ULIL ALBAB : Jurnal Ilmiah Multidisiplin
Vol.1, No.3, Februari 2022

Characterization And Antifungal Activity Evaluation Of Tamanu Seed Oil Based Liquid Soap Towards *Candida Albicans*

Devi Puji Rahayu[1], Nur Khanifah[2], Rofikoh[3], Mochammad Chasani[4]
[1,2,3,4]Chemistry, Mathematic and Natural Science Faculty, Jenderal Soedirman University
E-mail: moch.chasani@gmail.com

Article History:
Received: 28 Januari 2022
Revised : 02 Februari 2022
Accepted: 03 Februari 2022

Keywords: *Liquid soap, Tamanu Seed Oil, Candida Albicans, Bacang Leave, Nutmeg Leave*

Abstract: *Candida albicans fungal infection towards skin could be prevented by using the soap with antifungal activity. Material used for the soap making was tamanu oil which was potential as antifungal soap. This study aims to find out the best characteristic liquid soap and to evaluate its antifungal activity towards Candida albicans. The methods used in this research were maseration and followed by soap making using semi-boiled method. Tamanu seed oil based liquid soap was formulated with variety concentrations of bacang leave extract as an antifungal agent and nutmeg leave extract as foaming agent. Each soap were characterized following the SNI 4085:2017 standard. The liquid soap formula with the best characteristic was determined and its antifungal activity towards Candida albicans was tested using disk diffusion method. The result shows that the best soap formula is the tamanu oil based liquid soap with the addition of 0.9% bacang leave extract and 0,9% nutmeg leave extract (SFM5P5). The best formula had 9.25 pH value; 18,3017% total active agent, 3,3840% free fatty acid; 1,0235 g/mL density and 94,1176% foam stability. In conclusion, liquid soap has strong activity to inhibit the fungal growth of Candida albicans with 18.18 mm of inhibition zone diameter.*

INTRODUCTION

Candidiasis is a desease caused by *Candida albicans* fungal infection. *Candida albicans* could infect various organs include skin. *Candida albicans* infection towards skin is well-known as Cutaneous Candisiasis. Cutaneous Candisiasis is ranked *third* in dermatomycosis cases occured in Indonesia (Seru *et al*., 2013). Fungal infection towards skin could be treated using tropical or systemic drugs. Antifungal tropical drugs usually available in the form of cream or ointment. However, the use of tropical drugs is devoted for medications which means it only can be used when the skin is already infected. Therefore, the prevention effort is needed by using antifungal soap so that the damage caused by fungal infection on skin could be prevented (Seru *et al*., 2013).

Soap is a product used for cleaning. Tamanu seed oil can be used as alternative material to make soap. Tamanu (*Calophyllum inophyllum* L.) is categorized as potential producer of non edible oil for biofuels. The oil content of tamanu seed is very high, which is in the range of 40-73% (w/w)

(Musta *et al*., 2017). Tamanu seed oil is already developed as the basic ingredient to antibacterial soap (Chasani *et al*., 2015). Tamanu seed oil is known to have the weak antibacterial activity and have no antifungal activity towards *Mallasezia furfur* (Artanti *et al*., 2020). Therefore, this research aims to formulate liquid soap using tamanu seed oil as its basic material with the addition of bacang leave extract as antifungal agent and nutmeg leave extract as foaming agent.

Bacang leave (*Mangifera foetida* L.) contains mangiferin as active substance which is categorized as polyphenol and has antifungal. Beside of magniferin, bacang leave also contains another seondary metabolites, such as alkaloid, phenol, flavonoid, steroid, tannin, and saponin, which have strong antifungal activity. It was proven that 1000 ppm of bacang leave extract could inhibit the growth of *Candida albicans* with 22,8 mm inhibition zone diameter (Arifin *et al*., 2018).

The foaming agent used in this research was nutmeg leave extract (*Myristica fragnans* Houtt). Essential oil produced from nutmeg leave is proven to contain saponin so that nutmeg leave oil can be used as natural foaming agent (Puspa *et al*, 2017). The aim of this study is to find out the concentration of bacang leave extract and nutmeg leave extract that could produce nanoparticle liquid soap with the best characteristic and to evaluate its antifungal activity towards *Candida albicans*.

