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Optimization of serum formula active fraction of moringa leaf extract (Moringa oleifera Lambk) as antibacterial against Staphylococcus aureus

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Abstract---This study aims to determine the optimum formula for the serum active fraction of Moringa leaves with a combination of carbopol, NaCMC and propylene glycol as antibacterial. Material and methods the fractionation of the extract were tested on Staphylococcus aureus bacteria using the diffusion method. The ethyl acetate fraction which has antibacterial activity at a concentration of 15% was made into serum preparations with a combination of carbopol, NaCMC and propyleneglycol. Making serum formulas using the simplex lattice design method resulted in 14 serum formulas. Testing of the physical quality of the preparations carried out included organoleptic tests, homogeneity, pH, adhesion, viscosity, spreadability, and antibacterial activity. The results of the study on the ethyl acetate fraction of Moringa leaves with a concentration of 15% had antibacterial activity against S. aureus with an inhibition zone of 14.36 ± 1.1 mm. The TLC test the chemical compounds contained in the ethyl acetate fraction of Moringa leaves were flavonoids, saponins and tannins. The optimum combination proportions for serum preparations obtained variation values, namely carbopol 0.67% and NaCMC 3% and propylengliol 5.32%.

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Introduction

There are many plants that have medicinal properties and can practically be found all around us (Suriana, 2013). Plants that are used as medicines traditionally can be used as alternatives in the search for new antimicrobial substances (Ervizal, 2001). Moringa leaves (Moringa oliefera Lambk) are a plant in the Moringaceae family which is often found in tropical climates such as Indonesia. Moringa is known as The Miracle Tree or a miracle tree, which is scientifically proven to be the source of useful nutrients and medicines whose contents are beyond the normal content of other plants (Toripah, 2014). Identification of compound content using the TLC method from the n-hexane fraction and ethyl acetate fraction of Moringa leaves shows that Moringa leaves contain several chemical compounds, namely flavonoids, alkaloids, polyphenols, terpenoids and anthraquinones. Saponin, tannin and flavonoid compounds are known to have antibacterial potential (Primasari, 2016).

The water, butanol, ethyl acetate and chloroform fractions of Moringa leaf extract have antibacterial activity against S. aureus in acne. The ethyl acetate, butanol and chloroform fractions of Moringa leaf thick extract have a sequential inhibitory diameter of 13.6; 10.3 and 11.0 mm (Emad, M. Abdallah., 2015). The ethyl acetate fraction of Moringa oleifera leaves with a concentration of 50 mg/ml showed an inhibitory zone for S. aureus with an Rf value of 0.79 containing 10.17 mm of flavonoids and an Rf value of 0.94 containing 13.73 mm of polyphenols (Endarwati, 2016).

Serum is a preparation that uses active substances in high concentrations and low viscosity, thin films deliver active ingredients to the surface of the skin (Draelos, 2010). One of the advantages of using serum preparations is that there are more active substances in the serum compared to other cosmetic preparations, so the serum is more effective and more quickly treats skin problems. Serum is applied topically to the surface of the face, eyelids and neck (Thakre, 2017). Serum preparations require several gelling agents (bases), these bases can affect the physical quality of the serum preparations, these bases include NaCMC, Carbopol and Propylene glycol.

The use of NaCMC as a base includes providing a stable viscosity to the preparation. NaCMC is a natural polymer that is stable at pH 5-9 (Rowe, Sheskey, and Quiin., 2009). Carbopol is a base which, when formulated in preparation, will form a gel with a clear shape (Hasyim et al, 2011). The use of 1% to 2% propylene glycol provides a good gel effect and good physical properties (Patil et al, 2015). Based on the description above, this research aims to create a serum formula for the active fraction of Moringa leaf extract with a combination of Na CMC and carbopol bases and determine the optimal formula using the Simplex Lattice Design method and carry out physical stability tests including homogeneity tests, viscosity tests, pH tests, spreadability, adhesion test and antibacterial test against S. aureus bacteria in acne.

Method

Material

The main materials used in this research were Moringa leaves (Moringa oleifera Lambk) as well as other materials such as the bacterial culture Staphylococcus aureus ATCC 25923, ethanol 96%, Nutrient Agar (NA) medium, NaCMC, carbopol 940, Propylene glycol (Dow Chemical Company), Methyl paraben, TEA, propyl paraben, n-hexane, ethyl acetate, water or distilled water, concentrated HCl, gelatin, NaCl, alcohol 70%, safranin, H_2O_2, MR-Vp medium, alpha naphthol solution, KOH 40%, $FeCl_3$, BHI medium , Indol reagent, Methyl reagent, Voges proskarer reagent, TSIA test, magnesium, Design Expert software version 10.

