look for an attractive skincare soap, which has soft foam and leaves the skin moisturized, they usually look for transparent bar soap. Today, many soaps are produced with an addition of natural ingredients. Natural ingredient soaps are in great demand by the consumer, especially those that are beneficial for healthier skin.

Green betel (*Piper betel*) is one of the most common types of medical plants and has many benefits for daily life. Betel is reported to have 4.2% essential oil that has antibacterial effects [5]. Green betel
leaf extract has a strong effectiveness in inhibiting the growth of *Staphylococcus aureus* bacteria [6]. Red betel (*Piper crocatum*) is known in Indonesia as an ornamental plant. The utilization of this plant is not popular due to the lack of knowledge about its functional potential [7]. According to Juliantina et al. [8], 25% red betel ethanol extract can inhibit the growth of and kill *Staphylococcus aureus* and 6.25% red betel ethanol extract can inhibit the growth of *Escherichia coli*. Therefore, this study aims to identify the effects of red and green betel by applying its ethanol extract as an ingredient of soap. The use of red betel and green betel is expected to serve as a substitute of synthetic antibacterial substances which are commonly used nowadays.

2. Materials and methods

2.1. Material

The materials used in this study were fresh red betel and green betel leaves obtained from Ulee Kareng District, Banda Aceh. They were harvested on the day of use. The other materials included indicator phenolphthalein, *Staphylococcus aureus* culture *Mueller Hinton Agar* (Oxoid CM0337), water, and transparent bar soap making ingredients, which are listed in Table 1.

| Table 1. Material of 200 g glycerine soap bar. |
|----------------------------------------------|
| Material | Composition (g) |
| Stearic acid | 14 |
| Palm oil | 40 |
| NaOH 30% | 40.6 |
| Glycerine | 26 |
| Ethanol | 30 |
| Sucrose | 34 |
| Coco DEA | 6 |
| NaCl | 0.4 |
| Water | 9 |

2.2. Experimental design

This experiment was conducted according to a Factorial Completely Randomized Design (FCRD) with betel types as one factor, which are, red betel (S1) and green betel (S2) and concentrations of betel extract as another factor, which are, 2.5% (K1); 5% (K2); 7.5% (K3); and 10% (K4). Each experimental unit is repeated 3 times meaning that in total, there were 24 units of experiments. Data were analysed using the Analysis of Variance test (ANOVA) and examined further with the Duncan’s Multiple Range Test (DMRT). For organoleptic data, tabulations were done based on SNI 01-2346-2006 and continued to be statistically measured based on ANOVA.

2.3. Method of extraction

Fresh betel leaves were washed clean then dried using the oven at 40 °C for 24 hours, until the betel leaves became dry. Dried betel leaves were crushed into powder using a blender. Betel powder was soaked using 96% ethanol with a ratio of 1:5 for 72 hours. The separation of filtrate and residue was carried out by filtering using filter paper. Then, the filtrate was evaporated using a rotary vacuum evaporator to separate the extract from the solvent [6].

2.4. Glycerine soap bar production

Materials were weighed according to the amount in Table 1. Those were put in to a beaker glass with 14 g stearic acid then heated for 2 minutes. Then, 40 g of palm oil was added and it was heated again until the temperature reached 70 °C. Then, 40.6 g NaOH was added and stirred until homogeneous, producing soap stock. Then, 26 g of glycerine, 30 g of ethanol, 34 g of coco dea, and 6 g of NaCl were added. The temperature was then kept constant at 70–80 °C for 30 minutes or until all materials
dissolved. The temperature of the soap solution was then cooled to 40 °C. Then, the extract of betel is diluted using ethanol, according to specified concentrations (2.5%, 5%, 7.5%, and 10%). The next step was to add 10 ml of the extract to the soap solution and stir until the mixture was homogeneous, and then do soap bar moulding. The soap was left to harden for 30 minutes and cure for three weeks before being analysed [9].

2.5. Analysis of glycerine soap bar
The analysis of glycerine soap bar measured water content [10], free alkali level [11], pH [11], antibacterial activities on *Staphylococcus aureus* [11], and organoleptic (hedonic) preferences [12].

3. Result and discussion

Figure 1 shows glycerine soap bar with an addition betel extract, after 3 weeks of curing time. Based on SNI 3532-2016, all produced samples are qualified in accordance with SNI. The measured quality parameters are moisture content, free alkali level, pH, and antibacterial activities to see the effect of betel extract as an active substance on the resistance of bacteria.

