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Antibacterial Activity of Transparent Soap Added Avocado Oil and Telang Flower Extract (*Clitoria ternatea*)

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ABSTRACT
Telang flowers contain secondary metabolites, one of which is antibacterial because it has potential as an alternative to antibiotics. The purpose of the research was to increase the function of transparent soap moisturise the skin and inhibit the growth of bacteria on the skin's surface. The butterfly pea flower was extracted by maceration using 96% ethanol, and then the extract was added to the making of transparent soap according to the treatment. The treatments in this study were the addition of S0 (0 mL) butterfly pea flower extract as a control; S1 (0,10 mL); S2 (0,15 mL); S3 (0,20 mL); S4 (0,25 mL) into 100 mL of transparent soap formulation. The best formulation resulting from this study was the S4 treatment (6 g cocoa butter, 7 g stearic acid, 14 g palm oil, 5 g avocado oil, 48% NaOH 7 g, 13 g glycerin, 15 g ethanol, cocamide- DEA 5 g, 17 g sugar, 0.2 g NaCl, 10.45 g aquadest and 0.3 g perfume and 0.05 g dye. The results of the antibacterial activity produced from the butterfly pea flower extract and avocado oil resulted in an inhibitory effect on *P. acne* bacteria ranging from 8,1-20,1 mm, which had moderate to strong inhibition, and *E. coli* bacteria ranged from 21,1-28,8 mm which had very strong inhibition.

Keywords: Antibacterial, Avocado Oil, Telang Flower, Transparent Soap

ABSTRAK
Bunga telang memiliki kandungan senyawa metabolit sekunder salah satunya antibakteri, karena memiliki potensi sebagai alternatif pengganti antibiotik. Penelitian ini bertujuan untuk menambah fungsi dari sabun transparan selain dapat melembabkan kulit serta dapat menghambat pertumbuhan bakteri pada permukaan kulit. Bunga telang diekstrak dengan cara maserasi menggunakan etanol 96%, kemudian ekstrak bunga telang ditambahkan pada pembuatan sabun transparan sesuai perlakuan. Perlakuan dalam penelitian ini yaitu penambahan ekstrak bunga telang S0 (0 mL) sebagai kontrol; S1 (0,10 mL); S2 (0,15 mL); S3 (0,20 mL); S4 (0,25 mL) ke dalam 100 mL formulasi sabun transparan. Formulasi terbaik yang dihasilkan dari penelitian ini yaitu pada perlakuan S4 (cocoa butter 6 g, asam stearik 7 g, minyak kelapa sawit 14 g, avocado oil 5 g, NaOH 48% 7 g, gliserin 13 g, etanol 15 g, cocamide-DEA 5 g, gula 17 g, NaCl 0,2 g, aquadest 10,45 g dan parfum 0,3 g serta pewarna 0,05 g. Hasil aktivitas antibakteri yang dihasilkan dari ekstrak bunga telang dan avocado oil menghasilkan daya hambat pada bakteri *P. acne* berkisar antara 8,1-20,1 mm yang memiliki daya hambat sedang sampai kuat dan bakteri *E. coli* berkisar antara 21,1-28,8 mm yang memiliki daya hambat sangat kuat.

Kata Kunci : Antibakteri, Bunga Telang, Minyak Alpukat, Sabun Transparan

1. Introduction

Soap is an ingredient that cleans dirt and bacteria on the skin. Currently, the use of soap as a skin cleanser is increasing and becoming more diverse. The diversity of commercially sold soaps can be seen in the types, fragrances, colors and benefits offered. Bath soap is divided into two types: liquid and solid. Solid soap consists of 3 types, namely opaque, translucent and transparent. Opaque soap (ordinary solid soap) is a soap that is used daily. Translucent soap is soap whose properties are between opaque and transparent, while transparent soap is often used for facial beauty and skin health soap [1]. If dirty all day, the skin will develop bacteria that can infect if not cleaned. Adding efficacious ingredients to soap is expected to inhibit bacterial growth more

effectively. The addition of plant extracts to transparent soap can increase its function. One is by adding telang flower extract (*Clitoria ternatea L.*).

Telang flower (*Clitoria ternatea L.*) is one source of material used as a blue natural dye. The blue color produced from the flower petals of this telang plant comes from the anthocyanin compounds contained in it [2]. According to Purba (2020), seen from a phytochemical review, telang flowers have several active ingredients with pharmacological potential. The pharmacological potential of telang flowers, among others, is as an antioxidant, antibacterial, anti-inflammatory and analgesic, antipyretic and anticidal, anti-diabetic, anti-cancer, antihistamine, immunomodulator, and potential role in the central nervous system (CNS) [3]. Telang flowers have a class of tannin compounds, flobatanins, carbohydrates, saponins, triterpenoids, flavonoid phenols, flavonol glycosides, proteins, alkaloids, anthraquinones, anthocyanins and steroids. This class of compounds can inhibit bacterial growth on facial skin because it has a potential as an alternative to antibiotics [4].