Method

Making Moringa Leaf Powder

Fresh Moringa leaves are separated from the stems and washed thoroughly with running water until they are free from dirt or objects stuck to the leaves. Moringa leaves will be dried by airing them at room temperature (32-35°C) until dry for 5 days. Dried Moringa leaves will be made into powder by blending and sifting with a No. 60 mesh sieve to ensure that the powder is really fine and then weighed (Singh, G. P., et al., 2012).

Making Moringa Leaf Extract

800 g of Moringa leaf powder was weighed then put into a vessel and 96% ethanol was added in a ratio of 1:10 then macerated with 8 liters of 96% ethanol for 5 days. Storage must be at room temperature and protected from sunlight. After 5 days it is filtered to obtain macerate. The maserate was evaporated using a rotary evaporator at a temperature of 50°C at a stable speed until a thick extract was produced (Wigati et al, 2018).

Determination of total ash content

Weigh 2-3 g of Moringa leaf extract then put it in a silicate crucible that has been ignited and tared, then stir it slowly until finished, cool and weigh.

Determination of acid insoluble ash content

The ash from determining the total ash content was added with 25 ml of HCl LP then boiled for 5 minutes. The part that does not dissolve in acid is collected, filtered using ash-free filter paper, washed with hot water then heated in a crucible until the weight remains at a temperature of 800 ± 25 °. The acid insoluble ash content was calculated based on the weight of Moringa leaf extract and expressed as % w/w.

Determination of water content

Weigh 2 g of sample then put it in a container that has been tared, dry at 105° for 5 hours and weigh. Carry out drying and weighing at 1 hour intervals until there is a difference between two consecutive weighings of no more than 0.25%.

Identify chemical compounds

Identification of chemical compounds in Moringa leaf extract using a tube test, identification of chemical compounds including flavonoids, tannins and saponins from Moringa leaf extract.

Fraction creation

Fractionation uses the liquid-liquid extraction (ECC) method in n-hexane and ethyl acetate solvents. A total of 10 g of ethanol extract of Moringa leaves was added to ethanol solution and 20 ml of distilled water and homogenized, put into a separating funnel then extracted with 40 ml of n-hexane, shaken and left to stand until 2 layers were formed, namely the n-hexane fraction and the water fraction. The n-hexane fraction was collected and fractionated again using n-hexane solvent until a clear n-hexane fraction was obtained. The water fraction was added to 50 ml of ethyl acetate, shaken and allowed to separate. The ethyl acetate layer was separated and continued with fractionation until a clear ethyl acetate fraction was obtained. The resulting collection of n-hexane fraction and ethyl acetate fraction was evaporated until a thick extract was obtained (Hafizan, 2016).

Preparation of bacterial suspension

Bacterial cultures that have been aged for 1 x 24 hours and have been rejuvenated in slanted NA medium are suspended with physiological NaCl (NaCl 0.9%) then the turbidity is measured at 25% T on a UV-Vis spectrophotometer at a wavelength of 580 nm (Harmita, 2005).

Identify bacteria
Gram staining

The slide was cleaned with 70% alcohol and dried, sample preparations were made on the slide and dried near a fire. Crystal violet was dropped on the preparation then left for 60 seconds and rinsed with flowing distilled water. Iodine is dropped then left for 60 seconds then rinsed with flowing distilled water. The preparation was dripped with 96% alcohol and rinsed with flowing distilled water, the last was dripped with safranin then left for 60 seconds then rinsed with flowing distilled water. The preparations were dried using a tissue and then observed under a microscope. Results for Gram positive bacteria are shown in purple or blue and Gram negative bacteria are shown in red (Pratiwi, 2008).

Catalase test

This test was carried out by dripping H_2O_2 liquid on a glass object then adding 1 dose of S. aureus bacteria from MSA and then mixing. Catalase is said to be positive as indicated by the presence of gas bubbles (O_2) produced by Staphylococcus bacteria (Tolle and Lenda, 2014).

Mannitol sugar test

Bacteria that have been cultured are inoculated into the media incubated at 37°c for 24 hours. Positive results indicate a yellowish color change and negative results indicate no color change (Ibrahim, 2017).

Testing of the antibacterial activity of Moringa leaf extracts and fractions

Antibacterial activity testing was carried out by means of preliminary testing of the extraction and fractionation of Moringa leaves to see the dose that best inhibits the growth of S. aureus bacteria. The test bacterial suspension was inoculated in 0.1 mL of MHA media, then spread evenly with a sterile cotton bud, and left to dry. The paper disc was inserted into the vial which already contained the extraction solution, n-hexane fraction, ethyl acetate fraction, water fraction and the positive control used was clindamycin. The positive and negative controls were soaked for ± 5 minutes then placed on the surface of the media aseptically. A clear zone around the disc paper was observed.