MATERIALS AND METHOD

Materials

Instruments used in this study were Rotary Vacuum Evaporator (IKA RV 10 digital V, Germany) and Spektrofotometer UV-Vis (Shimadzu UV-1800, Japan). Materials used in this study were tamanu seed oil, nutmeg leave from Purbalingga, bacang leave from Banyumas, Na_2SO_4 unhydrous (Merck, Germany), aquadest, acetone (technical grade), n-hexane (technical grade), methanol (technical grade), etanol (technical grade), ethyl acetate (Bratachem, Indonesia), *Citrus X sinensis*, petroleum ether (Merck, Germany), methylene red, phenolphthalein (pp), potassium hydroxide (KOH) (Merck, Germany), ethanol 96% (technical grade), *Candida albicans* isolates, Sabouraud Dextrose Broth (SDB) (Merck, Germany), Sabouraud Dextrose Agar (SDA) (Merck, Germany), and commercial antiseptic liquid soap.

Methods

Bacang leave extraction

Bacang leaves were cleaned and wind-dried for 3 days to remove it water content. The dried leaves were pulverized. As much as 100 gram of pulverized leaves were macerated in 350 mL of methanol for 2 days. The mixture were then filtered using filter paper. The filtration residue were further macerated using 50 mL of methanol for 3 times. The filtrate were collected together and evaporated to get the concentrated extract and pondered.

Nutmeg leave extraction

Nutmeg leaves were cleaned and wind-dried for 3 days and pulverized. As much as 200 gram of pulverized leaves were macerated using methanol. Distillate obtained was further fractionated using ethyl acetate solvent and concentrated using rotary vacuum evaporator to obtain ethyl acetate concentrated fraction.

Antifungal soap formulation

The tamanu seed oil soap making was conducted using semi-boiled method. As much as 300 gram of tamanu seed oil was pured into a glass and stirred tith 70-80 °C temperature. As much as 150 gram of 30% KOH solution (w/v) were added. The mixture were stirred at 500 rpm for 1

hour until it reach homogenous phase. The temperature was lowered until it reach 60 °C. Some aquadest were added with 1:1 ratio and stirred again until it reached homogenous phase. The soap product was stored for 3 days in separatory funnel. After 3 days of storage, the soap was separated from the dirts. *Citrus X sinensis* fragrance was then added into the soap. Subsequently, bacang leave extract and nutmeg leave extract were added into the soap with the variety of concentrations 0; 0,1; 0,3; 0,5; 0,7 and 0,9 (%) from the total weight while being stirred for 15 minutes at 500 ppm. The mixture was then stored in the closed container.

Characterization

The characterization was conducted following the SNI 4085:2017. The characterization involve the measurement of acidity, the total of active substances, free fatty acids, density, and foam stability. To characterization acidity (pH), as much as 1 mL of liquid soap prepared was dissolved into 10 mL of aquadest and shaken. The acidity of the sample was measured using pH meter at 25 °C.

The measurement of total active substances was conducted by calculating the amount of the substances dissolve in ethanol reduced by the amounts of substances dissolve in petroleum ether. To calculate the ethanol-soluble substances, 1 gram of sample was added with 20 mL of 96% ethanol and heated and stirred for 30 minutes. The mixture was filtered and washed with 10 mL of 96% of ethanol and cooled down. The filtrate was poured into 50 mL volumetric flask and added ethanol 96% until the border line. As much as 20 mL of solution was poured into the beaker glass, pondered, and heated to evaporate the ethanol. After cooled down, the sampel was stored inside the desiccator and pondered. The amount of ethanol-soluble substances could be calculated using the equation 1.

$$C_{et}(\%) = \frac{A}{S \times \left(\frac{100}{250}\right)} \times 100\% = \frac{250 \times A}{S} \qquad (1)$$

where: C_{et} = the amount of ethanol soluble substances
A = the remaining substances after the drying (g)
S = the sample weight (g)
$\left(\frac{100}{250}\right)$ = the final pipetted volume divided by the final volume of the sample.

To calculate the petroleum ether-soluble substances, as much as 1 gram of sample was poured into 20 mL mixture of water-ethanol and filtered. The mixture was added with 0,5 mL NaOH 05 mol/L and a few drops of pp indicator to make sure that the solution is base. The mixture was then poured into separatory funnel and extracted with 15 mL of petroleum ether and washed twice with 3 mL of aquadest and dried using unhydrous Na_2SO_4. The sample was filtered and collected into the beaker glass, washed using petroleum ether and colled down. Subsequently, the sample was stored inside the desiccator and pondered. The amount of petroleum ether-soluble substances could be calculated using the equation 2.