Avocado oil has a similar content to olive oil. The vitamin content in avocado oil is very diverse, namely vitamin A, riboflavin, pyridoxine, pantothenic acid, folic acid, thiamine HCl, ascorbic acid, niacin, choline, biotin, and vitamin E. The vitamin content is very important in the cosmetic industry because it hydrates and regenerates. Hence, it is useful as an anti-aging and anti-wrinkle product, improves dry skin, and makes elasticity in the skin [5].

According to Dreher and Davenport (2013), avocado oil is rich in vitamin content and fatty acids consisting of oleic acid and linoleic acid, which are efficacious in increasing skin moisture. The avocado oil concentration is 2 - 10% in moisturising preparations [5].

2. Materials and Methods

2.1. Equipment

The tools used in this study include glassware, analytical balance, hotplate stirrer, magnetic bar, stative and clamps, triple neck flask, condenser, oven, thermometer, and sample cups.

2.2. Materials

The materials used in this study include palm oil, cocoa butter, sodium hydroxide (NaOH), glycerin, ethanol 96%, sugar, sodium chloride (NaCl), aquadest, Telang flower, avocado oil, fragrance oil, Dragendorff reagent, Bouchardat reagent, Mayer reagent, hydrochloride acid (HCl), $FeCl_3$ 1%, sulfuric acid, n-hexan, phenolphtalein indicator, methyl orange indicator, stearic acid, cocamide dea, dyes, metal Mg, concentrated HCl, methanol, paraffin, neutral ethanol, Liebermann-Burchard reagent.

2.3 Research Procedures

2.3.1 Preparation of Telang Flower

Telang flower samples were washed thoroughly with running water for three washes. Then, the washed samples were put into the oven at 50ºC for 4 hours.

2.3.2 Preparation of Telang Flower Simplisia Extract

Telang flower simplisia extract was prepared by maceration using 96% ethanol solvent. Dry powder of telang flower was put into a beaker, 96% ethanol solvent was added, and soaked for 24 hours while occasionally stirring. The macerate was separated by filtration and then repeated three times. (Pharmacopoeia, 2017).

2.3.3 Phytochemical Screening Test of Telang Flower Extract

2.3.3.1 Alkaloid Screening

A total of 0.5 g sample was added 1 mL of 2 N HCl and 9 mL of distilled water, heated in a water bath for two minutes, and cooled. Filtrate used for the following experiments:

(i) 3 drops of filtrate were added two drops of Mayer reagent solution to form a white or yellowish-white clumpy precipitate.

(ii) 3 drops of filtrate were added two drops of Bouchardat reagent solution to form a brown-to-black precipitate.

(iii) 3 drops of filtrate were added to two drops of Dragendorff reagent solution, forming a red or orange precipitate [6].

2.3.3.2 Flavonoid Screening

A sample of 0.5 g was added to 2 mL of 50% methanol. It was heated at 50℃ and then cooled. The magnesium and 5 drops of concentrated hydrochloric acid were added. If a red/orange color appears, it is positive for flavonoids [7].

2.3.3.3 Saponin Screening

A total of 0.5 g of sample was put into a test tube, and added 10 mL of hot distilled water, cooled, and then shaken vigorously for ten seconds. If a stable foam of 1-10 cm in height is formed for not less than ten minutes and does not disappear with 2 N HCl, it indicates the presence of saponins [6].

2.3.3.4 Tannin Screening

A total of 0.5 g of sample was put into a test tube, added 5 mL of distilled water and then added one to two drops of 1% iron (III) chloride reagent. If a blue-black or green-black color occurs, it indicates the presence of tannins [8].

2.3.3.5 Steroid and Terpenoid Screening

1 g of simplisia powder was macerated with 20 mL of n-hexane for two hours. Then, the obtained maserate was filtered, the filtrate was evaporated in a vaporizer cup, and Liebermann-Burchard reagent was added to the rest. If a greenish-blue or purple-red color is formed, it indicates the presence of terpenoids/steroids [8].

2.3.4 Transparent Soap Making

The essential ingredients for 100 g of transparent soap formulation were melted 6 g cocoa butter, 7 g stearic acid, 14 g palm oil and 5 g avocado oil in a beaker glass at 70-80°C. After that, a 48% NaOH solution of 7 g was added gradually while stirring until soap stock was formed. After soap stock was formed, 13 g of glycerin, 15 g of ethanol and 5 g of Cocamide DEA were added and stirred until homogeneous. Measured pH to 9, then 17 g sugar, 0.2 g NaCl, 10.45 g distilled water, 0.3 g perfume and 0.05 g dye while stirring slowly until homogeneous with the temperature that has been lowered, poured into the mold and allowed to stand at room temperature until frozen.

