OPTIMIZATION OF AFRICAN LEAF EXTRACT CREAM FORMULA (*Vernonia amygdalina* Del.) AS AN ANTIBACTERIAL *Staphylococcus aureus*

Iklila Zahra[1*], Ana Indrayati[1], Ilham Kuncahyo[1]
[1]*Ilmu Farmasi, Universitas Setia Budi, Surakarta*
**Email Corresponding: iklilazhr21@gmail.com*

Submitted: 30 July 2023 ***Revised: 21 September 2023*** ***Accepted: 9 October 2023***

ABSTRACT

The use of natural ingredients as active medicinal substances has been developed again, one of which is African leaf extract (*Vernonia amygdalina* Del.), which is used as an antimicrobial bacterium *against Staphylococcus aureus,* which causes skin disease. The aim of this study was to compare the antibacterial activity of African leaf extracts and fractions, as well as to optimize the cream preparation formula using a factorial design with a combination of stearic acid, triethanolamine (TEA), and Adeps lanae with the critical parameters of pH, viscosity, spreadability, Franz diffusion, and KBM value. This study used experimental methods to determine the highest antibacterial activity by disc diffusion from extracts and active fractions of African leaves using solvents of different polarities. The extract or fraction with the highest antibacterial activity was formulated using a combination cream of stearic acid, triethanolamine, and adeps lanae. After obtaining the cream formula, physical testing of the cream, penetration test, and antibacterial test using macrodilution were performed, and the most optimal formula was determined using a factorial design. The extract standardization test results met both specific and non-specific parameters. In the disc diffusion antibacterial test, the highest activity was found in the African leaf extract (13.23 ± 0.757 mm) against *Staphylococcus aureus* bacteria. Variations in cream composition affect pH, viscosity, spreadability, and Franz diffusion. After testing several tests, based on the contour plot of the factorial design, an optimum cream formula was obtained with a concentration of 10 g of stearic acid, 4 g of triethanolamine, and 4 g of adeps lanae, with a cumulative % penetration result of Franz diffusion of 12.53% and a KBM concentration of 25%.

Keywords : African leaf extract, cream , antibacterial , Staphylococcus *aureus bacteria* ATCC 25923 , factorial design

INTRODUCTION

Infectious diseases remain the main cause of high morbidity rates *in* Indonesia (Darmadi, 2011). Infectious diseases, one of which is found on the skin, are still the main cause of high morbidity rates in Indonesia (Darmadi, 2011). *Staphylococcus aureus* is a pathogenic bacterium that causes bacterial infections in the human skin. Several skin diseases caused by infection with *Staphylococcus aureus* include boils, impetigo, cellulitis, blistered skin, and abscesses (Leboffe & Pierce, 2011). To overcome diseases caused by bacteria, which are usually called microbes, antibacterial drugs are required. Antibacterial drugs are commonly used to treat microbial infections in humans. Antimicrobials are better bactericidal than bacteriostatic. Bactericidal agents kill microorganisms, whereas bacteriostatic agents only inhibit and paralyze the growth of microorganisms; therefore, the immune system is required to achieve a total anti-infective effect (Radji, 2019). The use of natural ingredients as active medicinal substances has been encouraged. Existing

technologies can be utilized to produce safer and more effective pharmaceutical preparations. Active drug substances in pharmaceutical technology are beginning to utilize natural sources (BPOM RI, 2012). The African leaf (Vernonia amygdalina Del.) is a plant used in traditional medicine.

Research conducted by Ghamba (2012) states that water and ethanol extracts of African leaves *(Vernonia amygdalina* Del.) have antibacterial effects against Staphylococcus aureus, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella spp ., and Candida albicans* with concentrations ranging from 12.5 and 50 mg/ml. Similarly, the ethanol extract of the stem has an antibacterial effect against *Staphylococcus aureus* at a concentration of 50 mg/ml (Akinyele et al., 2014). There are several phytochemical components in African leaves *(Vernonia amygdalina* Del.*)*, including flavonoids, triterpenoids, and tannins (Del et al. ., 2011) . According to Ana, (2012) , the advantages of using traditional medicine are that it is relatively safe, easy to obtain, cheap, does not cause resistance and is relatively harmless to the surrounding environment. One of the requirements for traditional medicine is that raw materials must meet quality requirements in accordance with the Indonesian Herbal Pharmacopoeia, Indonesian Materia Medika and/or scientific references, so that raw materials must be standardized according to requirements (BPOM, 2005). The use of African leaf extract directly on the skin is impractical; therefore, it is necessary to create a suitable preparation that is easy to use. One of the preparations commonly used for skin treatment is topical preparations, one of which is a cream preparation.

Cream is the preparation of half containing one or more solid drugs dissolved and dispersed on an appropriate material basis. Triethanolamine, sour stearate, and adeps lanae were used as the internal base preparation creams. We used emulgator anionic like triethanolamine and acid stearate, considering that cream made addressed for use outside. The combination of sour stearate and TEA forms an o/w emulsion, which is very stable when combined with free fatty acids. Varying concentrations of stearic acid, triethanolamine, and the adep lane will influence the characteristics of the cream preparation. Similar to the research conducted by Safitra and Sari (2014), enhanced concentration of sour stearate causes changes in characteristics, preparation cream covers organoleptic, pH, viscosity, and power spread before and after storage, as well as the cumulative amount of released curcumin. To formulate the best preparation cream from the fractionated extract of leaf Africa with varying stearic acid, TEA, and adeps lanae, the required optimization of formulation cream was optimized. Formula optimization was performed using an application expert design form design factorial. The analysis uses a factorial design because it evaluates the impact of a combination of two or more treatments on the dependent variable. A factorial design was used if the experiment consisted of two or more factors. Factorial design own-level high flexibility can explore or increase proportion treatment, and so on can effectively test the interaction between influence main and factors or level and can be used to determine proportion relative to the materials used in a formula (Aisyah and Michrun, 2015).

Based on the background and several studies that have been presented, the focus of this research was to test the formulation cream as an antimicrobial against *Staphylococcus aureus ATCC 25923* bacteria and assess the optimum formula for cream from extracts or fraction African leaf extract using the *Factorial Design method.*

RESEARCH METHODS

Tools and materials

The tools used were glassware (Pyrex), Incubator (Memert), Autoclave (SG41 46 280A), large oven (*Binder), small oven (Binder), scales (SCA 301), microscope (13A), distillation flask (Pyrex), pH meter, viscometer, rotary evaporator (Rotavapor II BUCHI), micropipette and microplate.*

The main ingredients in this research used African leaf simplicia (Vernonia amygdalina Del.) 074/240/102.20-A/2022, TEA 99 % (Petronas), propylene glycol, stearic acid 99% (PT. Indokemika Jayatama), adeps lanae (Wujang Jinyu Lanoline Corporation), Alcohol 96% (Bratako), methanol 99.9% (Merck), ethyl acetate 99.5% pa (Merck), n-hexane 99%

pa (Merck), distilled water (Rofa), *nutrient agar (NA)* medium, 0.9% NaCl (Merck), MHB medium (Himedia), *p-iodonitrotetrazolium* (INT) solution, *Staphylococcus aureus* ATCC 25923 obtained from the Surabaya Health Laboratory Center.