Testing the antibacterial activity of serum preparations

Testing the antibacterial activity of the serum preparation of the most active fraction of Moringa leaves using the well diffusion method, the best concentration of curcumin isolate was 2%. The test was carried out using the medium used Muller Hinton Agar (MHA) with 0.1 ml of S. aureus bacterial inoculum which is equivalent to 0 .5 mc of Farland was inoculated throughout the MHA medium evenly using a sterile cotton bud, holes were made in the media with a sterile Cork borer with a diameter of 6 mm and each hole was filled with various cream preparation formulas totaling 14 holes with a concentration of 2% and incubation was carried out at a temperature of 37 °C for 24 hours, repeat 3 times and measure the diameter of the resistance around the hole using a caliper (Ortez, 2005).

Formulation design

Design of a serum formula for the active fraction of Moringa leaves using a variety of NaCMC, carbopol and propylene glycol bases using the SLD method. Varying carbopol, NaCMC and propylene glycol components using the SLD method produces several runs or several formulas which will be made into Moringa leaf active fraction serum preparations with the addition of 15% Moringa leaf ethyl acetate fraction for each formula.

Testing the physical properties of serum preparations
Serum preparation stability testing

Physical stability testing of serum preparations is carried out using the stress condition method, namely storing serum preparations for a certain period of time (24 hours per cycle) with extreme temperature differences (5°C and 35°C). Testing will be carried out before and after 3 cycles of storage.

Organoleptic testing

This test is to determine the condition of the serum preparation by naked eye, including testing the shape, color, smell, and is carried out visually on the serum preparation being made.

Viscosity testing

This test uses a viscometer to test changes in viscosity. A total of 100 mL of Moringa leaf serum fraction was put into a beaker, then the appropriate spindle was lowered until the spindle was submerged in the preparation, the results were recorded on the spindle set at a speed of 50 rpm (Mardhiani, et al., 2020)

pH testing

This test is to observe the pH stability of the serum preparation. The pH requirement range for topical or non-topical preparations is (4.5-7), to ensure that the serum preparation cannot cause irritation to the skin. The pH of the preparation was measured using universal indicator paper (Martono, et al., 2018).

Adhesion testing

0.5 g of serum is smeared on a glass object marked with an area of 2x2 cm, on top of which another glass object is placed. A load of 500 g was placed on a glass object and left for 5 minutes. The object glass is mounted on an adhesion test tool that has been given a load of 80 g. Time is recorded after the two glass objects are separated or separated from each other (Tambunan and Sulaiman, 2018).

Spreadability Testing

0.5 g of serum was placed in the middle of the glass, covered with another glass which had been weighed and then left for 1 minute, then the diameter of the spread of the serum was measured. The load was added every 1 minute by 50 g, 100 g, and 150 g, then the serum distribution diameter was measured (Tambunan and Sulaiman, 2018). A good serum spreading power is between 5-7 cm, where the greater the spreading power given, the wider the ability of the active substance to spread in contact with the skin (Sayuti, 2015).

Formula optimization

Formula optimization was carried out with the Design Expert version 13 software using the Mixture Simplex Lattice method by entering the test results for pH, viscosity, spreadability, adhesion and inhibition power. One formula was chosen which was considered to have the highest desirability. The optimum formula chosen is the formula that produces the critical target parameters expected from the optimum formula for the activated Moringa leaf serum fraction which has strong antibacterial activity, high spreadability and appropriate viscosity. A good desirability value is close to one (Rahayu et al, 2016).

Results and Discussion

Identification results of Moringa leaf powder

Moringa leaf plants that have been taken with a wet weight of 8000 grams are separated from foreign objects and adhering dirt. Next, dry it by drying it in the sun at room temperature until it is completely dry. The yield of Moringa leaf powder is shown in table 1.

Table 1
Yield Of Wet Weight and Dry Weight

Wet Weight (grams)	Dry Weight (grams)	Yield (%)
8000	920	11.5

Identification results of Moringa leaf extract

The Indonesian Herbal Pharmacopoeia (FHI) edition II of 2017 wrote that the identification of Moringa leaf extract includes total ash, acid-insoluble total ash, and water content. The identification results of Moringa leaf extract are shown in table 2.

