Liquid Soap from Nyamplung Seed Oil (*Calophyllum inophyllum L*) with Ketapang (*Terminalia catappa L*) as Antioxidant and Cardamom (*Amomum compactum)* as Fragrance

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ABSTRACT

The non-edible oil from nyamplung seed (*Callophyllum inophylum L.*) has been widely used as a soap ingredient. The soap production from nyamplung seed oil with the addition of antioxidant and fragrance had been carried out in this research. *Ketapang* (*Terminalia catappa L*) fruit extract and Cardamom (*Amomum compactum*) were used as antioxidant and fragrance substances, respectively. The variation of concentration of antioxidant and fragrance has been carried out to obtain the high quality of soap. Characterization was done to identify the quality of soap based on SNI 06-4085-1996. The antioxidant activity was analyzed using DPPH (1,1-diphenyl-2-picrylhydrazyl). The results showed that the addition of 1% ketapang fruit extract and 3% cardamom fragrance exhibited the best characteristics. The antioxidant activity test showed that the IC_{50} value of the best antioxidant soap is 79.51 ppm, indicating that the soap is a strong category.

Keywords: antioxidant, cardamom, ketapang, liquid soap, nyamplung oil

INTRODUCTION

People use soap as a cleanser because it can remove dirt that is attached to the body. All kinds of soaps use the same main ingredient i.e oil or triglycerides. The types of oils that are used by soap industries are coconut oil and palm oil as the main ingredient. The oil that is used as the basic ingredient of soap is an edible oil which is also often used as cooking oil. This will lead to competition between the soap industries and the cooking oil industries so that eventually one of these industries will run out of raw materials. To overcome this problem, an alternative raw material of non-edible oil materials such as oil derived from the seeds of nyamplung (*Callophyllum inophylum L.*) is needed (Hadi, 2009). Nyamplung is a plant that can be found in coastal areas of Indonesia. Nyamplung seeds contain oil of about 40-70% (Chasani, Nursalim, Widyaningsih, Budiasih, & Kurniawan, 2014). These can be used as the basic material of biodiesel production (Alamsyah & Lubis, 2012). Chasani, Widyaningsih, & Mubarok (2015) have made a soap from Nyamplung seed oil which fulfills the SNI requirement, however, the results need to be developed.

People are very aware that health is very important. Antioxidants are compounds that are considered to improve health because of its ability to inhibit the oxidation of other molecules. Oxidation can produce free radicals that damage cells. People tend to buy products that contain antioxidants for health reasons. The soap containing antioxidants are preferred by consumers, especially the antioxidant from the natural ingredients. Omenna and Chukwuma (2015) reported that ketapang (*Terminalia catappa L.*) leaves has antioxidant activity. Krishnaveni (2014) reported that ketapang (*Terminalia catappa L.*) fruits also have antioxidant activity. Ketapang (*Terminalia catappa L*) is a plant that is found in the park or roadside. It has many benefits but the utilization of the antioxidants from ketapang fruits in soap has not been reported.

In this research, the formulation of soap is conducted by adding antioxidant additives from ketapang fruits. Ketapang fruits contain a compound of a phenolic group that could be used as a free radical scavenger. It has high antioxidant activity with the ES50 value of 66.87 ppm (Krishnaveni, 2014). To attract the people's interest to this product, the fragrance is added. One of the fragrances that have a natural fragrance is cardamom (*Amomum compactum)* essential oils (Tambunan, 2017).

Soap formulations were conducted with ketapang percentages of 1, 3, 5, 7, and 9% (w/w) and the cardamom (fragrance percentages of 1, 3, 5, 7, and 9%. The products were characterized according to SNI 06-4085-1996. The best-characterized soap was tested

for its antioxidant activity with DPPH (1,1-diphenyl-2-picrylhydrazyl).