2.3.5 Transparent Soap Characteristic Test

2.3.5.1 Water Content Test

As much as 4 g of sample was weighed and put into a porcelain cup with a known weight. The sample was heated in an oven for 2 hours at 105°C. Then, the sample was cooled in a desiccator, then weighed. This treatment is repeated until a constant weight is reached. The moisture content of transparent soap can be calculated by the formula presented in Equation 1:

$$\text{Water content (\%)} = \frac{(W1-W2)}{W}\ x\ 100\% \qquad \text{(Eq.1)}$$

Description:
W1 = Weight of sample + empty petri dish (g)
W2 = Weight of sample after drying (g)
W = Weight of sample (g) [9].

2.3.5.2 Free Fatty Acid Test

A sample of 1 g was weighed and put into 250 mL Erlenmeyer. Then, neutral ethanol was added 50 mL and heated until the sample dissolved. After the sample dissolves, three drops of Phenolphthalein indicator are added and titrated with 0.1 N NaOH drop by drop through the burette until a pink color appears, lasting 30

seconds. Free fatty acid content in transparent soap can be calculated using the formula presented in Equation 2:

$$\text{FFA (\%)} = \frac{V \; x \; N \; x \; BM}{W \; x \; 1000} \text{ x } 100\% \qquad \text{(Eq.2)}$$

Description:
V = NaOH 0.1 N used (mL)
N = Normality of NaOH used
W = Weight of the sample used
BM = NaOH equivalent weight [9].

2.3.5.3 Fatty Acid Content Test

Weighed carefully 10 g of sample prepared in a glass beaker 250 mL and added 100 mL of distilled water, heated in a steam bath. Dripped methyl orange pointer, then added 20% H_2SO_4 enough to red color. Stirred with a stirring rod to make it homogeneous, covered with a watch glass, then heated continuously until two transparent layers were formed. Put into it, 10 g of paraffin weighed carefully. It was heated for several hours until the whole mixture became clear again. Cooled quickly in a water bath. After the paraffin and fatty acid/fatty acid mixture became solid, it was removed from the glass beaker. Wax cake is weighed using a glass beaker of known weight [9].

$$\text{FA content (\%)} = \frac{W \; cake \; wax - W \; parafin}{W} \text{ x } 100\% \qquad \text{(Eq.3)}$$

2.3.5.4 Antibacterial Activity Test on Soap Preparations

- Dissolving Soap with Aquadest

A 1 g soap sample was weighed and dissolved with 1 mL of distilled water and homogenized with a vortex.

- Preparation of Suspension

Bacterial isolates are inserted into sterile distilled water, and then homogenized with vortex. They are then measured with a spectrophotometer with OD 0.5.

- Working on Bacterial Inhibition

Poured Muller Hinton Agar (MHA) media aseptically into a petri dish, left to solidify. Sterile cotton buds were taken aseptically and inserted into the bacterial suspension, then applied to the surface of the MHA media. Then, divided into four parts with a marker on the outside of the Petri dish, marked the part that will be given with the control +, control - and treatment samples, then took paper discs soaked in soapy water respectively, and placed on the part that has been marked according to the type of antibiotic, incubated for 24 hours. The inhibition zone around the disc paper was measured.

2.3.5.5 Hedonic Test

The test method used to measure the level of liking for the product using an assessment sheet by giving each sample of transparent solid soap preparation made to the panelist. The panelists used are random, namely panelists selected from a limited circle based on testing their level of sensitivity. The number of panelists needed to carry out the hedonic test is 25 people. The panelists will assess liking and dislike, aroma, color, skin impression, foaming, hardness, and soap clarity (transparency).

3. Results and Discussion

3.1 Phytochemical Screening Analysis of Telang Flower Extract

Fresh telang flowers that have been dried by heating the oven at 50ºC for 4 hours, producing 50 g of dried telang flowers, which were then soaked for one night using 96% ethanol. The thick extract obtained was 16.08 g with a yield of 32.16%, less than the results of Ganis's research (2022) of 40.375% with concentrated purplish blue characteristics [10].