Table 2
Yield of Wet Weight and Dry Weight

Extract weight	Ash content (%)	Total ash content is acid insoluble	Water content of Moringa leaf extract
2 grams	7.1	1	9.5
2 grams	9.7	1.5	14.5
2 grams	8.7	1	12
Mean ± SD	8.8 ± 1.3	1.2 ± 0.3	12 ± 2.5

The results of testing the total ash content of Moringa leaf extract obtained a value of 8.8 ± 1.3%, in 2 grams of the extract there was an ash content of 8.8% which contained the remaining amount of inorganic or mineral materials. The applicable standard parameter is that the total ash content in Moringa leaf extract is no more than 9.0% (FHI, 2017). The resulting value meets the requirements for the total ash content of Moringa leaf extract. If the total ash content value exceeds the provisions, it indicates that the mineral and internal

content of the extract is too little. The test results for acid insoluble ash content obtained a value of 1.2 ± 0.3%, in 2 grams of total ash there was 1.1% acid insoluble content. The requirement for acid insoluble ash content in Moringa leaf extract is more than 0.9% (FHI, 2017). The value of the acid insoluble content test results in Moringa leaf extract proves that it meets the requirements. If the acid insoluble content value is less than the provisions, it indicates that the amount of impurities in the extract is too large.

Determination of water content as a minimum limit for water content in extracts (Ministry of Health of the Republic of Indonesia, 2000). The water content requirement for Moringa leaf extract according to the Indonesian Herbal Pharmacopoeia (2017) is no more than 10.0%. The results of the water content test in Moringa leaf extract obtained a value of 12 ± 2.5%, the value did not meet the required limit, namely the water content in the extract exceeded the required limit. High water content can cause fungal growth in the extract so that it can damage the quality of the extract and can allow the growth of microorganisms which can trigger enzymatic reactions, namely decay of the extract (Puspita, 2009).

Results of identification of chemical compounds from Moringa leaf extract

After testing the identification of chemical compounds in Moringa leaves in the form of flavonoids, tannins and saponins, it was discovered that Moringa leaf extract contains chemicals in the form of flavonoids, tannins and saponins. These chemical contents can provide an antibacterial effect. Flavonoid compounds are lipophilic, where they work by damaging bacterial membranes. This compound has an antibacterial mechanism of action, namely forming complex compounds with extracellular proteins and soluble proteins so that it can damage bacterial cell membranes, followed by the release of intracellular compounds (Nuria et al., 2009).

The mechanism of saponin's action as an antibacterial is by causing leakage of proteins and enzymes from the cells of the Porphyromonas gingivalis bacteria (Madduluri et al., 2011). Saponin is an active substance that can increase membrane permeability resulting in hemolysis in cells. If saponin interacts with bacterial cells, the bacteria will burst or lyse (Poeloengan and Praptiwi, 2012). The mechanism of action of tannin as an antibacterial is by causing Porphyromonas gingivalis cells to lyse. This happens because tannins target the polypeptide walls of bacterial cell walls so that cell wall formation becomes less than perfect and then the bacterial cells die. Tannins also have the ability to inactivate bacterial enzymes and disrupt the flow of proteins in the inner layers of cells (Ngajow et al, 2013).

Results of fractionation of Moringa leaf extract

Fractionation using the maceration method using 96% ethanol solvent. 96% ethanol solvent can extract almost all of the simplicia content, both polar, semi-polar and non-polar. Fractionation for non-polar compounds uses n-hexane solvent, ethyl acetate solvent for semi-polar compounds so that it can estimate active compounds that are useful as antibacterials. The yield results of

fractionation with n-hexane are shown in table 14. The percentage yield of fractionation with n-hexane from Moringa leaf extract obtained an average result of 20.8 ± 1.3%. A total of 10 grams of the n-hexane fraction of Moringa leaves had an average yield of 20.8%. Fractionation using n-hexane which is non-polar which works by attracting non-polar compounds such as steroids, chlorophyll and terpenoids contained in Moringa leaf extract (Mahardika et al, 2020).

The ethyl acetate fraction of Moringa leaves obtained an average yield of 22.8 ± 1.1%, in 10 grams of Moringa leaf ethyl acetate fraction the yield was 22.8%. Fractionation with ethyl acetate has the aim of separating polyphenol or flavonoid compounds. Moringa leaves contain chemical compounds, one of which is flavonoids. Apart from that, Lutfiana (2013) reported the results of phytochemical tests on the ethyl acetate fraction of Moringa leaves containing flavonoid, saponin and tannin compounds. The total fractionation yield of n-hexane, ethyl acetate and water was 65.7% w/w from 100% w/w. The total fractionation value is less than 100% because there are several causes, namely in the fractionation process, such as imperfect separation in the separating funnel and a large amount of fractionation residue that is attached to the tool but is not removed.