![Figure 1. Glycerine soap bar extract red betel leaves and green betel leaves.](image)

3.1. Moisture contents
Moisture content of the glycerine soap bar in this study ranged from 1.24%–5.64% with an average of 3.03%. Based on ANOVA, only the betel type factor has a significant effect (P<0.01) on the moisture content of transparent bar soap. This is shown in Figure 2.

As shown in Figure 2, the red betel soap has a higher moisture content (3.78%) than the green betel soap (2.27%). This might be caused by the differences of moisture content from the betel extract. Green betel extract is reported to have a moisture content of 3.33% [13], and red betel extract has 6.52% [14]. Based on SNI, the maximum water content for soap is 15% [10]. The low moisture content of transparent bar soap produced in this study was caused by the evaporation of the water in the soap during the three weeks curing process. Within that time, the soaps were stored at room temperature and not covered in plastic packaging. Both transparent bar soaps were produced using the same method.
Figure 2. The effect of betel type on glycerine soap bar moisture content in DMRT<sub>0.05</sub> = 0.982, KK = 37.46% (Values followed by the same letter show an unreal difference based on LSD test).  

3.2. Free alkali level  
Free alkali formation is a cause of a failure of fatty acid to bind all alkali which is added during the soap production [15]. The safe amount of free alkali contained in sodium soap is a maximum of 0.1%, in accordance with SNI regulation 3532-2016. Soap with an amount of free alkali more than the prevailing SNI limitation will be considered poor quality and cannot be used as commercial soap [10].  
The free alkali value of this glycerine soap bar ranged from 0.03% to 0.44% with average of 0.17%. ANOVA shows that betel type (S) factor and the interaction between two factors have a very significant effect (P<0.01) on free alkali value, as presented in Figure 3. Figure 3 shows the effect of interaction between betel type and concentration of betel leaf extract towards alkali levels of glycerine soap bar. Figure 3 shows an increasing trend in free alkali value of soap bar as the extract betel concentration increased. This trend is clearly visible in both red and green betel glycerine soap bars.  

Figure 3 also shows that red betel, which had the highest amount of added extract concentration (10%), was also the soap with highest free alkali value (0.40%) which is very statistically noticeable when compared to the other treatments. This phenomenon might be influenced by the fact that the
amount of alkaloid compounds in red betel extract is than higher in the green one [16]. The alkaloids in red betel are reported as being in the weak alkaline primary amine group [17]. Because of this, red betel extract already has alkali present and it requires higher amounts of fatty acid in order to bind all the present alkali therefore decreasing the presence of free alkali.

3.3. pH

The result of pH glycerine soap bar with added betel extract on this study ranged from 10.72–11.31 with an average of 10.96. The pH of all produced soap bar meets the quality requirements. In accordance with applicable regulations [18], the best soaps have a pH range from 9 to 11. The ANOVA showed the interaction combination of betel type and betel leaf extract concentration treatment has a very significant effect on pH value of glycerine soap bar and can be seen in Figure 4.

![Figure 4](image)  

Figure 4. The effect of betel type interaction and betel leaf extract concentration on glycerine soap bar pH in DMRT<sub>0.05</sub> = 0.233, KK = 1.08% (Values followed by the same letter show an unreal difference based on LSD test).

Figure 4 shows the pH value of glycerine soap bar increases as the concentration of green or red betel extract is added to the soap making process. The soap with red betel extract 10% has the highest pH value. The pH value of the soap has the same tendency as the free alkaline levels: the higher the free alkaline content in the soap, then the higher the pH value. The pH value of the soap can be influenced by active compounds contained in betel, such as flavonoid, alkaloids, polyphenols, tannins and essential oils in red betel, and phenols, saponins, chavicol, terpenes, tannins, carotene, thiamine, vitamin C, starch sugar and amino acids in green betel. This difference in active compounds in both types of betel leaf can cause characteristic differences in each resulting soap product [19]. In addition, according to Widyasanti et al. [20], the addition of white tea to liquid soap will increase the pH value, this is because white tea contains alkaloids which are alkali.