$$Yield\ (\%) = \frac{Weight\ of\ extract\ obtained}{Weight\ of\ dried\ flower} \text{ x } 100\% \qquad \text{(Eq.4)}$$

$$= \frac{16{,}08\ g}{50\ g} \text{ x } 100\%$$

$$= 32.16\%$$

3.2 Transparent Soap Making

Cocoa Butter and avocado oil were chosen as raw materials in making transparent soap because they contain vitamin E and stearic acid, which are beneficial for skin health, such as moisturizing the skin, and can ward off free radicals because they contain tocopherols and polyphenols [11]. Based on SNI, cocoa butter has a distinctive aroma and melting point of 31-35 ° C so that it can directly absorb the skin but does not make it sticky. NaOH 48% serves to help the saponification process of oil in soap making. Ethanol 96% is a solvent and one of the ingredients used to make transparent soap. Glycerin also serves to increase transparency in soap. The use of cocamide-Dea serves to emulsify and add foam to the soap. The sugar solution is added at 50-60 °C so as not to caramelize the soap. Perfume is added at the end of the process because it is not volatile. The dyes are used to provide a color that matches the blue bay flower extract.

The formulation of transparent soap followed the formula by Prasetyo et al., (2020), which was then modified to achieve better results. The modified transparent soap produced a texture that was not too hard and had good transparency. The comparison between the formula by Prasetyo et al., (2020), and its modification can be seen in Table 1.

Table 1. Comparison of the Formula by Prasetyo et al., (2020), and Its Modification [12]

Material	Prasetyo et al., (2020)	Treatment				
		1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
Palm oil	20.00	14.00	14.00	14.00	14.00	14.00
Cocoa Butter	-	6.00	6.00	6.00	6.00	6.00
Stearic acid	7.00	7.00	7.00	7.00	7.00	7.00
Avocado Oil	-	5.00	5.00	5.00	5.00	5.00
NaOH 30%	20.30	-	-	-	-	-
NaOH 48%	-	7.00	7.00	7.00	7.00	7.00
Glicerin	13.00	13.00	13.00	13.00	13.00	13.00
Ethanol	15.00	15.00	15.00	15.00	15.00	15.00
Cocamide Dea	3.00	5.00	5.00	5.00	5.00	5.00
Sugar	17.00	17.00	17.00	17.00	17.00	17.00
NaCl	0.20	0.20	0.20	0.20	0.20	0.20
Aquadest	4.50	10.45	10.35	10.30	10.25	10.20
Dye	-	0.05	0.05	0.05	0.05	0.05
Perfume	-	0.30	0.30	0.30	0.30	0.30
Butterfly pea flowers	-	-	0.10	0.15	0.20	0.25

3.3 Soap Characteristics

3.3.1 Water Content

The results of the water content test produced from transparent soap ranged from 9.66 - 11.72%. This shows that the more telang flower extract is added, the lower the water content. Based on the results obtained, transparent soap with the addition of telang flower extract is following the quality standards set by the national standardization body in 2016, which is a maximum of 15%. The results obtained can be seen in Figure 1.

The amount of water content a product has, especially in soap, can affect the hardness and usability of the soap. The more water contained in the soap, the hardness of the soap will decrease, and the soap runs out quickly when used because the soap will dissolve easily in water. The quantity of water that is too much in soap will shrink easily and is uncomfortable when used [13].