Results of identification of Staphylococcus aureus bacteria
Identification by gram stain

Identification using gram staining has the aim of observing the morphology of the S. aureus bacterial cells and to determine the purity of the bacterial cells.

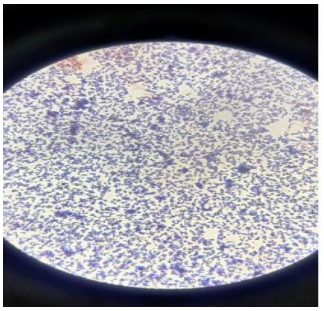

Figure 1. Identification of Staphylococcus aureus bacteria using gram staining

The results of gram staining on S. aureus bacteria are shown in figure 8, namely S. aureus is included in the Gram positive bacteria which are in the form of cocci arranged in irregular groups (resembling grapes), and can also be arranged in fours. The blue color is caused by bacteria retaining the first color, namely crystal violet. The outer cell wall of Gram-positive bacteria consists of thick peptodoglycan (Retnowati, Bialangi, & Posangi 2011).

Identification of catalase test

S. aureus bacteria produce bubbles due to the catalase enzyme which hydrolyzes hydrogen peroxide (H2O2) into water (H2O) and bubbles (O2). Positive catalase test results on S. aureus. Catalase is an enzyme that can catalyze the breakdown

of hydrogen peroxide into H_2O and O_2. Hydrogen peroxide has toxic properties to cells because this material can inactivate enzymes in cells as shown in figure 2.

Figure 2. Identification of Staphylococcus aureus bacteria using the catalase test

Identify the sugar mannitol

S. aureus bacteria have facultative anaerobic properties where they can ferment glucose in the absence of oxygen, so an anaerobic mannitol fermentation test was carried out on S. aureus. S. aureus bacteria is a pathogenic bacteria which shows a positive sample which is indicated by a yellow color change in the test medium (Maulitasari, 2014). The change in color of the medium to yellow is caused by the presence of the Phenol red indicator in the medium (Kateete et al. 2010). Where the addition of the Phenol red indicator to the medium undergoing carbohydrate fermentation becomes acidic in aerobic conditions, the pH will decrease and finally the Phenol red indicator will change color to yellow (Dewi, 2013). The test results are shown in figure 3.

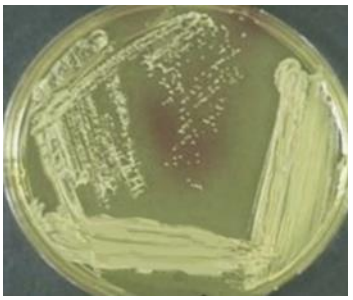

Figure 3. Identification of Staphylococcus aureus bacteria using the mannitol sugar test

Results of observation of antibacterial activity of extracts and fractions

The antibacterial activity test was carried out using the paper disc method with 3 replications on the extract and fractions. The paper disc method is a method commonly used to test the antimicrobial activity of an antibiotic against disease-causing pathogenic microorganisms. The sensitivity of pathogenic microorganisms to antibiotics can be seen from the size of the clear zone formed (Cappuccino & Sherman, 2001). The results of the antibacterial test of extracts and fractions with paper discs are shown in table 3. Based on the results of the antibacterial test in table 3, the best concentration is shown in the ethyl acetate fraction of Moringa leaves in a concentration of 15% which shows an inhibition

zone of 14.36 mm. At a concentration of 15% the ethyl acetate fraction shows that this concentration is included in the category of strong inhibitory power

Table 3
Antibacterial identification test results of fractions and extracts of 15% concentration

	Replication 1(mm)	Replication 2(mm)	Replication 3(mm)	Mean ± SD
Extract	14.5	11.7	12.3	12.8±1.4
n-hexane	8.9	10.2	9.5	9.5±0.6
Ethyl acetate	14.8	15.2	13.1	14.36 ± 1.1
Water	9.1	8.5	7.3	8.3±0.9
Control (+)	24.3	22.7	22.9	23.3 ± 0.8
Control (-)	0	0	0	0±0

Results of serum preparation formulation using the simplex lattice design method

The serum preparation was designed using an expert design application with the Simplex Lattice Design method by entering 3 factors including factor A, carbopol, factor B, NaCMC, and factor C, propylene glycol. These three factors produce 14 serum formulations with a variety of different concentrations. The proportions of the design formula will be determined automatically by the Design Expert Software version 13. The design results are shown in table 4.