$$C_{pe}(\%) = \frac{A}{S} \times 100\% \qquad (2)$$

where: C_{pe} = the amount of petroleum ether-soluble substances
A = he amount of extracted substances into petroleum ether (g)
S = the sample weight (g)

The determination of total active substances could be calculated using equation 3.

$$\text{Total bahan aktif } (\%) = C_{et} - C_{pe} \qquad (3)$$

where: C_{et} = ethanol-soluble substances, % mass fraction
C_{pe} = petroleum ether-soluble substances % mass fraction

To characterization Free fatty acid, as much as 20 mL of alcohol were added with 0,1 mL phenolphthalein indicator (pp) and boiled at 70° C. The mixture was neutralized using alcoholic KOH 0,1N. Subsequently, 0,5 gram of soap were refluxed for 30 minutes. If the solution was alkaline (not colored red), the mixture should be titrated with alcoholic KOH 0,1N until the color turned red and the color should last for 15 second. The total fatty acid could be calculated using equation 4.

$$\text{Total Fatty acid (\%)} = \frac{V \times N \times 282}{W} \times 100\% \quad (4)$$

where: V = volume of KOH 0,1 N used (mL)
N = normality of KOH
W = sample weight (mg)
282 = equivalent weight of oleic acid ($C_{18}H_{34}O_2$) (g/mol)

The determination of liquid soap density was conducted using picnometer at 25° C temperature. The density could be calculated using equation 5.

$$\text{Density } 25°C = \frac{W}{W1} \quad (5)$$

where: W = sample weight (g)
W1 = water weight (g)

The determination of liquid soap foam stability, as much as 1 mL liquid soap were poured into a reaction tube and added with 10 mL of aquadest. The sample was shaken for 1 minute and the height of the foam was measured. The foam height was measured again after 5 minutes after the first measurement. The foam stability could be calculated using equation 6.

$$\text{Foam stability (\%)} = \frac{\text{last measured foam height}}{\text{first measured foam height}} \times 100\% \quad (6)$$

Determination of the soap with the best characteristic

The soap with the best characteristic was determined using efectivity index method. The efectivity index method was conducted by sorting the order of the variable and given the score in the range of 0-1 following the contribution variable towards the good liquid soap characteristics. The best soap formula was determinated based on the highest score of the product. The score could be calculated by multiplying the effectivity value and weight value.

Antifungal activity evaluation

The antifungal activity test of the best character soap was conducted in a few steps involving the media formation to rejuvinate the *Candida albicans* fungi, the making of *Candida albicans* fungi suspension and inhibition test. The test was conducted using the disk diffusion method. Sabouraud Dextrose Agar (SDA) was poured into the petri dish and left until the media became solid. As much as 50 µL of *Candida albicans* culture was smeared evenly on the surface of solid SDA. Subsequently, the hole was made using cork borrer and 50 µL of the soap were poured into the hole. Negative control used was tamanu seed oil soap without the addition of extracts and positive control used was commercial liquid soap. Furthermore, the incubation was conducted inside the incubator at 37 °C and 48 hours. The appearance of clear zone around the hole indicated the fungal activity.

Data Analysis

Data of characterizations were analyzed using IBM Statistical Product Service Solution (SPSS) software version 25 using one way Analysis of Varian (ANOVA). The results were further tested using Duncan's Multiple Range Test (DMRT) with 95% of significance ($\alpha = 0,05$).

RESULT AND DISCUSSION

The extraction of bacang leave and nutmeg leave

The concentrated bacang leave extract obtained was 8,75 gram, which means 4,38% (w/w) of the total yield. Ethyl acetate extraxt of nutmeg leave obtained was 26,64 which means 15,67% (w/w) of the total yield.

Liquid soap formulation

The addition of bacang leave and nutmeg leave extracts was conducted with variety of concentration following the Table 1.

Table 1. The variation of bacang leave extract and nutmeg leave extract addition

Bacang leave extract (b/b)	Nutmeg leave extract (b/b)					
	P0 (0,0%)	P1 (0,1%)	P2 (0,3%)	P3 (0,5%)	P4 (0,7%)	P5 (0,9%)
M0 (0,0%)	SK	SKP1	SKP2	SKP3	SKP4	SKP5
M1 (0,1%)	SKM1	SFM1P1	SFM1P2	SFM1P3	SFM1P4	SFM1P5
M2 (0,3%)	SKM2	SFM2P1	SFM2P2	SFM2P3	SFM2P4	SFM2P5
M3 (0,5%)	SKM3	SFM3P1	SFM3P2	SFM3P3	SFM3P4	SFM3P5
M4 (0,7%)	SKM4	SFM4P1	SFM4P2	SFM4P3	SFM4P4	SFM4P5
M5 (0,9%)	SKM5	SFM5P1	SFM5P2	SFM5P3	SFM5P4	SFM5P5

Descriptions: SK = control soap, SF = formulated soap

Characterization and Data Analysis

The prepared soap was characterized following the SNI 4085:2017 standard involving pH, the total of active substances, the total of free fatty acid, density,and foam stability. The soap that tends to be alkaline will help the skin pores open and will ease the dirt removal on the skin surface. The pH value was measured using pH meter.