MATERIAL AND METHODS

Materials

The materials of the experiment are nyamplung seed oil, ketapang fruit, essential oil of cardamom, distilled solvents of methanol, ethanol, ethyl acetate, and n-hexane. Ammonium sulfate anhydrous, sodium hydroxide, potassium hydroxide, methyl red, phenolphthalein, sulfuric acid, and DPPH were purchased from Merck.

Isolation of nyamplung seed oil (Chasani et al., 2014)

The dried powder of nyamplung seeds are macerated with n-hexane solvent for 2x48 hours while occasionally stirring and then filtered. The filtrate is separated from the n-hexane solvent using a rotary evaporator. Evaporated oil is then added anhydrous ammonium sulfate to bind water that still contained in nyamplung oil.

Isolation ketapang fruits extract

Ketapang fruits were cut into small pieces, dried, mashed, and macerated with methanol for 2x24 hours while occasionally stirred and then filtered. The filtrate was filtered again using a Buchner funnel to obtain the filtrate and residue. The residue was macerated with methanol for 2x24 hours. The methanol extract was concentrated and weighed. The concentrated methanol extract was extracted with n-hexane to obtain an extracted methanol fraction and residue. The extraction residue with n-hexane was re-extracted using an ethyl acetate solvent to obtain an ethyl acetate fraction of methanol extract and residue. The fraction of the extraction with ethyl acetate was evaporated to obtain a concentrated ethyl acetate fraction of methanol extract then weighed. The ethyl acetate fraction of the ketapang fruit was used as an antioxidant in the soap formulation.

Isolation of essential oils of cardamom (Tambunan, 2017)

Dried cardamom powder was macerated with n-hexane solvent for 3x24 hours then filtered and concentrated under low pressure using a rotary evaporator. The extract was added anhydrous Na_2SO_4 to bind water and then used as perfume on soap formulation.

Antioxidant soaps production

The amount of 20 grams of nyamplung seed oil was stirred and heated to 70-80 °C, then 10 gram of KOH solution 30% (w/v) was added. The mixture continues to be heated while stirring at 500 rpm for 1 hour. The temperature was lowered to 60 °C, then added deionized water (1: 1 ratio with the soap formed) until homogeneous soap was formed. The ketapang fruits extract was added by stirring at 500 rpm for 15 minutes. Soap products were poured into containers and closed tightly. The variations of ketapang extract were 1, 3, 5,7, and 9%, while the variation of cardamom were 1, 3, 5, 7, and 9% a (see in **Table 1)**.

Characterization of soap

Total fatty acids (SNI, 1996)

The amount of 10 grams of soap dissolved in 50 mL distilled water, was added a few drops of methyl red and 20% of sulfuric acid until all fatty acids are free from potassium which are shown by the appearance of red color on the surface of the solution. The solution is poured into the separating funnel while the precipitate is extracted with n-hexane (aqueous solution is removed, while n-hexane is re-extracted). The procedure is repeated until the solvent does not contain free fatty acids. The solvent is shaken and washed with distilled water until it does not react acid (seen with congo paper).

Table 1. Variations of ketapang fruit extract and cardamom

Ethyl acetate fraction of ketapang fruits	Cardamom				
	P1 (1%)	P2 (3%)	P3 (5%)	P4 (7%)	P5 (9%)
A1 (1%)	A1P1	A1P2	A1P3	A1P4	A1P5
A2 (3%)	A2P1	A2P2	A2P3	A2P4	A2P5
A3 (5%)	A3P1	A3P2	A3P3	A3P4	A3P5
A4 (7%)	A4P1	A4P2	A4P3	A4P4	A4P5
A5 (9%)	A5P1	A5P2	A5P3	A5P4	A5P5

Each washing used 10 mL of distilled water. The solvent is added anhydrous ammonium sulfate, filtered and placed into a round flask. The weight of the flask with boiling stone noted as W1. The solvent is distilled and the flask is dried at 102-105 °C until a constant weight (W2) is obtained. Fatty acid levels were calculated by equation (1).

$$\text{Total fatty acids} = \left(\frac{W2 - W1}{\text{sample}}\right)x100\% \quad (1)$$