Research Procedure

1. **Making African Leaf Extracts and Fractions (*Vernonia amygdalina* Del.)**
   a. Preparation of extract: To prepare the ethanol extract of African leaves (*Vernonia amygdalina* Del.), a ratio of 1:10 was used use solvent 96% ethanol.
   b. Making fraction: Fractionation was performed with extraction liquid-liquid use solvent n-hexane, ethyl acetate, and water.
2. **Identification Extract Chemical Compounds**
   Content chemistry extracts were tested in the form of alkaloids, flavonoids, tannins, saponins, triterpenoids, and steroids.
3. **Activity Test Antibacterial African Leaf Extracts and Fractions (*Vernonia amygdalina* Del.)**
   Antibacterial activity was tested using the diffusion paper disc method. The paper discs were then soaked in the test solution at a concentration series of ethanol extract and active fraction of African leaves (*Vernonia amygdalina* Del.) concentration of 2 0.4 0.6 0.80 % for ± 15 minutes (Zeniusa *et al.* ., 2019) . Paper discs placed on the surface of the MHA media containing bacteria with number on one cup contained four concentrations of extract, and each fraction was active and repeated in triplicate (Ningsih *et al.* ., 2016) . Compared with the negative control (DMSO 10%) and positive control (gentamicin), 30 µg/disc. The Petri dishes were incubated at 35 ± 2°C for 24–48 hours. The zone of inhibition was measured based on the clear area around the disc, which indicated the absence of bacterial growth. The inhibition zone was measured in millimeters.
4. **Cream Formula Making**
   Extract or fraction active leaf Africa with Power resistor will be used as an active substance in making cream. The cream formulation used a combination of TEA, acid stearate, and adeps lanae at different concentrations for optimization.

Table I. Formulation cream extracts and fractions leaf Africa

Material Name	F1	F2	F3	F4	F5	F6	F7	F8
Extract / fraction (g)	8	8	8	8	8	8	8	8
Stearic Acid (g)	15	15	10	10	15	10	10	15
TEA (Triethanolamine)(g)	4	4	2	4	2	4	2	2
Adeps lanae (g)	4	3	3	3	4	4	4	3
Propylene glycol (g)	10	10	10	10	10	10	10	10
Methyl Paraben (g)	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Aquades ad (ml)	100	100	100	100	100	100	100	100

In this table there are 8 possible runs done optimization with different amount Its composition is stearic acid, TEA and adeps lanae

5. **Cream Physical Quality Testing**
   a. Organoleptic Test
      Organoleptic tests of the cream preparations were performed by observing the color, odor, and shape of the cream (Cortegiani *et al.* ., 2020) .
   b. Homogeneity Test
      Apply the cream made to the glass object, touch it with another glass object, and determine whether the base applied to the glass object is homogeneous and whether the surface is smooth and even (Ministry of Health of the Republic of Indonesia 2008).
   c. Measurement Viscosity
      The viscosity of the preparation cream in the RION VT 04F viscotester was then read in accordance with the rotor used (Ministry of Health RI, 2008).
   d. pH measurement
      pH measurement using a pH meter. Skin pH is in the range of 5-8 (Saryanti *et al.* ., 2019).
   e. Spreadability Test
      The cream was weighed as much as 0.5 grams, after which the cream was placed immediately below the glass round at the bottom, accompanied by a diameter scale; the other glass was then weighed and left for One minutes, after which the diameter of the spread was measured. After 1 minute, 50 g of load was added and left for 1 minute, after which the diameter of the spread was measured. The testing power spread was carried out in three replicates (Ministry of Health of the Republic of Indonesia, 2008).
6. **Diffusion Test .**
   Diffusion studies were performed using the Franz diffusion cells. The recipient fluid compartment was filled with a phosphate buffer solution pH 7.4 to full volume (50 ml). A total of 2 g of cream was smeared evenly on the cellophane membrane 250 nm pore were used as the replacement skin. The temperature was set at 37 ± 1°C, and the speed was 120 rpm. Samples were taken at intervals of 5, 10, 15, 30, 45, 60 and 90 minutes, amounting to 3 mL. The samples were analyzed using a UV-Vis spectrophotometer at a maximum wavelength of 378 nm. The cumulative amount of flavonoids that penetrated per unit was calculated from the regression equation obtained from the standard u curve in the form quercetin (Sapra *et al.* ., 2019).
7. **Activity Test Antibacterial By Macrodilution**
   a. Making Solution Parent: Solution parent used 10% DMSO solvent at a concentration of 1000 ppm.
   b. Creation of a concentration series: Variation-concentration test solutions were prepared at 100, 200, 400, and 800 ppm of the solution parent, as much as 10 ml with 10% DMSO solvent.
   c. Making Solution Control Positive: A total of 10 mg of cream gentamicin was weighed and then dissolved in 100 ml of sterile distilled water.
   d. Making Solution Control Growth: A total of 100 µL of MHB media was added to 100 µL of bacterial suspension.
   e. Making Solution Negative: A total of 100 ml of growth medium was added to 100 ml of cream-based test solution.
   f. Macrodilution Test: Research method used was serial dilution (dilution multilevel) with a ratio of 1:2 (w/v). The test method, which uses turbidimetric and measured the mark absorbance or mark turbidity, uses a spectrophotometer.

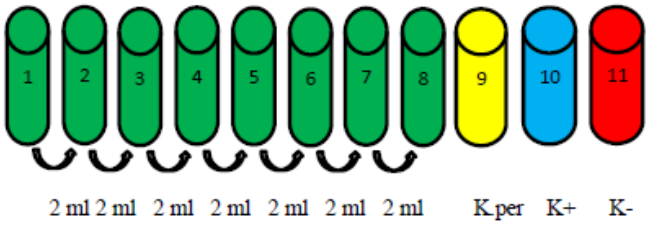


Figure 1. Dilution method for macrodilution testing.