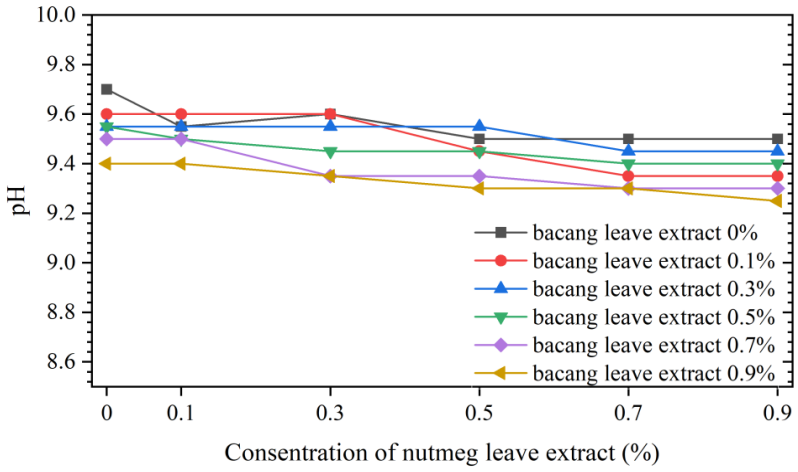

Figure 1. pH value of tamanu seed oil liquid soaps

Based on the Figure 1, the tamanu seed oil liquid soap has the pH value in the range of 9.25-9.70. The pH value of the prepared soaps were following the SNI 4085: 2017 standard, which is between 4-10. This shows that the tamanu seed oil liquid soap categorized safe to be used on skin. Too high pH value will cause the skin dry and too low pH value will cause the irritation of the skin (Adventi, 2018). The result of one way ANOVA analysis showed that the addition of bacang leave extract and nutmeg leave extract did not affect pH value of the soap significantly. The ascorbic acid contained in the bacang leave extract and flavonoid contained in nutmeg leave extract has weak acid characteristic and cause the decrease of pH value of the soap but not very significant (Pohan *et al.*, 2013; Puspa *et al*, 2017).

The total of active substances in surfactant contained in soap help to decrease the surface tension and tied up the dirts on the skin surface.

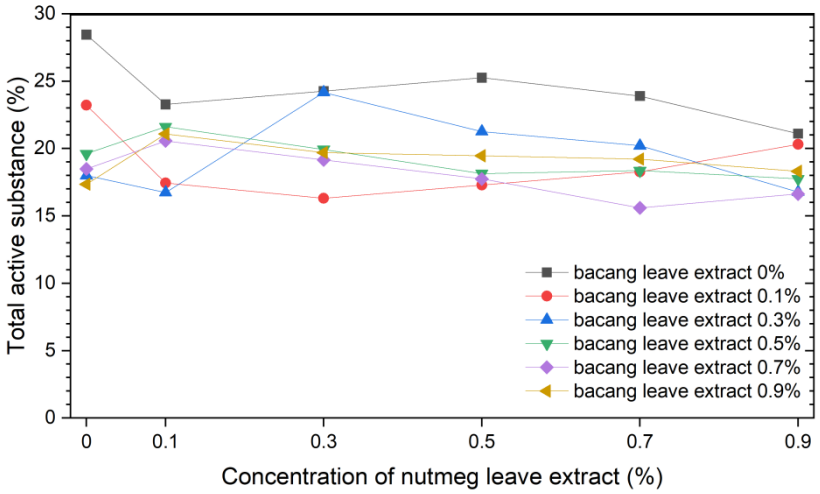

Figure 2. the total of active substances in tamanu seed oil liquid soap

Based on the Figure 2, it shows that control soap (SK) has the highest total active substances which was 28,4450%. The total of active substances of tamanu seed oil liquid soap should not be more than 15% based on SNI 4085:2017. The result of duncan test showed the addition of bacang leave extract with the concentration of 0,1% and 0,7%, and the addition of nutmeg leave extract with the concentration of 0,7% and 0,9% did significantly affect the total of active substances in soap. Surfactant is the main product of saponification reaction between KOH and triglyceride (Dinata *et al.*, 2018). The acid compunds in both extract, ascorbic acid and flavonoid, are able to bind with KOH base and cause the inhibition of saponification reaction and make the decrease of the product.