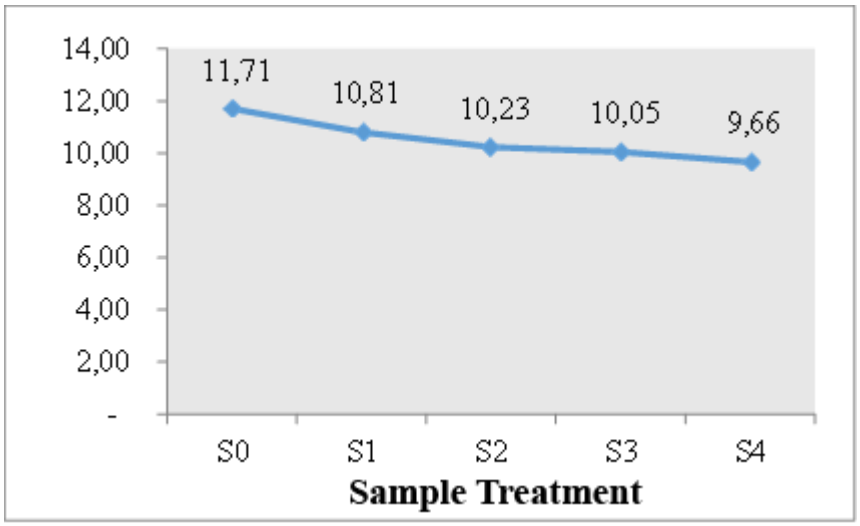

Figure 1.The graph of water content's soap

3.3.2 Free Fatty Acid Content

The free fatty acid test results ranged from 0.074-0.101% (Figure 2). The more telang flower extract added, the more fatty acids increased and the higher the free fatty acid value of the transparent bun. This is because the more telang flower extract added, the more free fatty acids increase due to hydrolysis reactions in the presence of water added during the transparent soap-making process. According to Prasetyo et al., (2020), the high-free fatty acids can reduce the binding power of soap to dirt, oil, fat, or sweat. Free fatty acids cannot bind to dirt because they are more polar than oil, fat, or non-polar dirt [12]. The free fatty acid content of the oil used can also influence soap's free fatty acid content. The high levels of free fatty acids can also be caused by adding stearic acid and citric acid. The use of acidic compounds that are not proportional to the amount of alkali will cause acidic compounds to become excess and remain in the soap [14].

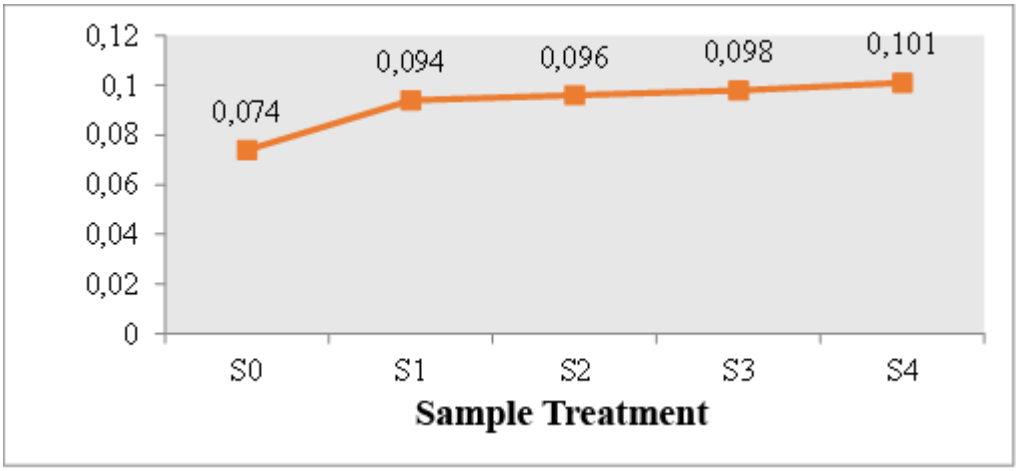

Figure 2. The graph of free fatty acid's soap

3.3.3 Fatty Acid Content

The amount of fatty acids are all fatty acids, both fatty acids bound to sodium and free fatty acids and neutral unsaponified fat [9]. SNI of solid bath soap (1994) requires the amount of fatty acids in solid bath soap to be more than 70%, while SNI of solid bath soap (2016) does not require the provision of more than 70% fatty acids but at least 65% [15].

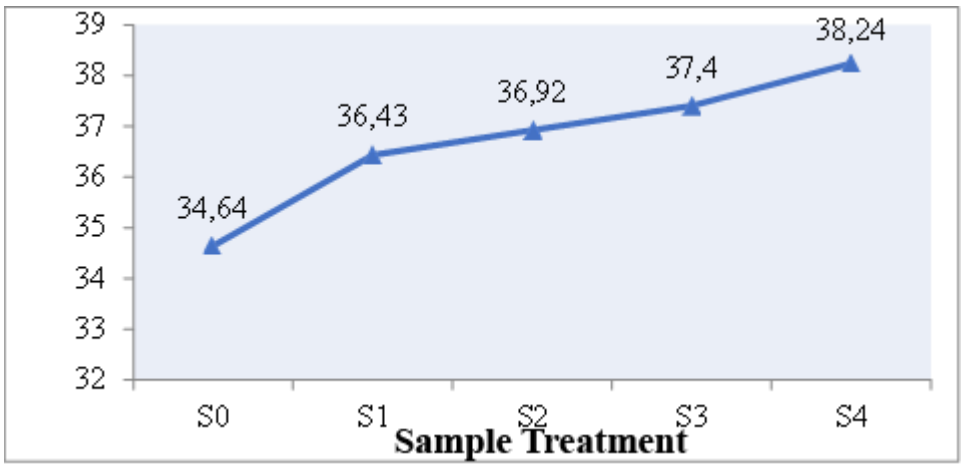

Figure 3. The graph of fatty acid's soap

The results measuring the amount of fatty acids in transparent soap ranged from 34.64 to 38.24% (Figure 3). This range of values does not meet the minimum limit of soap quality set by SNI solid bath soap (1994) [9]and SNI solid bath soap (2016) [15]. The more telang flower extract added, the higher the fatty acids in transparent solid soap. The low amount of fatty acids in transparent soap is due to the formulation of transparent soap, which is added with several other additives, such as glycerin and alcohol, which can increase transparency. Alcohol can also form transparency, besides functioning as a solvent for fatty acids. This is because alcohol is very polar, thus reducing the amount of fatty acids in transparent soap. According to Prasetyo et al., (2020), fatty acids in transparent soap come from stearic acid and palm cooking oil used as raw materials.