Description :
Tube 1 = contains 4 ml of 100% concentration + 0.5 ml of Mc Farland standard microbes
Tube 2 = contains 2 ml from tube 1 + 2 ml solvent + 2 ml media + 0.5 ml microbes
Tube 3 = contains 2 ml from tube 2 + 2 ml solvent + 2 ml media + 0.5 ml microbes
Tube 4 = contains 2 ml from tube 3 + 2 ml solvent + 2 ml media + 0.5 ml microbes
Tube 5 = contains 2 ml from tube 4 + 2 ml solvent + 2 ml media + 0.5 ml microbes
Tube 6 = contains 2 ml from tube 5 + 2 ml solvent + 2 ml media + 0.5 ml microbes
Tube 7 = contains 2 ml from tube 6 + 2 ml solvent + 2 ml media + 0.5 ml microbes
Tube 8 = contains 2 ml from tube 7 + 2 ml solvent + 2 ml media + 0.5 ml microbes
Tube 9 = Growth control = 2 ml media + 0.5 ml microbial suspension
Tube 10 = Control -, contains 2 ml of media + 0.5 ml of microbial suspension + 1 ml of 100% cream base
Tube 11 = Control +, contains 2 ml of media + 1 ml of microbial positive control + 0.5 ml of bacterial suspension

g. Determination Concentration Minimum Barrier

Before incubation, the test tube was tested on a UV-Vis spectrophotometer at a maximum wavelength of 625 nm (McFarland standard) using a microbial media blank, which was carried out in triplicate. Each tube with a known initial absorbance value was then placed in an incubator for antibacterial incubation at 35 ± 2˚C for 18-24 hours. After incubation, the absorbance values of all the treatment tubes were measured again using a spectrophotometer, and the final absorbance values were measured in triplicate. MIC is the lowest concentration at which there is no microbial growth in the tube used, based on the difference in well clarity when compared with the control (Fajrina *et al.* ., 2019) .

h. Determination Concentration Minimum Kill

A certain amount of solution was taken using a loop needle from the well in the test tube, which shows the MIC value, as well as from all other wells that are above the MIC value, which is characterized by the absence of growth of bacterial colonies at the smallest concentration. The solution was then streaked onto the surface of the previously prepared agar media. Petri dishes were incubated at 35 ± 2 °C. Agar media that show the presence or absence of bacterial colony growth and media that do not show bacterial growth are determined as the minimum kill concentration (KBM) (Fajrina *et al.* ., 2019) .

Data Analysis

The data were obtained and then optimized based on method design factorial data results experiments that included viscosity, power spread, ability diffusion Franz, and antimicrobial activity, entered into equality mathematical for design model factorial. Optimization data processing using " *Design Expert" software.* The equation was obtained using the factorial design method with a one-sample t-test (*one sample t-test*) with a confidence level of 95% for the parameters tested (spreadability, Franz diffusion viscosity test, and antimicrobial) between theoretical values and experimental results.

RESULTS AND DISCUSSION

1. Extracts and Fractions leaf Africa

A total of 2000 grams of African leaf simplicia powder (*Vernonia amygdalina* Del.) 257.9 grams of the thick extract was obtained. The yield obtained was 12.89%. The thick extract obtained was then fractionated using the liquid-liquid method. Fractionation was performed to separate the active components from the resulting extract. Separation by liquid-liquid partition must have a difference in solubility between the solvent and the solute, and the two solvents used must not mix with each other (Nuria *et al.* ., 2014) . The fractionation results of the ethanol extract of African leaves are shown in table below.

Table II. Fractionation results extract ethanol leaf Africa

Test Fraction	Weight fraction thick (grams)	Randemen (%)
Ethyl acetate	35.1	13.61
Water	62.7	24.31
N- Hexane	49.5	19,19

2. Content Extract Chemical Compounds

Organoleptic tests were carried out on African leaf extract (*Vernonia amygdalina* Del.) to determine the physical characteristics of the plant, including shape, color, smell, and taste. The results of organoleptic examination of African leaf extract (*Vernonia amygdalina* Del.) showed that it had a dark green-black color, a characteristic extract smell, a slight hangover, a bitter taste, and was in the form of an extract. thick. The next parameters were tested in the form of a content test chemistry extract. Testing the chemical content of extracts or what can be called phytochemical screening is one way that can be used to identify the content of secondary metabolite compounds in natural materials. Phytochemical screening aims to provide an initial picture of the content of certain compounds in the natural ingredients being studied (Vifta & Advistasari, 2018). Data from testing the African leaf extract (Vernonia amygdalina Del.) using phytochemical methods are shown in the table below.

Table III. Test Results Chemical Content Using Phytochemical Methods

No.	Compound	Reactor	Results	Information	
				I	II
1.	Alkaloids	Dragendrof	Brick red/ orange	+	+
2.	Flavonoids	HCl and FeCl 3	Red	+	+
3.	Saponins	Shake strong , HCl	Froth No is lost	+	+
4.	Tannin	FeCl 3 1 N	Blackish green	+	+
5.	Triterpenoids / Steroids	Ether, H 2 SO 4	Bluish green	+	+

Note:

Testing of the chemical content of the ethanol extract of African leaves (*Vernonia amygdalina* Del.) was performed twice, namely on results I and II, which were repeated.

3. Activity Test Antibacterial African Leaf Extracts and Fractions (*Vernonia amygdalina* Del.)

Antibacterial activity test of *Staphylococcus aureus* ATCC 25923 *with the Kirby Bauer* diffusion method using *Mueller Hinton Agar* (MHA) media. The inhibition zone is also influenced by incubation temperature, disk installation time, and antimicrobial disk distance (Nathan & Scobell, 2012). The results of measuring the antibacterial inhibition zone of *Staphylococcus aureus* ATCC 25923 are shown in table below.

Table IV. Test Results Antimicrobial against *Staphylococcus aureus bacteria* by diffusion

Preparation	Control + (mm)	Control – (mm)	20% (mm)	40% (mm)	60% (mm)	80% (mm)
African Leaf Extract	26.6± 0.404	0	6.9 ± 0.208	7.9 ± 0.916	10.07 ± 0.896	13.23± 0.757
N- Hexane Fraction	25.23 ± 1,604	0	4.1 ± 0.4	5.77± 0.702	7.2± 0.755	9.93± 1.8239
Ethyl acetate fraction	23.53± 1,750	0	4.67± 1,159	5.7±0.4	7.77 ± 0.737	11.8± 0.557
Water Fraction	24.57 ± 1,332	0	4.87± 0.862	6.37 ± 0.451	8.4 ± 0.889	12.42± 0.562

Information:
Control + using 10 µg gentamicin antibiotic disk
Control – using dmso 10%

The African leaf extract (*Vernonia amygdalina* Del.) has the largest zone of inhibition, which is caused by the presence of chemical compounds in the extract, which are still complete, and there has been no separation of compounds based on polarity. Therefore, the content of compounds with antibacterial properties was complete.