Free fatty acid is fatty acid that not bond as triglyceride nort with the alkaline of the soap. Free fatty acid could reduce the ability of the soap to tie up the dirts (Ayu *et al.*, 2010). The fatty acid determination was conducted by alkalimetric titration. The result of feee fatty acid determination is shown in the Figure 3.

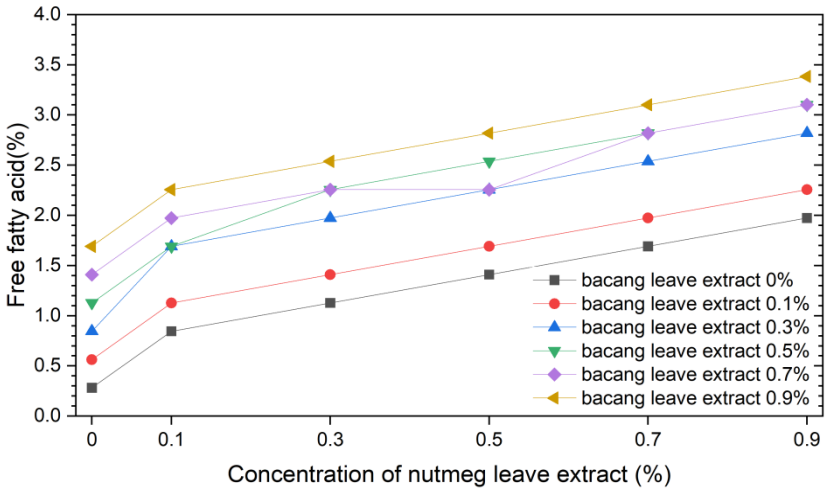

Figure 3. free fatty acid of tamanu seed oil liquid soaps

Based on the Figure 3, the SFM5P5 sample has the highest percentage of free fatty acid which was 3,3840%. The free fatty acid of the soap prepared was following the SNI 4085:2017 which was 4% maxium. The result of duncan test showed the addition of bacang leave and extract nutmeg leave extract did significantly affect the total of active substances in soap towards the increase of the fatty acid content of the soap. The aminoundecanoid omega acid in bacang leave extract and the myristic acid content in nutmeg leave extract caused the increas of the fatty acid content of the soap (Fitmawati *et al.*, 2019; Puspa *et al*, 2017).

The density measurement was conducted using picnometer to find out the ratio between soap and water with the constant volume and temperature.

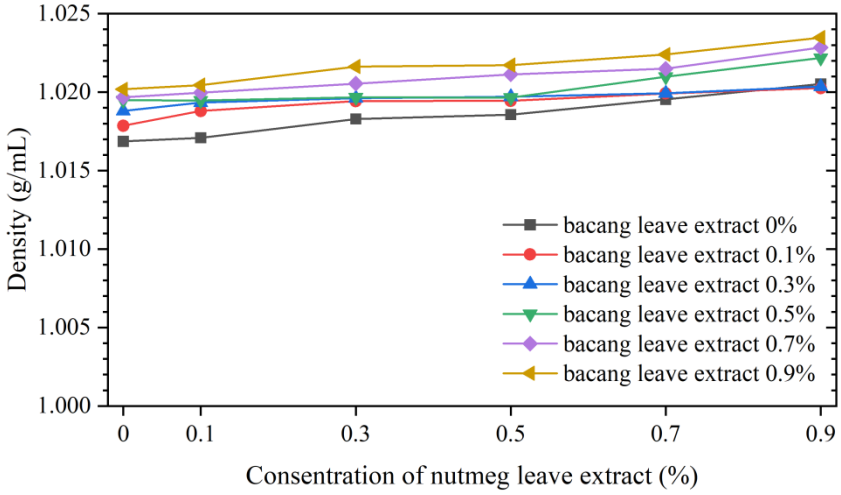

Figure 4. the density of tamanu seed oil liquid soaps

Based on the Figure 4, the SFM5P5 sample has the heaviest density value which was 1,0235 g/mL. The density of tamanu seed oil soap was following the SNI 4085:2017 which was in the range of 1,01-1,1 g/mL. The result of duncan test showed the addition of 0,5%; 0,7%; and 0,9% bacang leave extract and the addition of 0,7% and 09% nutmeg leave extract did significantly affect

the increase of the soap density. The added substances caused the difference of the soap density (Nurhadi, 2012).