3.4. Antibacterial activities

An antibacterial activity test was conducted to determine the ability of the transparent bar soap to inhibit bacterial growth. In this study, an antibacterial activity test was conducted on only the soap with the addition of red betel extract 2.5%, because it was determined to be the best treatment based on quality characteristic and organoleptic preferences. The antibacterial activity test results of the soap, following the diffusion method can be seen in Table 2.
Table 2. Antibacterial activity test of transparent bar soap against Staphylococcus aureus bacteria.

| Test Material                                      | Diameter of Bacterial Inhibition (mm) |
|--------------------------------------------------|--------------------------------------|
| Commercial antibacterial soap                     | 8                                    |
| Glycerine soap bar with 2.5% red betel extract    | 7.7                                  |
| Commercial soap (without antibacterial)           | 6                                    |
| Aquadest                                          | -                                    |
| Red betel extract                                 | 3                                    |

Table 2 showed that glycerine soap bar with 2.5% red betel extract can inhibit Staphylococcus aureus bacterial growth. The used of red betel extract is thought to increase the inhibitory power of soap against the growth of Staphylococcus aureus bacteria so that it can match the inhibitory power of commercial antibacterial soap. The active compounds in red betel have antibacterial properties, namely alkaloids, flavonoids, tannins and essential oils. Alkaloids inhibit bacterial growth by inhibiting DNA synthesis by intercalating. Flavonoids can increase the permeability of cell membranes so that they can inhibit bacterial activity. Tannins play a role in disrupting bacterial cell membranes and it form the complex compounds using enzymes that inhibit bacterial growth. Essential oils play a role in disrupting the formation of cell walls so that cell walls are not formed or are formed imperfectly [21].

3.5. Organoleptic (Hedonic) references of Glycerine Soap Bar
3.5.1 Colours. Organoleptic preferences were measured by the five hedonic scale, where 1 is weighed as strongly disliked and 5 is strongly liked. The colour of produced glycerine soap bar based on the panellists’ favourite levels range from 1.54–4.06 (dislike it to like it) with average score of 2.82 (neutral). The ANOVA showed betel type and concentration of betel leaf extract treatment have a significant effect on the attitude of the panellists about the various colours of the soap. The results can be seen in Figure 5.

![Figure 5](image-url)

**Figure 5.** Effect of betel extract type on transparent bar soap colour based on panellist acceptance in DMRT<sub>0.05</sub> = 0.166 advanced test, KK = 6.82% (Values followed by the same letter show an unreal difference based on LSD test). Scale values: 1 (strongly disliked), 2 (disliked), 3 (neutral), 4 (liked) and 5 (strongly liked).

Figure 5 showed that panellists preferred the colour of the red betel soap over the colour of the green betel soap. The difference in likeness can be caused by the pigments contained in both types of betel. Carotene, anthocyanins, chlorophyll a, chlorophyll b and xanthophyll are some of the types of pigments.
found in betel leaves. However, the amount of pigment content differs in each type of red and green betel. The pigments in the two types of betel cause different colours of soap, explaining the difference in consumer preferences. Carotene, anthocyanins, chlorophyll a, chlorophyll b and xanthophyll are some of the types of pigments found in betel leaves. The amount of these pigments differs in red and green betel. Red betel contains higher anthocyanins (7.643 mg/g), carotene (0.00059 mg/g) and xanthophyll (0.00027 mg/g). These pigments are the sources of the orange and red colours and they are the reason for the brighter colours preferred by the panellists [22]. The effects of extract concentration on the favourite colour of soap can be seen in Figure 6.

Figure 6. The effect of betel leaf extract concentration on glycerine soap bar colour based on panellist acceptance on DMRT_0.05 = 0.253 advanced test, KK = 6.82% (Values followed by the same letter show an unreal difference based on LSD test). Scale values: 1 (strongly disliked), 2 (disliked), 3 (neutral), 4 (liked) and 5 (strongly liked).

The panellists preferred the colour of the soap with the lowest concentration (2.5%) of betel leaf extract. It was perceived as a 3.73 (liked), which differs from other treatments. This shows that as the level of betel extract leaf concentration increases, the hedonic colour preferences decreased. This might be because the bar soap has darker colour as the concentration increases. According to Kanifah et al., [23] red betel extract has a flavonoid compound that can produce a brownish-red colour index so that the higher the concentration, the darker the resulting colour will be (Figure 1). This is similar to research by Hernani et al., [24] that the addition of high concentrations of Alpinia galanga extract produces soap with a colour that panellists disliked, which is because the extract has a brownish colour. The darker colour comes from the higher concentration of the added extract.

3.5.2 Transparency. The transparency of the soap is based on the panellists’ favourite level range from 1.56-4.02 (very dislike-like) with average of 2.82 (neutral). The ANOVA shows both of these factors have a very significant effect on the panellists’ preference for transparency and this can be seen in Figure 7.
Figure 7. The effect of betel type on glycerine soap bar transparency based on panellist preference on DMRT<sub>0.05</sub> = 0.210, KK = 8.63% (values followed by the same letter show an unreal difference based on LSD test). Scale values: 1 (strongly disliked), 2 (disliked), 3 (neutral), 4 (liked) and 5 (strongly liked).