4. Cream Physical Quality Testing

The use of stearic acid and TEA in cream preparations can lead to the formation of stable oil-in-water emulsions (Saryanti *et al.* ., 2019) . Based on a study by Cahyati *et al.* ., (2015) showed that creams prepared using stearic acid and TEA are stable during storage. A pH test of the cream was performed to determine whether the resulting cream formula was acidic or alkaline. A pH that is too acidic or too alkaline can cause irritation to the skin; therefore, it is necessary to match the pH of the cream preparation with the skin's pH, namely, in the range of 5-8 (Saryanti *et al.* ., 2019) . The results of the pH testing of the cream formula are shown in the table below.

Table V. pH Test Results Against All Cream Formulas

Formulas	pH data (mean) ± SD		Sig. (2-tailed)
	Before *cycling test*	After *cycling test*	
Formula 1	6.98 ± 0.005	6.40 ± 0.1	0,000
Formula 2	7.06 ± 0.05	6.71 ± 0.105	0.058
Formula 3	6.99 ± 0.02	6.27 ± 0.085	0.005
Formula 4	6.42 ± 0.01	6.03 ± 0.02	0.001
Formula 5	6.95 ± 0.02	6.66 ± 0.015	0.001
Formula 6	7 ± 0.01	6.75 ± 0.068	0.035
Formula 7	7.09 ± 0.005	6.83 ± 0.036	0.006
Formula 8	6.9 ± 0.005	6.61 ± 0.025	0.001

The pH testing performed for all formulas showed that there was a significant difference in the average pH between the formulas before and after the *cycling* test with a sig value. < 0.05, but the pH of the cream is still safe to use because it is still within the pH requirements accepted by the skin, namely, in the range of 5-8 (Saryanti *et al.* ., 2019) . A pH that is less than the requirements causes skin irritation, whereas a pH that is greater than the requirements causes the skin to become scaly (Sharon *et al.* ., 2013) .

Viscosity tests of the cream preparations were performed to determine the thickness of the cream. The viscosity results are shown in the table below.

Table VI. Test Results Viscosity To All Cream Formulas

Formulas	Viscosity data (d.Pa. s) (mean) ± SD		Sig. (2-tailed)
	Before *cycling test*	After *cycling test*	
Formula 1	200 ± 0	178.33 ±3.21	0.007
Formula 2	250 ± 0	233 ± 4.58	0.023
Formula 3	243.33 ± 11.54	236 ± 3.60	0.429
Formula 4	186.67 ± 5.77	181.33 ± 3.21	0.411
Formula 5	243.3 ± 5.77	200.33 ± 5.51	0.012
Formula 6	186, 67 ± 5.78	178 ± 3.61	0.184
Formula 7	220 ± 17.32	210 ± 10.53	0.590
Formula 8	210 ± 17.3	193 ± 2.64	0.184

The viscosity data obtained from the 8 formulas fall within the viscosity range of semi-solid preparations. The higher the volume of the cream preparation, the higher the viscosity; therefore, the preparation will be more stable because particle movement tends to be difficult as the preparation becomes thicker. The measurement results show that there are differences in viscosity between the formulas due to differences in emulsifier concentration. The greater the variation in the triethanolamine concentration and the less stearic acid, the lower the resulting viscosity.

The spreadability test was carried out to determine whether the cream preparation could spread evenly and was easy to apply or use within 5-7 cm (Edy et al., 2016). The spread power results are listed in the following table:

Table VII. Spreadability Test Results To All Cream Formulas

Formulas	Power data scatter (cm) (mean) ± SD		Sig. (2-tailed)
	Before *cycling test*	After *cycling test*	
Formula 1	5.16 ± 0.05	5.43 ± 0.153	0.094
Formula 2	5.46 ± 0.05	5.8 ± 0.3	0.300
Formula 3	5.2 ± 0.1	5.17 ± 0.15	0.808
Formula 4	5.7 ± 0.2	5.93 ± 0.152	0.020
Formula 5	5.8 ± 0.17	6.17 ± 0.153	0.173
Formula 6	6.2 ± 0.1	6.43 ± 0.153	0.073
Formula 7	5.7 ± 0.1	6.1 ± 0.1	0.057
Formula 8	5.76 ± 0.15	6.23 ± 0.153	0.107

The results of the spreadability test performed on all formulas show that only Formula 4 has a sig value. <0.05, indicating that the dispersion power did not show a significant difference before and after *the cycling test.* The higher the concentration of stearic acid and the lower the concentration of triethanolamine, the higher the viscosity of the cream and the lower the spreadability. The poor spreadability is probably caused by the unbalanced ratio between the oil emulsifier and water emulsifier, thus decreasing

the viscosity. This is also related to the amount of formulation between water and oil used, so that the viscosity between formula 1 and formula 8 has a different spreadability but is still within the required range, namely 5-7 cm.

5. Franz Diffusion Test

The results of penetration testing of African leaf extract cream (*Vernonia amygdalina* Del.), which shows the cumulative amount of active substance penetrated per area in formulas 1–6 through a 250 nm pore cellophane membrane as a skin substitute membrane, are shown in the table below.

Table VIII. Cumulative % Cream Penetration Per Unit Area

Minute	Cumulative % release substance per unit area ± SD (%)							
	F1	F2	F3	F4	I	F6	F7	F8
5	1.97±0.08	2.1±0.04	2.08±0.06	2.49±0.15	2.04±0.13	2.50±0.08	1.91±0.04	2.06±0.08
10	2.81±0.07	3.19±0.17	3.87±0.18	3.75±0.51	2.92±0.14	3.92±0.09	2.98±0.06	3.00±0.08
15	3.73±0.11	4.46±0.42	5.50±0.15	5.17±0.64	4.13±0.50	5.54±0.10	4.23±0.13	4.29±0.06
30	5.72±0.12	6.15±0.34	7.19±0.22	7.08±0.98	5.68±0.52	8.05±0.30	5.65±0.20	5.65±0.12
45	7.23±0.09	7.91±0.45	9.30±0.05	8.97±1.10	7.33±0.54	9.92±0.43	7.11±0.13	7.07±0.16
60	9.37±0.34	10.01±0.33	11.28±0.13	10.61±1.50	9.33±0.50	12.26±0.28	8.76±0.15	8.78±0.15

The cumulative % amount of penetrated substances in all cream formulations showed that the sample increased each time. Data from analysis using *One-Way* ANOVA from eight formulas show that the average value percentage. The cumulative penetration ability of each formula has a significant difference, namely 0.000 ($p<0.05$).

6. Antibacterial Cream Activity Test By Macrodilution

Determination of antimicrobial MIC was carried out on all cream formulations using the macrodilution method with spectrophotometry to determine the absorbance. If the final absorbance value (after incubation) of each tube is greater than the initial absorbance value (before incubation), it can be concluded that bacterial growth is still occurring. The test tube was tested using a UV-vis spectrophotometer at a wavelength of 625 nm (Mc. Farland standard). The minimum inhibitory concentration results obtained from the clarity of the solution are shown in Table 24.