Where :
W_1: weight of flask + boiling stone
W_2: weight of flask + boiling stone + fatty

Free fatty acids and free alkali (SNI, 1996)

The 100 mL of alcohol was added 0.5 ml of phenolphthalein and boiled at 70 °C. The mixture was neutralized with a 0.1 N alcoholic KOH. 5 g of soap was poured into the alcohol mixture and refluxed for 30 minutes. If the solution is not alkaline (not colored red), the solution is titrated with a 0.1 N alcoholic KOH, until a red color lasts up to 15 seconds. Determination of free fatty acids using equation (2).

$$\text{Free fatty acids} = \left(\frac{VxNx0.205}{W}\right)x100\% \quad (2)$$

Where:
V: volume of KOH 0,1 N that was used
N: normality of KOH that was used
W: sample weight (g)
0,205: a weight that equivalent to lauric acid

When the sample contains many insoluble parts, it is filtered before it is titrated. If the solution is alkaline (red) then the test is alkaline free. The solution was titrated with 0.1N HCl in alcohol until the red color disappeared. The free alkali content is calculated by equation (3)

$$\text{Free alkaline content} = \left(\frac{VxNx0.04}{W}\right)X100\% \quad (3)$$

Where:
V: volume of HCl 0,1 N that was used
N: normality of HCl that was used
W: sample weight (g)
0,04: a weight that equivalent with NaOH

Neutral fat (SNI, 1996)

A solution of free fatty acid or alkali-free acid procedure is added 5 mL of alcoholic KOH 0.5N, refluxed for 30 hours, and titrated with 0.5N HCl until red color disappears (V1). Titration was carried out again using a blank solution of 0.5 N alcoholic KOH (V2). The amount of neutral fatty can be determined using equation (4)

$$\text{Neutral fatty} = \left(\frac{(V2 - V1)xNx0.0561}{0.1981xW}\right)x100\% \quad (4)$$

Where:
$V_{1:}$ volume of HCl to neutralize KOH + sample (mL)
$V_{2:}$ volume of HCl to neutralize blanko KOH (mL)
N: normality of HCl that was used
W: sample weight (g)
0,0561: a weight that equivalent with KOH
0,198: average saponification numbers of nyamlung oils (g)

The pH measurement (SNI, 1996)

The pH measurements were carried out using pH-meters calibrated with buffer solutions of pH 4, 7 and 9. The electrodes were cleaned with distilled water then dipped in 10% (w / w) soap solution at 25.

Type weight (SNI, 1996)

The sample is inserted into the pycnometer until the top of the line is closed. The pycnometer is inserted into the thermostat until it is temperatures of 25 °C and allowed to stand for 30 minutes then settled at room temperature and weighed. The experiment was repeated using deionized water. The calculation of type weight is given by equation (5).

$$\text{Density} = W_2 / W_1 \quad (5)$$

Where:
W_1: weight of water (g)
W_2: weight of the sample (g)

Foam stability

Soap solution of 1 mL was put into the reaction tube and 9 mL of distilled water was added. The samples were shaken for 1 minute and the foam height was measured. Samples were allowed for 1 hour and recalculated the foam height. The stability of the foam is determined by equation (6)

$$\text{Foam stability} = \frac{\text{the final height}}{\text{the initial height}}x100\% \quad (6)$$

Antioxidant activity determination with DPPH

Preparation of DPPH solution

The amount of 1.97 mg DPPH was dissolved with methanol in a flask up to 100 mL to obtain a solution with a concentration of 0.05 mM.

Preparation of antioxidant soap solution

The amount of 0.5 mg of antioxidant soaps was dissolved in 100 mL of methanol in a 100 mL flask to obtain a concentration of 5 ppm.

Determination of maximum absorption wavelength of DPPH

The amount of 4 mL of 0.05 mM DPPH solution was allowed 30 minutes in darkness, then the absorption of the solution was measured by a UV-Vis spectrophotometer at a wavelength of 400-600 nm.