The panellists preferred transparent soap (Figure 7). When transparency is the same, red betel soap bar was preferred by panellists over the green soap. The amount of pigment contained in each type of extract produces its own colour. Since red betel extract has brighter colour pigments than the green betel, the produced soap bar is likely more transparent and was more liked by the panellists. This can be seen in Figure 8.

Figure 8. Effect of betel leaf extract concentration on glycerine soap bar transparency based on panellist preference in DMRT<sub>0.05</sub> = 0.321 advanced test, KK = 8.63% (values followed by the same letter show an unreal difference based on LSD test). Scale values: 1 (strongly disliked), 2 (disliked), 3 (neutral), 4 (liked) and 5 (strongly liked).

Figure 8 shows that panellists had a stronger preference for the betel soap bar with the lowest concentration (2.5%). As the concentration increases, the soap tends to have a darker colour and is less transparent. This finding is similar to previous research from Putri and Suhartiningsih [25], which reported that moringa leaf extract has a blackish green colour, and when added to soap, produces a bright green, more transparent product, which is preferred by the panellists.

3.5.3 Aroma. Panellist hedonically measured the aroma of glycerine soap bar based in the range from 2.58 to 3.23 (neutral-neutral) with average of 2.91 (neutral). The ANOVA showed betel type (S) treatment has a very significant effect on panellist preferences of the aroma of the soap and this can be seen in Figure 9.
The effect of betel type on the aroma of glycerine soap bar based on panellist acceptance on advanced test DMRT₀.₀₅ = 2.99, KK = 4.49% (values followed by the same letter show an unreal difference based on LSD test). Scale values: 1 (strongly disliked), 2 (disliked), 3 (neutral), 4 (liked) and 5 (strongly liked).

The red betel soap had a higher aroma preference value (3.04), and the green betel soap was less preferred by panellists (2.78). The aroma of the soap comes from essential oils in the betel extract. The different aromas of these essential oils caused the panellists to have a difference in preference. Green betel essential oil has a yellowish-white colour with a strong aroma while the red betel essential oil has a clear colour with a soft aroma [26].

3.5.4 Hardness. Panellist measured the hardness of glycerine soap bar hedonically range from 2.68-3.39 (neutral-neutral) with average of 3.05 (neutral). The ANOVA betel leaf extract (K) treatment has a very significant effect on panellist preferences of hardness glycerine soap bar and this can be seen in Figure 10.

From Figure 10, it can be shown that the addition level of 10% betel leaf extract produces a soap that is less hard and more preferred, in comparison to higher levels of betel extract. The soap bar with 10% added extract betel leaf tends to have a harder texture than the soap bar with lower concentration. Because betel extract contains alkaloid compounds, it has a significant influence on soap hardness. Glycerine soap bar with the addition of betel extract up to 10% will increase alkali levels in the soap, which causes the free alkali value and pH to increase. As the free alkali level increases, the soap is denser [27]. In addition, according to Agustini and Winarni [28], one of the factors that can increase the hardness of soap is the curing process, but in this study the curing process was the same for all soaps used.

3.5.5 Lots of foam. In this research, panellists were asked their preferences towards the amount of foam produced from the glycerine soap bar. Panellist tend to score the soap foam hedonically in range from 2.26 to 3.71 (dislike-like it) with average of 3.10 (neutral). Based on ANOVA, it can be shown that betel type and betel extract concentration has no significant effect on the soap foam production. According to Rozi [29], foam production is influenced by the addition of palm DEA to the soap making process. In this study, the same amount of palm DEA was added to all soaps. This was intentional so as to control the amount of foam produced by the soap. Palm DEA serves as a foam stabilizer which is very important in increasing the amount of foam and forming the texture of foam.

3.5.6 After used. After used parameter is a sensation received by panellist after using the soap. Panellist measured the after used sensation in hedonic scale, which range from 2.98-3.49 (neutral) with average of 3.23 (neutral). Based on ANOVA, it can be shown that both of independent variables, the betel type and betel extract concentration have no significant effect on the panellist’s preferences towards its after
used effects. The rough or soft sensation on the skin produced by the soap is influenced by the free alkali properties and pH. In this study, all the resulting soap bar had a pH in the same range of 10.72–11.31, so it is reasonable that panellists tend to receive similar sensation and impression after using the soap. Continuous use of glycerine soap bar containing high pH and free alkali will irritate the skin.