3.3.4 Antibacterial Activity of Prepared Soap

The test results show that the average diameter of the inhibition zone of telang flower extract in transparent soap measured using disc paper against *P. acne* bacteria ranges from 8.1-20.1 mm, which has moderate to strong inhibition and *E. coli* bacteria ranges from 21.1-28.8 mm which has very strong inhibition data (Figure 4.6). Based on the results of research by Khumairoh (2020), it is said that telang flower extract has antibacterial activity against P. acne bacteria, producing inhibition of 8.57-13.55 mm [16], and Nurgustiyanti (2021) reported that telang flower extract has antibacterial activity against *E. coli* bacteria producing inhibition of 11.2-12.3 mm [17]. The inhibition zone on *P. acne* and *E. coli* bacteria can be seen in Appendix 3. The results of this study indicate that the more telang flower extract is added, the more antibacterial activity there is in transparent soap. Adding 0.25% telang flower extract to the transparent soap formulation is the best treatment, giving the highest inhibition zone against *P. acne* bacteria of 20.1 mm and *E. coli* of 28.8 mm.

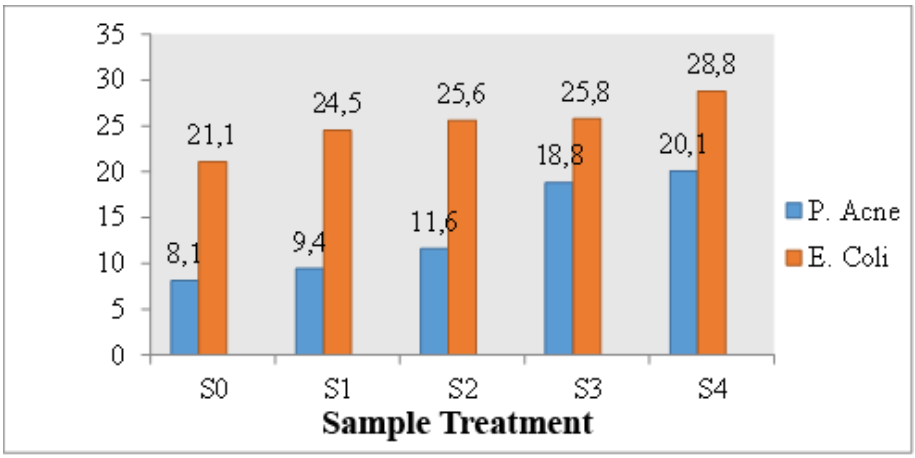

Figure 4. Clear zone graph of transparent solid soap for *P. acne* and *E. coli* bacteria

3.3.5 Hedonic Test

Smell is one of the attributes that consumers consider when choosing soap. Many consumers will certainly choose a soap that has an attractive odor. One of the determining factors for creating an odor impression on the soap from the samples in this study is the cocoa butter concentration of perfume used. Cocoa butter has a distinctive cocoa odor that is inherent in soap products. The test results show no difference between the five test samples based on the aroma of transparent soap shown in Figure 5.

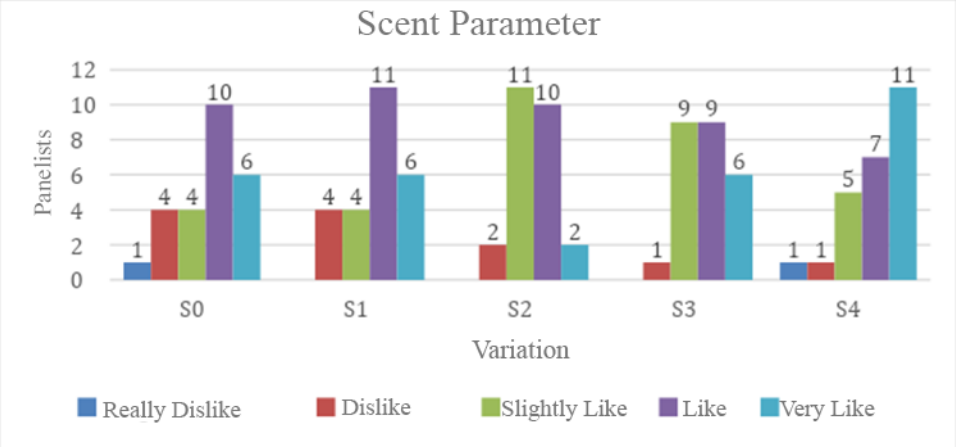

Figure 5. Hedonic test chart for smell parameters

In addition, the color test is a test to assess the appearance of the transparent soap produced. Panelists were asked to assess the appearance of the soap starting from the consistency of the color and shape of the soap produced and then give an assessment according to the predetermined value category. The test results show that there is no difference between the five test samples based on the color of the transparent soap (Figure 6).