Table IX. MIC results of antibacterial *Staphylococcus au reus ATCC 25923* seen from the clarity of the solution

Formulas	concentration (%)
Formula 1	25
Formula 2	25
Formula 3	25
Formula 4	50
Formula 5	25
Formula 6	12.5
Formula 7	12.5
Formula 8	12.5

Research conducted by Murhadi (2019) stated that stearic acid has inhibitory activity against *Staphylococcus aureus* at concentrations >100 mg/mL. Another cream with antimicrobial activity is propylene glycol, although there was no concentration variation in the cream composition. In addition to being a humectant, propylene glycol can also be used as a solvent, preservative, disinfectant, and antimicrobial agent (Tsabitah *et al.*., 2020) .

Determination of antimicrobial KBM is carried out by examining the agar media, which shows whether there is growth of bacterial colonies, and media that do not grow bacteria is determined as the minimum kill concentration (KBM). The results of the minimum kill level test, observed from the absence of bacterial colonies growing on the petri dish, are shown in the table and image below.

Table X. Results K B M Antibacterial *Staphylococcus aureus ATCC 25923* seen from the absence of bacterial colony growth in the Petri dish

Formulas	Concentration (%)
Formula 1	50
Formula 2	50
Formula 3	50
Formula 4	100
Formula 5	50
Formula 6	25
Formula 7	25
Formula 8	25

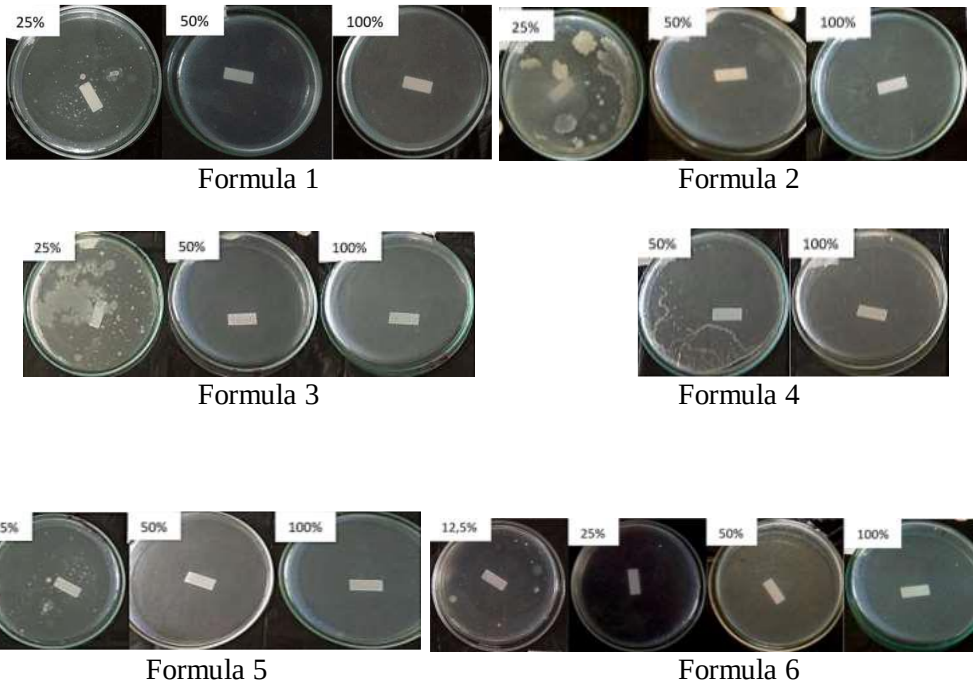


Figure 2. KBM test results for extract formula leaf Africa to bacteria *Staphylococcus aureus*

The results of the antimicrobial ability of KBM against S. *aureus* in all cream formulations showed that only at a minimum concentration of 25%, the formula can kill microbes.

7. Optimization Cream Formulation Using Factorial Design

The results of the data test optimization cream extract of leaf Africa, including viscosity, pH, power spread, diffusion fraction, and KBM values against S. *aureus*, *were included* in the equality mathematical model with *the design* factor model. Results of data experiments on *the Layout of Design* (*actual*) using *Design Expert ® software* 13. Analysis data obtained from results testing viscosity for know big effect of each factor and interaction between factor in determine value response viscosity, the obtained equation as following:

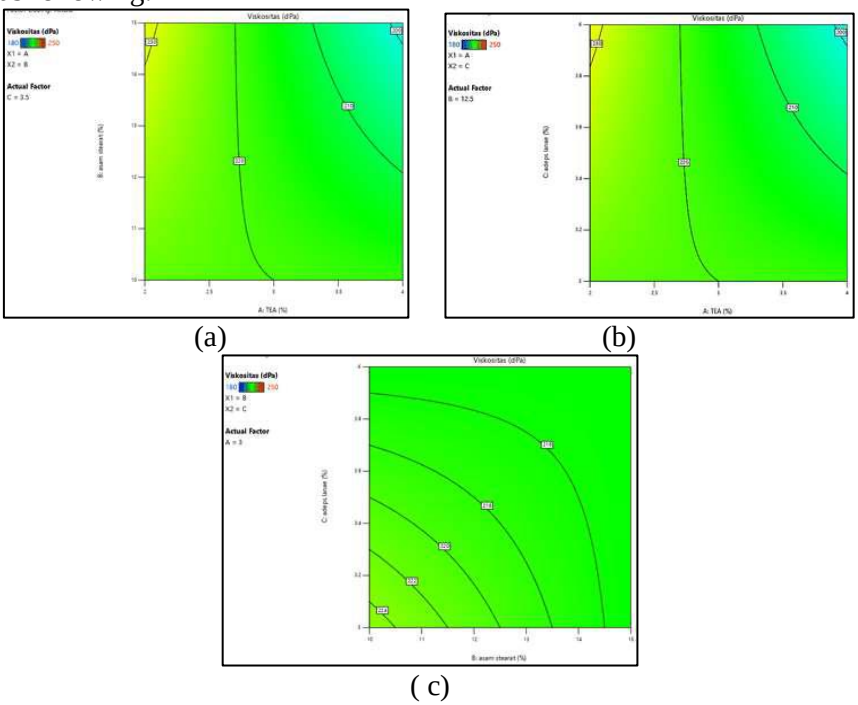


Figure 3. *Contour plot* of the interaction of TEA, stearic acid and *adeps lanae* to viscosity

The contour plot is shown in figure 3 (a), which shows that the amount of stearic acid and TEA have an effect on the lower viscosity cream. This thing shown on The *contour plot* of the blue young color is at the upper limit of TEA and stearic acid, indicating a low viscosity of 200 dPaS. Likewise, *the contour plot of* the TEA interaction with *the adeps lanae* (b) shows that the enhancement amount *of adeps lanae* and the amount of TEA has an effect in lower viscosity cream, where the color blue young is at the upper limit of TEA with *adeps lanae*, which signifies a low viscosity of 200 dPaS. The interaction results between sour stearate and *adeps lanae* (c) also showed a decline in the mark viscosity at the upper limit of stearic acid and *adeps lanae* 216 dPaS. Testing the can concluded that addition *adeps Lanae* and sour stearate with the same amount can stabilize mark viscosity of the preparation. The test results are in accordance with the research conducted by Siti Sunari in 2020, which compared addition *adeps lanae* with addition *ghee*. The results showed that the mark viscosity of adeps lanae is 18, 200 cps larger than that of ghee, which is 16,453.