Table 4
Results of Moringa leaf serum fraction formulation using the Simplex lattice design method

	F1(g)	F2(g)	F3(g)	F4(g)	F5(g)	F6(g)	F7(g)	F8(g)	F9(g)	F10(g)	F11(g)	F12(g)	F13(g)	F14(g)
Ethyl acetate fraction	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Carbopol	1	0.5	0.5	0.5	0.75	0.58	0.75	0.83	0.5	0.5	1	0.66	0.75	0.58
NaCMC	3	3.5	3	3	3.25	3.33	3.25	3.08	3.25	3.5	3	3.16	3	3.08
Propylene glycol	5	5	5.5	5.5	5	5.08	5	5.08	5.25	5	5	5.16	5.25	5.3
TEA	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Methyl paraben	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Aquadest ad	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Based on the results of the Moringa leaf fraction serum formulation produced by the simplex lattice design software, physical quality testing of the cream preparation will be carried out and from the results of the physical quality testing it was found that the serum preparation has a slightly thick shape and a slightly yellow color from the results of organoleptic observations, and from the results of pH testing It was found that the pH of the serum preparation was still in the

range of 4-7, so the Moringa leaf fraction serum preparation was said to be safe to use on the surface of the skin because it did not cause skin irritation.

The results of the adhesion test on the Moringa leaf fraction serum preparation had an average value of ±2 seconds so it can be concluded that the serum preparation has met the requirements. Good adhesion power is in the range of 2.00 -300.00 seconds (Betageri and Prabhu, 2002). Higher levels of carbopol cause an increase in adhesive power. High adhesion affects drug delivery, because the longer the drug is in contact with the skin, the more optimal drug delivery will be so that the therapeutic effect can be maximized (Hidayati, 2014). The test results are shown in table 5.

Table 5
Results of testing the physical properties of serum preparations

Formulas	Organoleptic	pH	Stickiness
1	Thick	6.6±0.28	2 ± 0.35
2	Thick, slightly runny	5.1 ± 0.13	2.2 ± 0.18
3	Thick, slightly runny	5.05±0.29	2.2±0.20
4	Thick, slightly runny	4.95 ± 0.34	2.4 ± 0.24
5	Thick	6.81 ± 0.14	1.2 ± 0.16
6	Thick	5.99±0.33	1.4 ± 0.46
7	Thick	6 ± 0.82	1.4 ± 0.54
8	Thick, slightly runny	6.45 ± 0.24	1.6 ± 0.23
9	Thick, slightly runny	6.3 ± 0.12	1.5 ± 0.44
10	Thick	6.3 ± 0.51	1.8 ± 0.35
11	Thick	6.82 ± 0.6	1.7 ± 0.49
12	Thick, slightly runny	6.5±0.32	1.4 ± 0.32
13	Thick, slightly runny	5.82 ± 0.78	1.9 ± 0.29
14	Thick, slightly runny	5.22 ± 0.64	1.5 ± 0.63

After testing the physical quality including organoleptics, pH and adhesion, the next step is to observe the critical parameters which will be entered into the design expert software to determine the optimum formula, including visosity, spreadability and antibacterial activity. The viscosity value of a serum preparation is said to be good if it is in the range of 230 – 1150 cPas (Yani, D, 2018). The results of viscosity testing of Moringa leaf serum preparations were in the range 62 - 235 dPas (620-2350 cPas). Most of the Moringa leaf fraction serum preparations had a high viscosity or above the good serum viscosity range so they did not meet the viscosity requirements for serum preparations. This may be due to a decrease in the water content of the serum preparation due to evaporation during testing, making the consistency high.

The spreadability value is inversely proportional to the viscosity value, the greater the viscosity value of a serum preparation, the thicker the consistency, so the spreadability value will be smaller. The results of the serum spreadability test showed that it was in the range of 3-7.2 cm, so it could be concluded that the Moringa leaf fraction serum preparation was a semifluid preparation with a high spreadability value.

The inhibitory power value of the serum preparation decreased from the fractional inhibitory power value. The decrease in inhibitory power values could be caused by serum quality and viscosity which is influenced by the addition of carbopol which varies for each formula. The greater the carbopol content, the viscosity of the serum preparation will increase and the greater the resistance (Sinko, 2011), thus preventing the release of the active substance and resulting in a decrease in the resistance of the serum formulation against Staphylococcus aureus bacteria. The critical parameter test results are shown in table 6.