Determination of operating time (OT) of antioxidant soap solution

The amount of 4 mL of 0.05 mM DPPH solution was added with 1 mL of test solution. The solution was measured at a maximum wavelength at intervals of 5 minutes until a stable absorbance is obtained and no absorbance decreases.

Determination of inhibition percentage and antioxidant activity (IC50)

The best antioxidant soap is determined by its antioxidant activity by adding 4 mL of 0.05 mM DPPH solution to 1 mL of antioxidant liquid soap. The mixture was allowed during operating time then the absorbance was measured at the maximum wavelength. As a comparison, a 5 ppm of the ascorbic acid solution is treated similarly to the test solution. The best formula is determined by percent of the inhibition that can be calculated by equation (7).

$$\text{Inhibition (\%)} = \frac{A_1 - A_2}{A_1} \text{x}100\% \qquad (7)$$

Where
A_1: control absorbance
A_2: sample absorbance

The best concentration of liquid soap antioxidant formula and percent of inhibition were plotted on the x and y-axes respectively on the linear regression equation to find IC50 (Inhibitor Concentration 50%) of the best formula. IC50 expresses the concentration of the sample solution that is required to reduce 50% DPPH.

RESULTS AND DISCUSSION

The yields of isolation and purification of nyamplung seed oil are 41.50% (w/w). The yields of ketapang fruit are 8.34% (w/w), while the yield of essential oil of cardamom is 5.26% (w / w).

Liquid Soap Antioxidant

The resulted soap is a yellow viscous liquid for soap control (0%) while the soap with the addition of ketapang fruits extract is brown. The yellow color of the soap product matches to the color of nyamplung oil that is used as the raw material. While the color brown on soap comes from ketapang extract.

Total fatty acids

The total fatty acid is the total amount of fatty acids that are present in the antioxidant liquid soap, both fatty acids which are bound to alkali and free fatty acids or neutral fatty acids (triglycerides or unabsorbed fat). The fatty acid content in soap acts as a regulator of consistency. This is because fatty acids have a limited ability to dissolve in water thereby making durable soaps in use, as well as to improve the efficiency of dirt cleaning processes. Determination of total fatty acid is one condition of liquid bath soap based on SNI 06-4085-1996. The soap should have a total fatty acid between 60-70% (SNI, 1996).