Chart interaction between TEA, stearic acid and adeps lanae are produced to pH response can seen in figure 3 below This

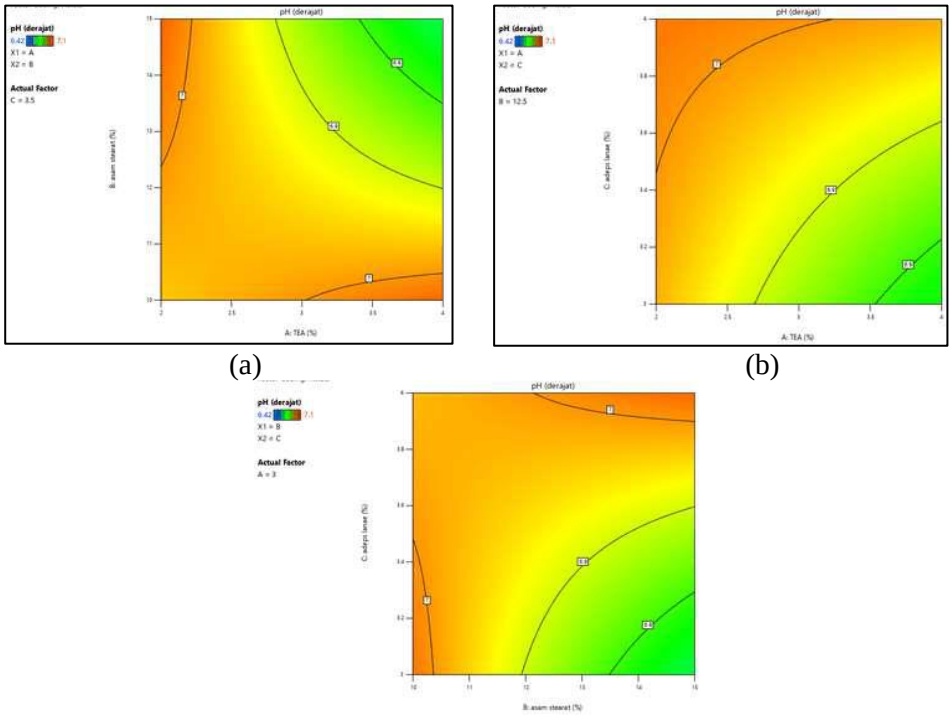
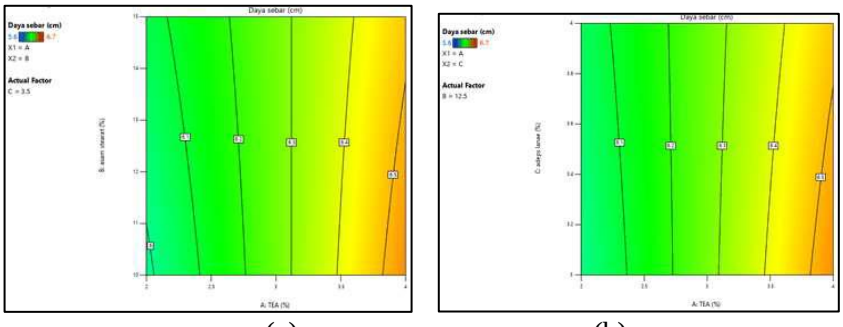
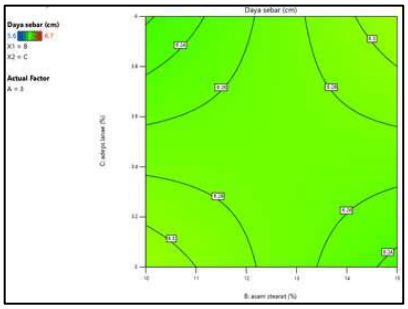


Figure 4. ***Contour plot*** **of the interaction of TEA, stearic acid and** ***adeps lanae*** **to pH**

The contour plot in Figure 4 (a) shows that the higher the amounts of TEA and stearic acid, the lower the pH value of the preparation. A high pH value is observed at the lower limit of stearic acid with an upper limit of TEA, as well as an upper limit stearic acid with a lower limit of TEA with a pH value of 7. This shows that the concentrate on one factor must be larger to obtain a high pH value. *Contour plot* (b) shows that the highest pH value was at the upper limit *of the lanae* with the lower limit of TEA. The difference in color showed an increase in pH with the addition *of adeps lanae* and reduction of TEA with a pH value of 7. This proves that the concentration of A. *lanae* influences the pH value of the cream extract of leaf Africa (*Vernonia amygdalina* Del.). *Contour plot* (c) shows that the more lots sour stearate and *adeps lanae* are given, then add the pH value of the preparation. The highest pH was found at the upper and lower limits of stearic acid with *Adeps lanae,* with pH values of 7. This shows that the number of intermediate factors, stearic acid and *adeps lanae,* which were balanced, had high pH values.

Chart interactions between TEA, stearic acid, and adeps lanae were produced to respond to the power spread, as shown in figure 4.