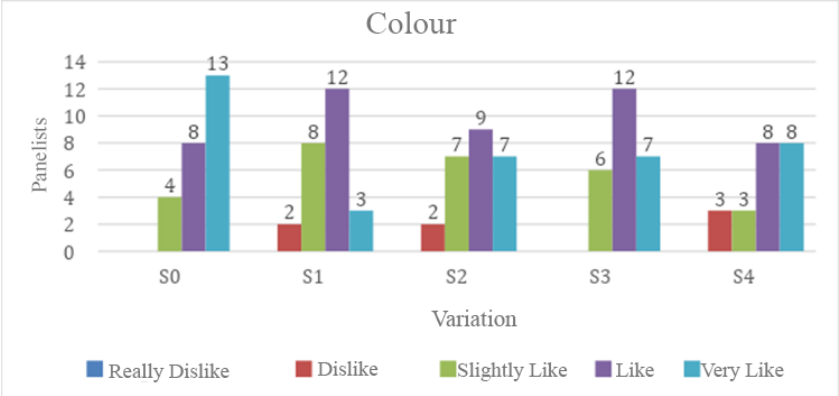

Figure 6. Hedonic test chart for color parameters

The impression left on the skin after using soap is one of the reasons consumers return to using soap. Consumers generally like the impression of being soft and clean after using soap. In this test, panellists were asked to use the soap on the desired body part and then assess the soft impression caused after using the soap. The test results showed no difference among the five test samples based on the skin feel of the transparent soap (Figure 7).

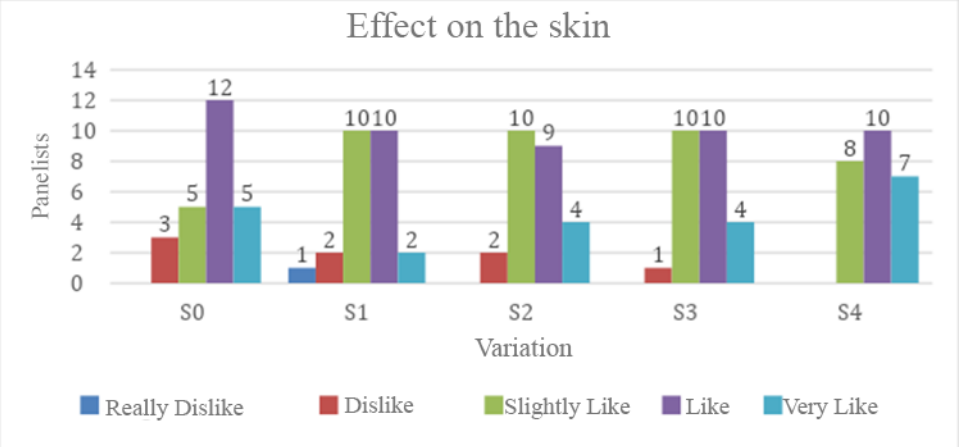

Figure 7. Hedonic test chart for impression left on the skin

Generally, panellists like soaps that have a lot of foaming. In this foaming test, panellists were asked to assess the foaming process of the soap, which included the foam formation process length, the amount of foam produced, and the consistency of the foam. Panelists were then asked to give their assessment according to a predetermined value. The test results showed no difference among the five test samples based on the foaming of the transparent soap (Figure 8).