The foam stability was measured based on the resistance of the foam in the constant state for 5 minutes.

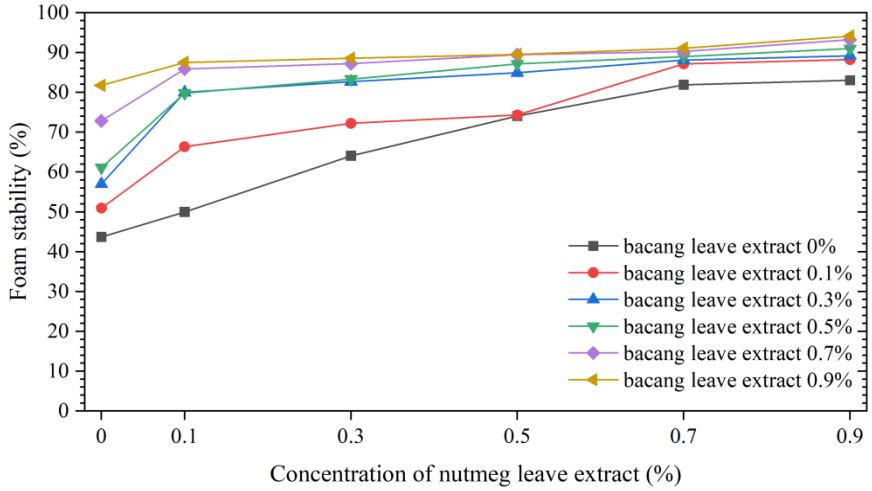

Figure 5. the foal stability of tamanu seed oil liquid soaps

Based on the Figure 5, it shows that SFM5P5 has the most stable foam which was measured 94,1176%. The soap foam of tamanu seed liquid soap has the good stability. During the 5 minutes the formula could maintain the 60-70% of the foam height. (Anggraeni *et al.*, 2020). The duncan test result shows that the result of duncan test showed the addition of bacang leave extract nutmeg leave extract did significantly affect the total of active substances in soap towards the increase of the soap's foam stability. The saponin content of the both extracts could produce the stable foam and helps the stability of the tamanu seed oil liquid soap's foam (Minarno, 2016).

The determination of the best soap formula

The best formula should show the nearest characteristic required based on SNI 4085: 2017.

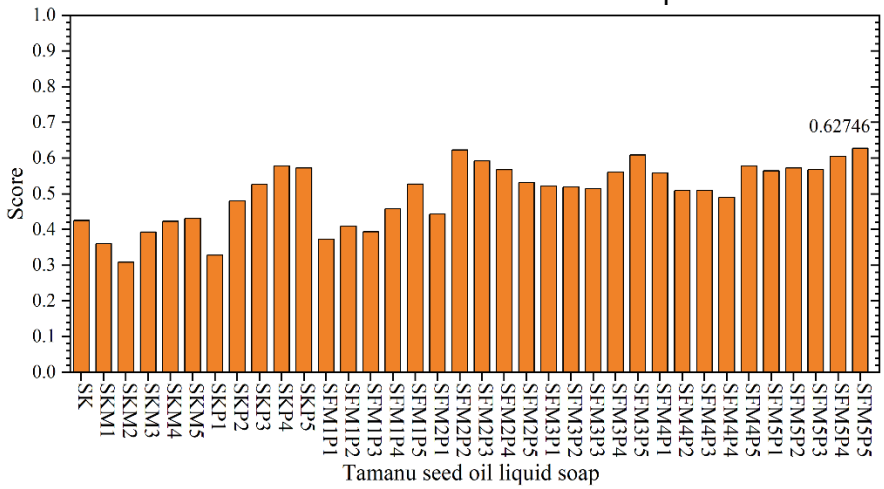

Figure 6. the product value of liquid soaps

Based on the Figure 6, the best soap formula is the soap with the addition of 0,9% bacang leave extract and 0,9% nutmeg leave extract (SFM5P5) with the product value of 0,6275. The data of the best soap formula characterization following the SNI standard are shown in Table 2.