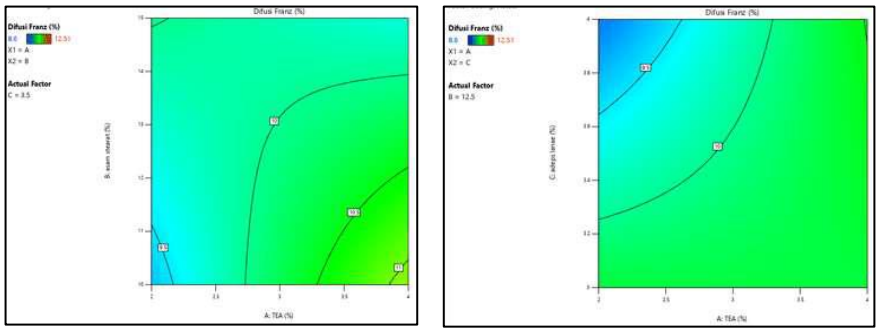


(c)

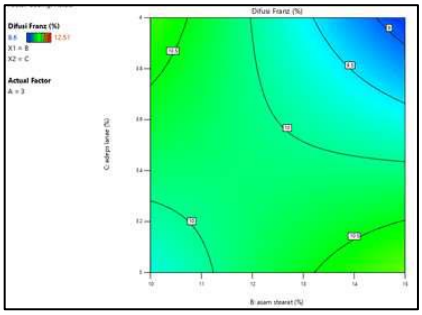
Figure 5. *Contour plot* of the interaction of TEA, stearic acid and *adeps lanae* to Power spread

The contour plot in Figure 5 (a) shows an orange color at the position of the minimum limit for stearic acid and the maximum limit for TEA, with a mark power spread of 6.5 cm. Contour plot (b) color picture show orange color at the position of the minimum border of the adeps lanae with a maximum limit of TEA with mark Power spread of 6.5 cm. This color indicates that the addition of TE increases the mark power spread, whereas stearic acid and adeps lanae can lower the mark power spread. Contour plot (c) shows color green in a way overall description that combination with the same amount between stearic acid with adeps lanae give mark Power stable distribution.

Chart interaction between TEA, stearic acid and adeps lanae are produced to response ability diffusion Franz can seen in figure 5 below This



(a) (b)



(c)

Figure 6. *Contour plot* of the interaction of TEA, stearic acid and *adeps lanae* to diffusion Franz

Contour plot (a) color picture shows color green at the lower limit position of stearic acid and the upper limit of TEA with the highest mark being 11%. The green color image shows that the combination of stearic acid and TEA with amount bigger

increased the diffusion ability of Franz. Contour plot (b) color picture show color green in position middle adeps lanae and TEA with mark by 10%. This indicates that the addition of adeps lanae and TEA with the same composition can increase the diffusion ability of Franz. Contour plot (c) shows green color at the respective positions of the upper and lower boundaries of the adeps lanae and stearic acid. This color indicates that stearic acid with adeps lanae, if the same formula composition, will give mark diffusion high frequency with a mark of 10.5%.

Chart interaction between TEA, stearic acid and adeps lanae are produced to response ability diffusion Franz can seen in figure 6

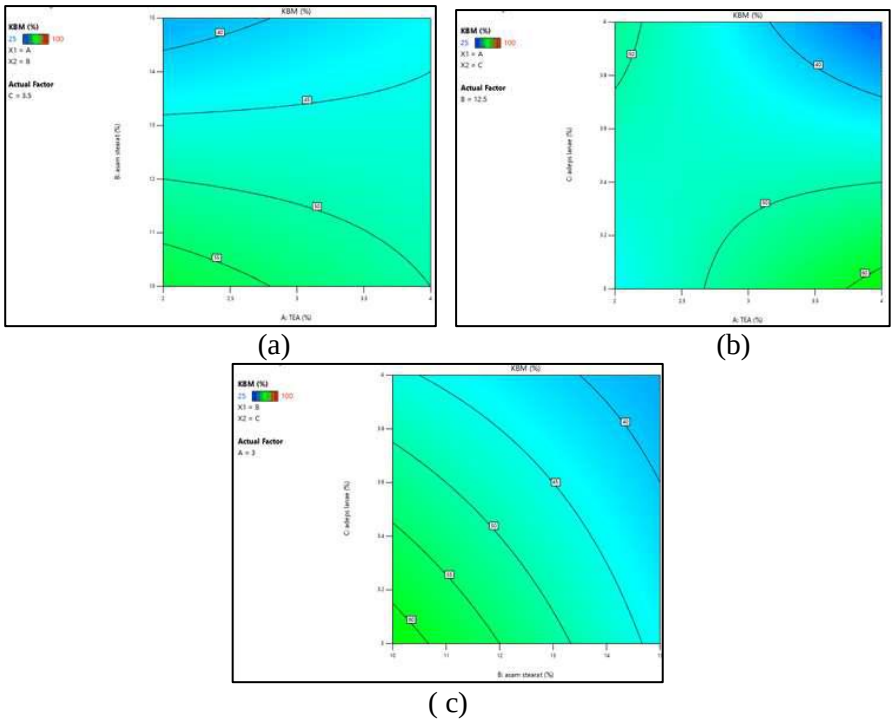


Figure 7. *Contour plot* of interaction between TEA, stearic acid and *adeps lanae* to KBM.

The contour plot in Figure 6 (a) shows the blue color at the upper limit position of stearic acid and the lower limit of TEA with a value of 40%. The color image shows that the combination of a small amount of stearic acid and TEA increased the ability of KBM to produce the lowest value. Contour plot (b) color picture shows blue at the position of the upper border of the adeps lanae and TEA with a value of 40%. This color indicates that the addition of adeps lanae and deep TEA can increase the ability cream in determining KBM, with proof that the value obtained is the lowest. The contour plot (c) shows the blue young color, which is in the upper limit position of the adeps lanae and stearic acid with a value of 40%. This color indicates that the addition of adeps lanae and stearic acid increases the ability of the cream to determine KBM with the smallest value. These results indicate that the ability of the extract hinders the growth of microbes at the smallest dose.

Equations obtained from parameters that were optimized using factorial design obtained the optimum formula for cream preparation of African leaf extract (*Vernonia amygdalina Del.)* in formula 6 with a composition of 10 g stearic acid, 4 g TEA, and 4 g adeps lanae. The verification results of viscosity, pH, spreadability, Franz diffusion and KBM parameters show that there is no significant difference with the sig value. > 0.05. The parameters of the physical properties of the cream preparation of African leaf

extract (*Vernonia amygdalina* Del.) during 6 storage cycles with changing temperatures showed a sig value. <0.05 in viscosity, pH and adhesion values, which means the values for these parameters cannot be stable. Meanwhile, the spread power gets a sig value. >0.05 which indicates stable results during storage.

CONCLUSION

Studies have been carried out on extracts of African leaves, with results from pregnancy chemistry extracts in the form of alkaloids, flavonoids, saponins, tannins, triterpenes, and steroids. Between extracts and fractions, African leaf extract (*Vernonia amygdalina* Del.) has antimicrobial activity bacteria *Staphylococcus aureus* is most effective than polar, semi-polar and non-polar fractions. Variations in stearic acid, TEA, and adeps lanae had a specific influence on the physical quality properties of cream, as indicated by ANOVA testing on *Tukey* HSD with a significance value of <0.05. The composition of the cream formula varies in concentration of 10 g stearic acid, 4 g TEA and 4 g adeps lanae with a viscosity value of 187 dPas ± 0.035, pH 7.02 ± 0.011, spreadability 5.9 ± 0.043 cm, adhesive power 11.98 ± 0.016 seconds, cumulative % penetration of 12.07 ± 0.28 % and KBM at 25%.