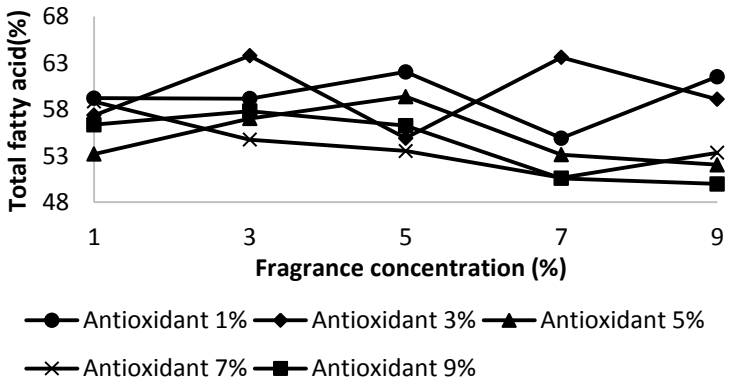

Figure 1. Total fatty acid at various antioxidant and fragrance concentration

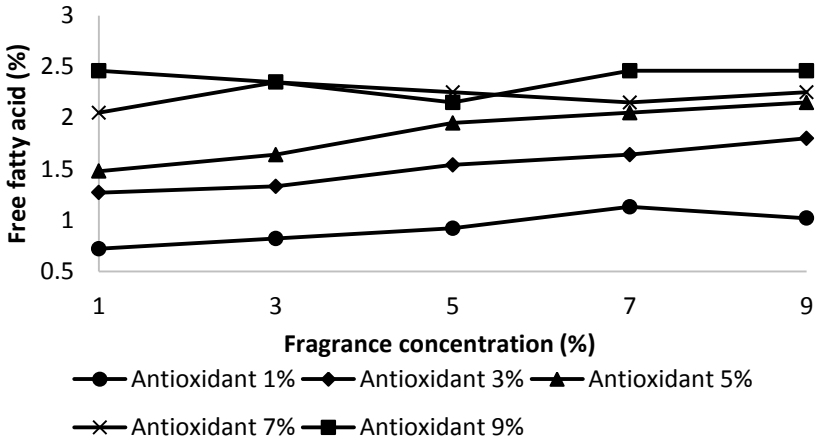

Figure 2. Free fatty acid at various antioxidant and fragrance concentration

Figure 1 showed that total fatty acid is in ranged 49,95–63,76%. Addition of antioxidant from ketapang fruits and fragrance from cardamom decrease the total fatty acid. The results did not qualify to SNI 06-4085-1996. According to SNI 06-4085-1996, the total fatty acid for liquid soap are 64-70%.

Free fatty acid and free alkaline

The free fatty acid is fatty acid in soap that is not bonded as sodium, potassium, or triglycerides (neutral fatty) compound. The content of free fatty acid will affect to cleansing ability. The high free fatty acid will decrease cleansing ability. Free alkaline is numbers of alkaline that is not bonded as a compound. The excess of alkaline on a soap can cause skin irritation. The free fatty acid or free alkaline determination is one of the quality requirement of liquid bath soap. The free fatty acid for liquid bath soap according to SNI 06-4085-1996 have to <2,5 % and free alkaline is counted as KOH maximum is 0,4%. The result can be seen in **Figure 2**. The free fatty acids of liquid soap are in the range of 0,72-2,46%. The highest free fatty acid is a soap with an addition 9% of an antioxidant. The value qualifies to SNI 06-4085-1996. The average of free fatty acid on formulation soap is higher than control soap. OH group on ethyl acetate extract of ketapang fruits react with KOH that was used when titration process. It will cause the KOH that was used is more and increase the value of free fatty acid.

Neutral fats

Neutral fat is triglyceride or other fat that is not saponified by alkaline. The more the fat that is unsaponified the more decreasing of cleansing ability. This is because soap will bond the fat from the soap itself. Determination of neutral fat is one of the qualify according to SNI 06-4085-1996. The soap has to have neutral fat <2,5% (SNI, 1996). The results can be seen in **Figure 3**. The neutral fats on the soap formulation are in the range of 4,24-13,87%. The highest value is 13,87% for soap with an addition 9% of an antioxidant. The results did not qualify to SNI 06-4085-1996 because there are so many compounds that cannot be saponified by alkaline. Nyamplung oils have a high unsaturated fatty acid such as oleic acid and some unsaponified component such as fat alcohol, sterol, xanthone, coumarin derivate, calophylate, isophthalate, capillariate, pseudo-bracid acid, and triterpenoid compound as much as 0.5 - 2%. Ketapang has terpenoid compound that can be considered as lipid. The terpenoid is one of unsaponified fatty. The more ketapang fruit extracts the more unsaponified fatty acid.

The pH parameter

The acidity, pH, is one parameter of soap quality that has to be concerned. The high and low of soap pH can cause skin irritation. The soap product must have pH that similar to skin pH. Usually, soap is alkaline (pH>7) because of alkaline use in production. The concentration of alkaline in production determines soap pH. The more concentration of alkaline the higher pH of soap. According to SNI 06-4085-1996 pH of liquid bath soap are in ranged 8 - 11. The pH value of the soap can be seen in **Figure 4**. The pH of formulation soap is in ranged 7,80-9,35. The lowest pH is 7.8 for soap with 9% of an antioxidant. The result qualifies with SNI 06-4085-1996 except for 9% of an antioxidant.