Table 6
Test results for critical parameters of serum preparations

Formulas	Viscosity(dPas)	Spreadability (cm)	Resistance(mm)
1	235	3,2	7
2	217	3	6.5
3	65	5.9	6.9
4	62	6.6	12
5	225	3.8	7.5
6	185	4.1	8.3
7	231	3	6.1
8	194	3.8	6.5
9	112	6,7	11
10	228	3,4	6.6
11	218	3.6	7.2
12	152	6.2	10.5
13	125	7.2	14.3
14	73	6.5	11.7

Serum preparation optimization results

The critical parameters in the optimum formula in this study are viscosity, spreadability and antibacterial. Expert design version 13 software which uses a mixture simplex lattice design provides a formula that matches the desired optimum serum formula target. The results of determining the optimum formula with critical parameters are shown in table 7. For viscosity with a target response in range, the expected minimize and maximize viscosity values are expected. The target response for spreadability is maximized with the degree of importance (++++), for antibacterial the target response is maximized with the degree of importance (+++++) meaning that a high degree of importance can provide an optimum formula that has strong inhibitory power as an antibacterial. .

Table 7
Optimization data for Moringa leaf serum formula using the Design expert application

Name	Goals	Lower limits	Upper limits	Importance
Carbopol	*Is in range*	0.5	1	3
NaCMC	*Is in range*	3	3.5	3
Propylene glycol	*Is in range*	5	5.5	3

Viscosity	*Is in range*	62	235	3
Spreadability	*Maximixe*	3	7.2	4
Antibacterial	*Maximixe*	6.1	14.3	5

In determining the optimum formula, the one with the highest desirability value is selected, namely with a desirability value of 0.897. The optimum formula chosen in this study was a formula with a carbopol composition of 0.67%; NaCMC of 3.00%; and Propylene glycol of 5.32%. The optimum formula is predicted to have a viscosity of 98.94 dPas; spreadability of 6.99cm; and antibacterial of 13.11. Desirability values are shown in table 8.

Table 8
Composition and prediction of physical quality test of optimum formula

carbopol	NaCMC	Propylene glycol	Viscosity	Spreadability	Antibacterial	*Desirability*
0.675	3.00	5.32	98.94	6,999	13,11	0.897

Based on the table above, it shows the composition and prediction of the physical quality test using the optimum formula which produces a desirability value shown in the yellowish green area which in this study provides the optimum response. The graphical results of the desirability values of the optimum formula are shown in figure 14 and the overlay graph plot of the optimum formula for Moringa leaf serum is shown in figure 4.

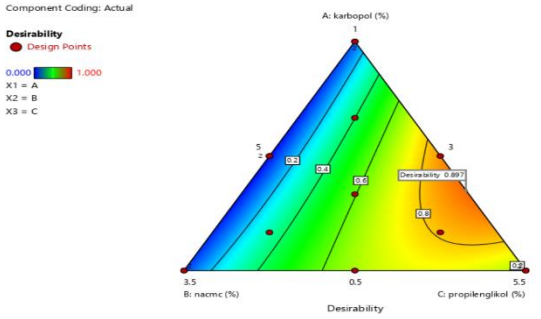

Figure 4. Desirability graph of the optimum formula for Moringa leaf serum

Based on the superimposed contour plot, it shows that the formula that has the most optimum physical properties is the yellow area consisting of X1, namely carbopol, X2, namely NaCMC and X3, namely propylene glycol. The yellow area is the area that has the optimum composition to obtain a serum preparation of the ethyl acetate fraction of Moringa leaves with a critical parameter value, namely a viscosity value of 98.95 dPa.s; the spreadability value is 6.99cm and the antibacterial value is 13.10 mm.

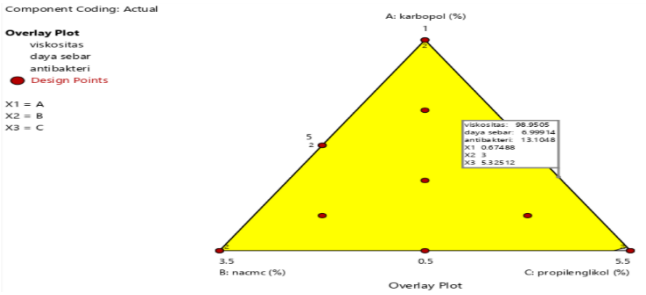

Figure 5. Contour plot superimposed on the optimum formula for Moringa leaf serum

Optimum formula verification results

The results of verifying the optimum formula with software predictions show that there is no significant difference, so these results state that the simplex lattice design method with Design expert software version 13 can predict serum formulas with viscosity, spreadability and antibacterial responses. The results are shown in table 9.