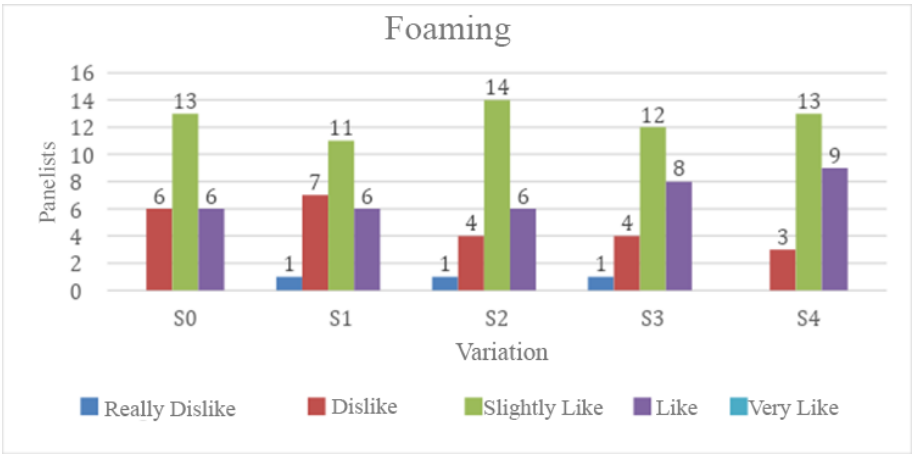

Figure 8. Hedonic test chart for foaming

A good soap has a sufficient level of hardness in the sense that it is not too hard or too soft. The level of hardness here includes the texture attribute attached to the soap. The test results show no difference between the five test samples based on the hardness level of transparent soap (Figure 9). Furthermore, the advantage of transparent soap is that it is attractive and looks transparent (translucent). The attribute of soap transparency or soap clarity indicates this property. In this test, panellists were asked to rate the transparency properties of the soap based on predetermined value categories. The test results show no difference between the five test samples based on the clarity or transparency of the transparent soap (Figure 10).

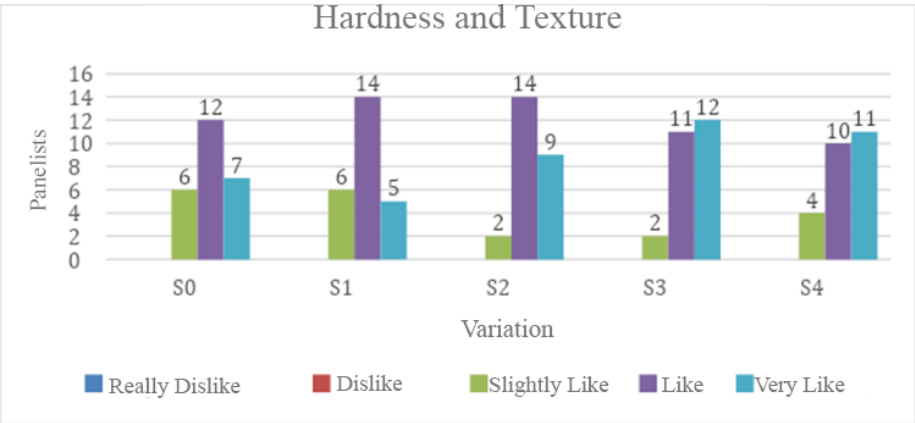

Figure 9. Hedonic test chart for hardness

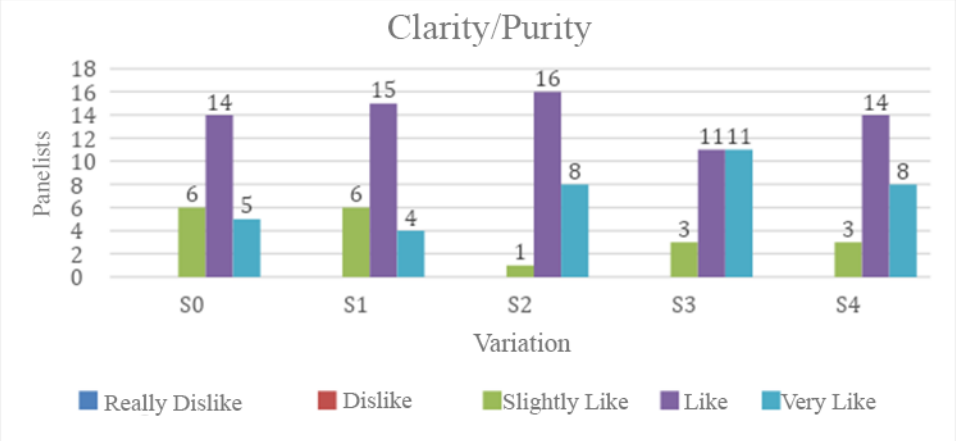

Figure 10. Hedonic test chart for transparant

4. Conclusion

The characteristics of transparent soap added with bayang flower extract and avocado oil produce a distinctive aroma, soft foam on the skin, and good transparency and color. In addition, the antibacterial activity of transparent soap produced from telang flower extract and avocado oil inhibits *P. acne*, ranging from 8.1-20.1 mm with moderate to strong inhibition and *E. coli* bacteria, ranging from 21.1-28.8 mm with very strong inhibition. Therefore, the best formulation produced in this study is sample S4, where the added telang flower extract can inhibit bacterial growth best in *P. acne* and *E. coli* was found to be 20.1 mm and 28.8 mm.

5. Acknowledgements

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6. Conflict of Interest

Authors declare no conflicts of interest

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