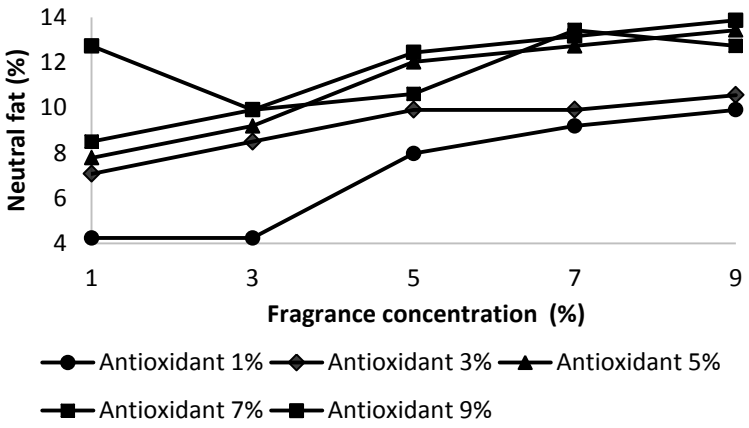

Figure 3. Neutral fat at various antioxidant and fragrance concentration

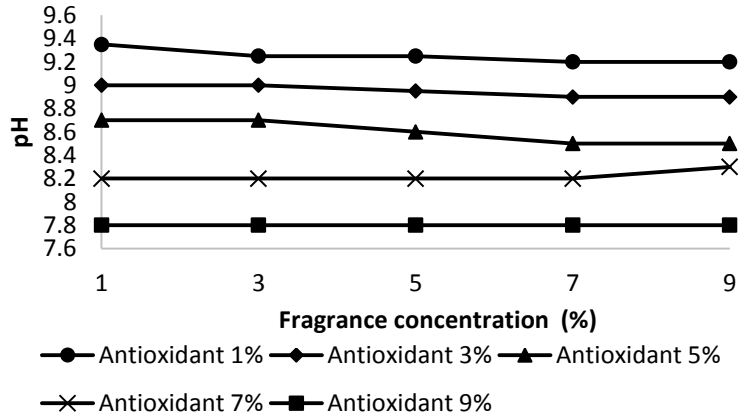

Figure 4. pH at various antioxidant and fragrance concentration

Type weight

Type weight is the mass ratio of a compound and water at the same volume and temperature. Type weight of soap product depends on each components mass. The result of type weight determination can be seen in **Figure 5**. The type weight of formulation soap is in ranged 1,0250-1,0460. According to SNI 06-4085-1996, the density for liquid soap are 1,0100-1,1000. The result has qualified SNI 06-4085-1996.

Foam stability

Foam is gas that dispersed in a liquid which has a stable structure. It contains air pockets which wrapped in a thin layer (Hambali, Suryani, & Rifai, 2005). Foam in soap play a role in the cleansing process and give fragrance to the skin (Hernani, Bunasor, & Fitriati, 2010). People prefer soap with abundant foam and stable. Foam formation can happen when surfactant was at the surface between water and air and the hydrophobic group at gas phase. The result of foam stability can be seen in **Figure 6**. The values of foam stability are in the range of 53,48-83,06%. The highest value is 83.06 found in the soap with 1% of an antioxidant.

Determination of the best soap antioxidant used the method of effectiveness index. This method is carried out by sequencing the antioxidant soap liquid characteristic parameters according to the priority and contribution to the results. The priority order is from the highest to the low value. The characteristic sequences are total fatty acids, neutral fats, free fatty acids, pH, density and foam stability. The most important characteristic of liquid antioxidant soap in determining formulation is a total fatty acid. Total fatty acids are a number of fatty acids in soap that can bind the non-polar dirt. The greater the total fatty acid in the soap the higher cleansing power the soap. The best liquid antioxidant soap is a soap with an addition 1% of antioxidant and 3% of fragrance. The characteristic is 59.14% of total fatty acid, 0.82 of free fatty acid, 4.24% of neutral fat, 9.25 of pH, 83.06% of foam stability, and 1.0262 g of type weight