![Figure 10. The effect of betel leaf extract concentration on the hardness of glycerine soap bar based on panellist acceptance on DMRT_0.05 = 0.170, KK = 4.24% (values followed by the same letter show an unreal difference based on LSD test). Scale values: 1 (very disliked), 2 (dislikes), 3 (neutral), 4 (likes) and 5 (very likes).](image)

4. Conclusion

In conclusion, the types of betel used in the glycerine soap bar production have a significant effect on soap bar moisture content, free alkali and pH values, as well as consumer preference for aroma, colour, and transparency. Red betel soap appears to have higher moisture content, free alkali level, and pH, as well as consumer preference for its aroma, colour, and transparency. Moreover, higher concentrations of betel leaf extract added to the soap led to higher free alkali level and pH, but a weaker preference from consumers for its colour and transparency. Based on all the measured parameters and its antibacterial activity with *Staphylococcus aureus*, the addition of red betel extract up to 2.5% is considered to be the optimal formula to produce a glycerine soap bar which is qualified to meet the national standards for soaps.

Reference

[1] Agung P and Fatma P D 2017 Rekayasa Ind. dan Inf. 210–216
[2] BPS 2019 Statistik Perdagangan Luar Negeri Impor juni (Jakarta: Badan Pusat Statistik)
[3] Prihandana R, Noerwijari K, Gamawati P and Setyaningsih D 2017 Bioetanol Ubi Kayu: Bahan Bakar Masa Depan (Jakarta: Agomedia Pustaka)
[4] Momuat L I and Wuntu A D 2017 *J. Ilmu. Sains* 17 169–174
[5] Sastroamidjojo S A 2001 *Obat Asli Indonesia* (Jakarta: PT Dian Rakyat)
[6] Inayatullah S 2012 *Skripsi* (Jakarta: Universitas Islam Negeri Syarif Hidayatullah)
[7] Hermiati, Naomi Y M and Mersi S S 2013 *J. Tek. Kim.* 2 37–43
[8] Juliantina F, Citra D A and Nirwani B 2009 *J. Kedokt. dan Kesehat. Indones.* 1 1-10
[9] Hambali E, Bunasor T K Suryai A and Kusumah G A 2012 *J. Agroindustrial Technol.* 15 46–53
[10] BSN 2016 SNI Sabun Mandi Padat (Jakarta: Badan Standarisasasi Nasional)
[11] Ayu D F, Nadi B S and Ali A 2018 *J. Teknol. Ind. Pertan.* 28 210–218
[12] Rohaeti E 2018 Skripsi (Bogor: Institut Pertanian Bogor)
[13] Wijaya W A, Paramita N L P and Susanti N M P 2018 J. Kimia 12 36-42.
[14] Suhermanto 2013 Skripsi (Bogor: Institut pertanian Bogor)
[15] Widyasanti A and Rohani J M 2017 J. Penelit. Teh dan Kina. 20 13-29
[16] Sudewo B 2005 Basmi Penyakit Dengan Sirih Merah (Surabaya: Agomedia)
[17] Azzahra F, Lukmayani Y, and Sadiyah E R 2015 Pros. Penelit. Spes. Unisba. 45–52
[18] ASTM 2001 Standard Guide for pH of Aqueous Solutions of Soap and Detergents (USA: ASTM)
[19] Shalini T, VermaN K, Singh D P, Chaudhary S K and Asha R 2012 Int. J. of Pharm Res and Dev 4 216-223
[20] Widyasanti A, Septianur A S and Rosalinda S 2019 J. Tekn. dan Ind. Pert. Ind 11 11–18
[21] Farida J R, Marshananda M A, Sheila H P, Zulfa N and Afivudien M 2018 Mutiara Med. J. Kedokt. dan Kesehat. 18 13–19
[22] Muthoharoh L 2011 Skripsi (Malang: Universitas Negeri Malang)
[23] Kanifah U, Lutfi M and Susilo B 2015 Bioproses Komod. Trop. 3 73–79
[24] Hernani T, Bunasor and Fitriati 2010 Buletin Littro 21 192-205
[25] Putri W E S and Suhartiningsih 2016 e-Journal Unesa 5 96-104.
[26] Widiyastuti Y, Haryanti S and Subositi D 2013 Balai Besar Litbang Tanaman Obat dan Obat Tradisional. 6 86-93
[27] Gunawan H 2010 Skripsi (Makasar: UIN Alauddin Makassar)
[28] Agustini W and Winarni A H 2017 J. Pascapanen dan Bioteknol. Kelaut. dan Perikanan. 12 1-12
[29] Rozi M 2013 Skripsi (Surakarta: Universitas Muhammadiyah Surakarta)