Table 2. characterization data of the best soap formulas

Analysis	SFM5P5 soap	Standard value based on SNI
pH	9,25	4,0-10,0
Total of active substances	18,3017%	> 15%
Fatty acid	3,3840%	< 4%
Density	1,0235 g/mL	1,01-1,10 g/mL
Foam stability	94,1176%	

Based on the Table 2, the result oh the characterization of SFM5P5 soap are in accordance with the quality of soap requaired by SNI.

Antifungal activity evaluation

The soap evaluated was the control soap withouth the addition of the extracts (SK), the control soap with the addition of 0,9% bacang leave extract (SKM5), the control soap with the addition of 0,9% nutmeg leave extract (SKP5), and the best formulated soap SFM5P5. The negative control used was aquadest and the positive control used was commercial soap.

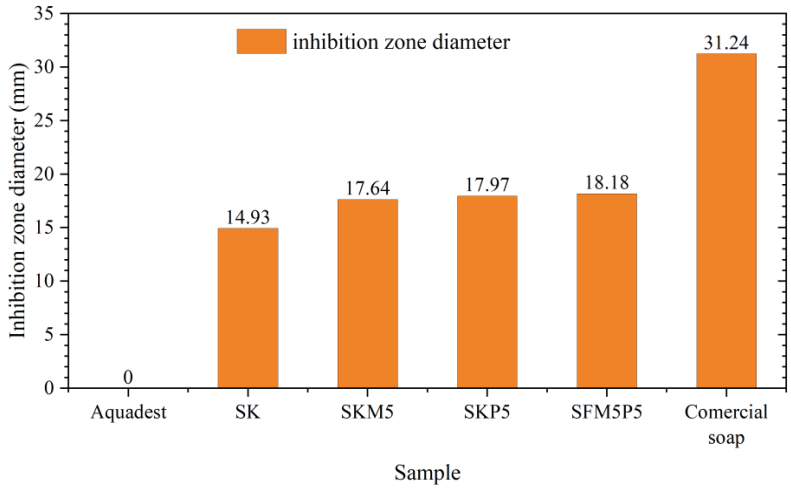

Figure 7. the result of antifungal activity evaluation

Based on the Figure 7. SKM5 soap shows the wider inhibition zone diameter than SK soap. Bacang leave extract contains active substance magniferin and the other secondary metabolytes such as alkaloid, phenol, flavonoid, tannin, and saponin that could inhibit the growth of the fungi. The inhibition mechanism was by denaturate the cell proteins and break the cell permability (Arifin *et al.*, 2018). The saponin and flavonoid content in nutmeg leave extract also caused the wide inhibition zone od SKP5 soap. The commercial soap as positive control shows the highest antifungal activity towards *Candida albicans* with the inhibition zone diameter of 31,24 mm. The

commercial soap contains sodium lauryl sulfate (SLS). SLS is a surfactant with fungal growth inhibitory ability by disrupting the cell permability of the fungi and caused the cell death (Fitria *et al.*, 2020). The SMFP5 soap shows the highest antifungal activity compared to all evaluated soap with the inhibition zone diameter of 18,14 mm. The inhibition zone diameter of all evaluated soap shows the high antifungal activity because the inhibition diameters resulted is in the range of 10-20 mm (Oroh *et al.*, 2015).

CONCLUSION

The tamanu seed oil soap with the addition of 0,9% bacang leave extract and 0,9% nutmeg leave extract (SMF5P5) is the best formulated soap, SFM5P5 has the nearest characteristic which have been specified by SNI 4085:2017. The SFM5P5 nanoparticle soap shows the high activity to inhibit the *Candida albicans* growth with the inhibition zone diameter of 18,14 mm.

ACKNOWLEDGEMENTS

We would like to thank Direktorat Pembelajaran dan Kemahasiswaan, Direktorat Jenderal Pendidikan Tinggi, Kementerian Pendidikan, Kebudayaan, Riset, dan Teknologi for providing PKM program reasearch funds.

REFERENCES

Adventi, F. (2018) Pengaruh Suhu Reaksi, Kecepatan Pengadukan, dan Waktu Reaksi pada Pembuatan Sabun Padat dari Minyak Kelapa. *Skripsi.* Medan: Universitas Sumatera Utara.

Anggraeni, Y., Nisa, F., & Betha, O. (2020). Physical Characteristics and Antibacterial Activity of Patchouli Oil (*Pogostemon cablin* Benth.) Liquid Soaps Based on Sodium Lauryl Ether Sulphate Surfactant. *Jurnal Kefarmasian Indonesia*, 10(1): 1-10.