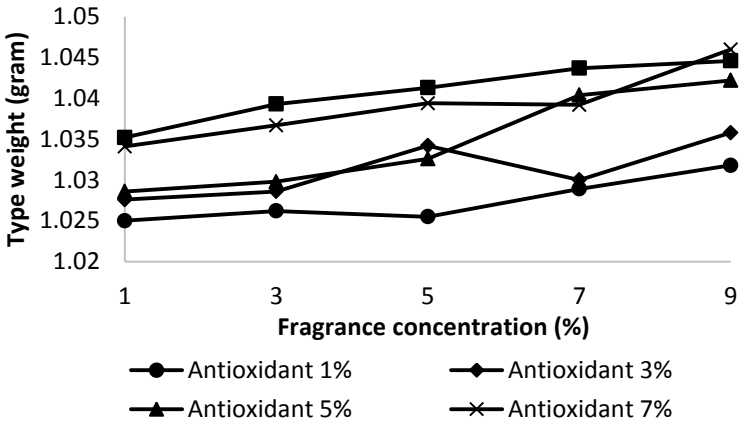

Figure 5. Type weight at various antioxidant and fragrance concentration

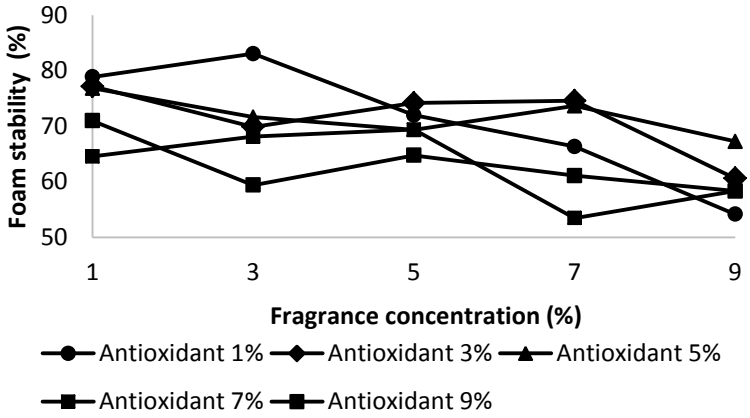

Figure 6. Foam stability at various antioxidant and fragrance concentration

Antioxidant Liquid Soap Activity

The antioxidant activity test is conducted to find out how much the soap ability in reducing free radical of DPPH. DPPH serves as a free radical compound that will react with antioxidant soaps. The DPPH method is an easy, fast and sensitive method for testing the antioxidant activity of a particular compound. The DPPH method measures the ability of an antioxidant compound in capturing free radicals. The ability to capture free radicals is related to the ability of a compound to donate electrons or hydrogen. Any molecule that can donate electrons or hydrogens will react and fade the DPPH color from purple to yellow to form the 1,1-diphenyl-2-picrilhidrazine compound.

The analysis was performed at 516.8 nm of maximum wavelength and operating time 35-40 minutes. The results showed that the inhibition percentage value of the best antioxidant soap liquid were 20.18; 20.86; 21.99; and 22.90%. The value of IC_{50} is determined by plotting the concentration of the solution with the percentage of DPPH inhibition on the linear regression equation. The graphic can be seen in **Figure 7**.