BIBLIOGRAPHY

Aisyah Fatmawaty, Michrun Nisa, et all. (2015). Pharmaceutical Preparation Technology. In *Pharmaceutical Preparation Technology* .

Akinyele, B. O Oladejo, B. I aAkinyemi, A. O Ezem, L. (2014). Comparative Study of the Antibacterial Effect of Mouth Washes and Vernonia amygdalina (del.) on Some Tooth Decay Causing Bacteria. *British Microbiology Research Journal*.

Ana, Y. (2012). Traditional medicine. *Jambi University Nursing Journal* .

BPOM. (2005). Guidelines on how to make good traditional medicine. *Guidelines on how to make good traditional medicines* .

BPOM RI. (2012). Technology Guidelines for Formulating Extract-Based Preparations. In *RI POM AGENCY* .

Cahyati, N., Ekowati, D., & Harjanti, R. (2015). Optimization of the combination of stearic acid and triethanolamine in the cream formula of legetan leaf extract (Spilanthes acmella l.) as an antioxidant using a simplex lattice design. *Indonesian Pharmacy Journal* .

Cortegiani, A., Ingoglia, G., Ippolito, M., Giarratano, A., & Einav, S. (2020). A systematic review on the efficacy and safety of chloroquine for the treatment of COVID-19. *Journal of Critical Care* . https://doi.org/10.1016/j.jcrc.2020.03.005

Darmadi. (2011). Nosocomial infections: Problems & Control. In *Nosocomial Infections: Problems & Control* .

Del, V., Ijeh, II, & Ejike, CECC (2011). Current perspectives on the medicinal potentials of. *Journal of Medicinal Plants Research* .

Republic of Indonesia Ministry of Health. (2008). Indonesian Herbal Pharmacopoeia. *Indonesian Herbal Pharmacopoeia* .

Fajrina, RFN, Rahayu, IG, Wahyuni, Y., & Rahmat, M. (2019). Antibacterial Activity of Ambon Banana Peel Extract (Musa Acuminata Colla) Against Staphylococcus Aureus In-Vitro. *Bandung Department of Health Polytechnic Health Research Journal* . https://doi.org/10.34011/juriskesbdg.v11i1.744

Formulation and Sterility Test of Herbal Hydrogel Ethanol Extract of Tagetes erecta L. Leaves (2016). *PHARMACON* . https://doi.org/10.35799/pha.5.2016.12163

Leboffe, M. J., & Pierce, B. E. (2011). A Photographic Atlas for the Microbiology Laboratory. In *Anti-Cancer Agents in Medicinal Chemistry* .

Lubis, ES, & Reveny, J. (2012). Natural Skin Moisturizer From Grapefruit Juice [Citrus maxima (Burm.) Osbeck]. *Journal of Pharmaceutics and Pharmacology* .

Murhadi. (2009). Compounds and Antimicrobial Activity of Fatty Acids and Their Esters from Plants. *Journal of Industrial Technology and Agricultural Products* .

Nainggolan, MT, Simaremare, ES, & Pratiwi, RD (2018). Evaluation of the Stability of Cream Preparations of Ethanol Extract of African Leaves (Vernonia amygdalina Delile) with Vanishing Cream (VC) Base. *Papuan Biology Journal* . https://doi.org/10.31957/jbp.129

Nathan, A. J., & Scobell, A. (2012). Prescott Microbiology. In *Foreign Affairs* .

Nuria, M.C., Chabibah, Z., Banu, S., & Fithria, R.F. (2014). Exploring the Potential of N-Hexane and Ethyl Acetate Fractions from Methanol Extract of Ketapang Deciduous Leaves (Terminalia Catappa L.) as Antidiarrhea. In *Scientific e-Publication of the Faculty of Pharmacy, Unwahas Semarang* .

PE Ghamba. (2012). In vitro antibacterial activity of crude ethanol, acetone and aqueous Garcinia kola seed extracts on selected clinical isolates. *African Journal Of Biotechnology* . https://doi.org/10.5897/ajb11.1956

Radji, D. . DM (2019). Microbiology textbook: pharmacy and medical student guide. In *EGC Medical Publishers* .

Riana Ningsih, D., Zusfahair, & Kartika, D. (2016). Identification of Secondary Metabolite Compounds and Testing of Soursop Leaf Extract Activity as an Antibacterial. *Molecular Journal* .

Safitra, D., & D, S. I. (2014). Pengaruh Konsentrasi Asam Stearat Terhadap Karakteristik Sediaan dan Pelepasan Krim Kurkumin. *Jurnal Pharmascience*.

Sapra, A., Riski, R., Hidayah, N., Tinggi, S., Pharmacy, I., Perintis, J., Km, K., & Selatan, S. (2019). Diffusion and Irritation Test of Solid Lipid Microparticle Cream Loaded Ethanol Extract of Kersen Leaf (Muntingia calabura L.) *Pharmaceutical and Medicinal Science* , *4* (1), 6–9.

Saryanti, D., Setiawan, I., & Safitri, RA (2019). Optimization of O/W Cream Formula from Kepok Banana Peel Extract (Musa acuminata L.). *Indonesian Pharmaceutical Research Journal* .

Sharon, N., Anam, S., & Yuliet. (2013). Formulation of Forest Onion (Eleutherine palmifolia L. Merr.) Ethanol Extract Antioxidant Cream. *Online Journal of Natural Science* .

Siti Sunari, AAM, Artini, NPR, & Apsari, DP (2020). Comparison of Cream Quality Between Adeps Lanae Base and Ghee (Cow's Milk Butter). *Widya Health* . https://doi.org/10.32795/widyakesehatan.v2i2.962

Tsabitah, AF, Zulkarnain, AK, Wahyuningsih, MSH, & Nugrahaningsih, DAA (2020). Optimization of Carbomer, Propylene Glycol, and Triethanolamine in the Formulation of Ethanol Extract Gel of Moonflower Leaves (Tithonia diversifolia). *Pharmaceutics Magazine* . https://doi.org/10.22146/farmaseutik.v16i2.45666

Types of Acne and Associated Therapy: A Review. (2017). *American Research Journal of Pharmacy* . https://doi.org/10.21694/2380-5706.16001

Vifta, RL, & Advistasari, YD (2018). Phytochemical Screening, Characterization, and Determination of Total Flavonoid Contents of Parijoto Fruit Extracts and Fractions (Medinilla speciosa B.). *Unimus National Seminar Proceedings* .

Zeniusa, P., Ramadhian, MR, Nasution, SH, & Karima, N. (2019). Inhibitory Test of Green Tea Ethanol Extract against Escherichia coli In Vitro. *Majority* .