Arifin, Z. (2018). Aktivitas antijamur ekstrak etil asetat daun mangga bacang (*Mangifera foetida* L.) terhadap *Candida albicans* secara in vitro. *Jurnal Mahasiswa PSPD FK Universitas Tanjungpura*, 4(3).

Artanti, A., Rahmawati, K., Rakhmawati, R., & Prihapsara, F. (2020). Uji Aktivitas Antibakteri dan Antijamur dari Kombinasi Minyak Nyamplung (*Callophyllum Inophyllum* L.) dengan Virgin Coconut Oil dan Pengembangannya sebagai Face Oil. *Jurnal Farmasi Indonesia*, 17(2): 17-29.

Ayu, D., Ali, A., & Sulaiman, R. (2010). Evaluasi Mutu Sabun Padat dari Minyak Goreng Bekas Makanan Jajanan di Kecamatan Tampan Kota Pekanbaru dengan Penambahan Natrium Hidroksida dan Lama Waktu Penyabunan. Prosiding, Pekanbaru: *Konferensi dan Seminar Nasional XX Badan Kerjasama Pusat Studi Lingkungan - Seminar Nasional Lingkungan.*

Chasani, M., Widyaningsih, S., & Mubarok, A. (2015). Sintesis dan Karakterisasi Sabun Natrium dari Minyak Biji Nyamplung (*Calophyllum inophyllum* L.) Serta Uji Aktivitas Antibakteri terhadap *Staphilococus aureus*. *Jurnal Molekul*, 10(1): 66-73.

Dinata, A., Rosyandi, A., Hamid, S., & Zainul, R., 2018, *A Rieview Chemical Vapor Deposition: Proses and Aplication.* Retrieved Juli 2, 2020, https://osf.io/preprints/inarxiv/yfeau/. was accesed on January 13, 2021.

Fitmawati, F., Fauziah, R., Hayati, I., Sofiyanti, N., Inoue, E., & Matra, D. (2017). Phylogenetic analysis of Mangifera from central region of Sumatra using trnL-F intergenic spacer.

Biodiversitas Journal of Biological Diversity, 18(3): 1035-1040.

Fitria, L., Shahib, M., & Sastramihardja, H. (2020). Perbedaan Penurunan Jumlah Koloni *Candida albicans* antara Pemberian Cebokan Rebusan Biji Manjakani dan Daun Sirih Merah pada Wanita Usia Subur (WUS) yang Mengalami Keputihan. *Jurnal Medikes*, 7(1): 185-196.

Minarno, B., 2016, Analisis Kandungan Saponin pada Daun dan Tangkai Daun *Carica pubescens* Lenne & K.Koch. *El-Hayah*, 5(4): 394-401.

Musta, R., Haetami, A., & Salmawati, M. (2017). Biodiesel of The Transesterification Product of *Callophyllum inophyllum* Seed Oil from Kendari Using Methanol Solution. *Indonesian Journal of Chemical Reasearch*, 4(2): 394-401.

Nurhadi, S. (2012). Pembuatan Sabun Mandi Gel Alami dengan Bahan Aktif Mikroalga *(Chlorrela pyrenoidosa Byerinck)* dan Minyak Atsiri *(Lavandula lativolia Chaix). Skripsi*. Malang: Universitas Ma Chung.

Oroh, S., Kandou, F., Pelealu, J., & Pandiangan, D. (2015). Uji daya hambat ekstrak metanol *Selaginella delicatula* dan *Diplazium dilatatum* terhadap bakteri Staphylococcus aureus dan Escherichia coli. *Jurnal Ilmiah Sains*, 15(1): 52-58.

Pohan, A., Purwaningsih, E., & Dwijayanti, A. (2013). Efek Kelasi Ekstrak Etanol Daun *Mangifera foetida* pada Feritin Serum Penderita Talesmia di RS Cipto Mangunkusumo. *Journal of The Indonesian Medical Association*, 1(1): 45-52.

Puspa, O., Syahbanu, I., & Wibowo, M. (2017). Uji Fitokimia dan Toksisitas Minyak Atsiri Daun Pala (*Myristica fragnans* Houtt) dari Pulau Lemukutan. *Jurnal Kimia Khatulisiwa*, 6(2): 1-6.

Seru, R., Suling, P., & Pandeleke, H. (2013). Profil Kandidiasi Kutis Poliklinik Kulit dan Kelamin RSUP Prof. Dr. R.D Kandou Manado Periode 2009-2011. *Jurnal e-Biomedik*, 1(1): 561-565.

.