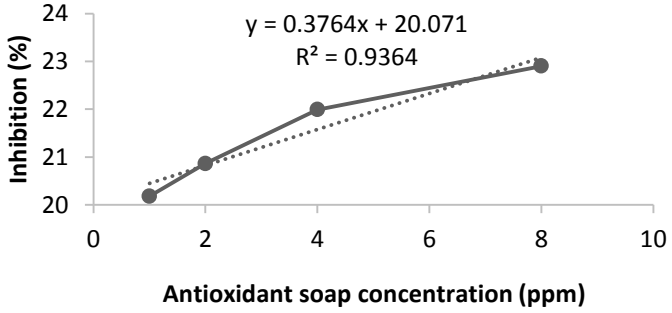

Figure 7. Inhibition percentage of antioxidant soap concentration

There are several categories of compounds as antioxidants (Isdinar, Wahyuono, & Setyowati, 2011). A very strong if it has IC50 value less than 0.05 mg/mL (<50 ppm); strong if IC50 values between 0.05-0.10 mg/mL (50-100 ppm); medium if IC50 value between 0,10-0,15 mg/mL (100-150 ppm); and weak if IC50 values between 0.15-0.20 mg/mL (150-200 ppm). IC50 value of liquid antioxidant soap the best formula is 79,51 ppm. These values indicate that the best antioxidant soap formula is included in the strong category.

CONCLUSION

The high quality of soap from nyamplung seed oil containing an antioxidant and fragrance was successfully made. The best characteristic could be achieved by the formulation of 1% *ketapang* fruit extract as an antioxidant and 3% cardamom as a fragrance. The IC50 value of the best antioxidant soap is 79.51 ppm, indicating that the antioxidant is a strong category.

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REFERENCES

Alamsyah, R., & Lubis, E.H. (2012). The Biodiesel Processing from Nyamplung Seed (Calophyllum inophyllum L) using the Dried Purification. *Jurnal Kimia dan Kemasan, 34(2),* 294-302.

Chasani, M., Nursalim, V.H., Widyaningsih, S., Budiasih, I.N., & Kurniawan, W.A. (2014). Synthesis, Purification, and Characterization of Methyl Ester Sulfonate (MES) as a Detergent Essential Material from Nyamplung Seed Oil (*Calophyllum inophyllum* L.). *Jurnal Molekul. 9(1),* 63-72.

Chasani, M., Widyaningsih, S., & Mubarok, A. (2015). Synthesis and Characterization of Sodium Soap from Nyamplung Seed Oil (Callophyllum inophyllum L) and Antibacterial Activity Test on Stphilococus aureus. *Jurnal Molekul, 10(1),* 66-73.

Hadi, A.W. (2009). Utilization of Nyamplung Seed Oil (Calophyllum inophyllum L.) as an Alternative of Petroleum Oil. *Jurnal Riset Daerah, 8(2),* 15-17.

Hambali, E., Suryani, A., & Rifai M. (2005). *Making Transparent Soap for Gift and Beauty*. Jakarta: Penebar Swadaya.

Hernani, T. K., Bunasor, & Fitriati. (2010). Antifungal Transparent Soap Formulation with Active Material of Galangal Extract (*Alpinia galangal* L). *Bulletin Litro, 21(2),* 197-200.

Isdinar, Wahyuono, S., & Setyowati, E.P. (2011). Isolation and Identification of Persimmon Leave Antioxidant Compounds (Diosyros Kaki Thund.) with DPPH (2,2-Difenyl-1-Pikrilhidrazil) Method. *Majalah Obat Tradisional, 16(3),* 157-164.

Krishnaveni, M. (2014). In vitro antioxidant activity of Terminalia catappa nuts. *Asian Journal of Pharmaceutical and Clinical Research 7(4),* 33-35.

Mohale, D.S., Dewani, A.P., Chandewar, A.V., Khadse, C.D, Tripathi, A.S., & Agrawal, S.S. (2009). Brief Review of Medicinal Potential of Terminalia catappa L. *Journal of Herbal Medicine and Toxicology, 3(1),* 7-11.

Omenna & Chukwuma, E. (2015). Antioxidative Activity of the Almond Leaves (Terminalia catappa). *International Journal of Nursing, Midwife, and Health Related Cases, 1(2),* 29-40

SNI (Indonesian National Standard). (1996). Liquid Bath Soap. Jakarta: Badan Standarisasi Nasional.

Tambunan, L. R. (2017). Isolation and Identification of Essential Oils from Seeds of Cardamom Plants (Amomum cardamomum Willd). *Jurnal Kimia Riset, 2(1),* 57-60.