Table 9
Comparison of physical properties test results for the optimum formula of Moringa leaf serum with program predictions

Response	Program calculation predictions	Serum physical properties test results	Conclusion
Viscosity	98,944	98.2	Not meaningfully different
Spreadability	6,999	6.73	Not meaningfully different
Antibacterial	13,106	13.03	Not meaningfully different

Conclusion

Based on the research results, it can be concluded that:

- The ethyl acetate fraction of Moringa leaves has antibacterial activity against S. aureus with a concentration of 15%, there is an inhibition zone of 14.36 ± 1.1 mm.
- Based on the TLC test on the ethyl acetate fraction of Moringa leaves, it contains flavonoid chemical compounds with an Rf value of 0.8; Saponin with an Rf value of 0.5; and Tannin with an Rf value of 1.7.
- The results of the optimum formula for Moringa leaf serum preparations showed the proportion value of the combination of additional ingredients, namely carbopol 0.67%, NaCMC 3.00% and propylene glycol 5.32% and with a viscosity value of 98.2 dPas, spreadability of 6.73 cm and antibacterial 13. 03mm.

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References

Betina. Edisi ke III. Kares, V, 1972. " Antibiotic" dalam Phermaceautical Applications of Thin layer and Paper Chromatography, Edisi ke III, Karel Macek (ed), 503-505, Elsevier Publishing Company, Amsterdam.

Bibah D, A. 2020. Uji Aktivitas Antibakteri sediaan Gel Ekstrak Daun kelor (Moringa oleifera Lambk.) terhadap bakteri Staphylococcus aureus ATCC 25923.

Choma., Irena, M., Edyta, M, G., 2010. Bioautography Detection in Thin Layer Chromatography. Journal of Chromatography.

Christianto, C.W., 2012. Efek Antibakteri Ekstrak Biji Alpukat (Persea Americana Mill) Terhadap pertumbuhan Streptococcus mutans, Oral Biol Dent., 4 (2) : 40-44.

Cowan, M. M. 1999. Plant Product as Antimicrobial Agent. J. Microbiology Reviews 12(4):564-582.

Damayanti, L. 2001. Uji Aktivitas Antibakteri tumbuhan Patikan Kebo (Euphorbia hirta L.) Terhadap Staphylococcus aureus ATCC 25923 Dan Escherichia coli ATCC 25922 Serta Profil kromatografi Lapis Tipisnya. Yogyakarta:Fakultas Farmasi universitas Gadjah Mada.

Davis, W. W., dan Stout, T.R., 1971. Disc Plate method of Microbiological Antibiotic Assay, Applied Microbiology, 22 (1) : 659-665.

Depkes RI. 2000. Parameter Standar umum Ekstrak tumbuhan Obat. Cetakan Pertama. Jakarta:Depkes RI. Halaman 10-11.

Draelos, Z.D., 2010. Cosmetic Dermatology Products and Prosedures. USA : Blackwell Publishing, Ltd.

Dunn, J. A., Robert, A. C., Richatd, T., Enas., Robert, J. G., 2008. Novel Topically Active Antimicrobial and Anti- Inflammatory Compounds For Acne. In: Walters, Kenneth A, editor. Dermatologic, Cosmoceutic And Cosmetic Development. New York: Informa Healthcare.h 243.

Efendi, Z., 2003. Peranan kulit Dalam Mengatasi terjadinya Acne Vulgaris Fakultas Kedokteran Universitas Sumatera Utara. Pp : 1-5.

Emad M. Abdallah. 2015. Antibacterial Properties of leaf Extracts of Moringa oleifera Lam. Growing in Sudan. SCIENCEDOMAIN International.

Emma, S. k., Iskandarsyah., Praptiwi., 2014. Evaluasi uji Stabilitas Sineresis Sediaan gel Yang Mengandung Monoksidil, Apigenin dan Perasan Herba Seledri (Apium graveolens L). Jurnal Bul. Penelitian Kesehatan. Vol 4 : 213-222.

Ervizal; Rahayu, W.P; Wijaya, C.H;dan Sari, P.P. 2001. Aktivitas Antimikroba Ekstrak Kedawung (Parka roxburghii G. Don) Terhadap Bakteri Patogen (Online). Buletin Teknologi dan Industri Pangan. Vol XII no. 1. Dakses 5 oktober 2013.

Fatmawati N, Anwar E, Azizahwati. 2014. Formulasi Serum penghambat kerja Tirosinase yang Mengandung Fitosom Ekstrak Biji Lengkeng (Dimocarpus longan Lour) Menggunakan Eksipien Koproses Kasein- Xanthan Gum. FF UI.

Farooq F, Meenu r, Avinash T, Abdul AK, Shaila F. 2012. Medicinal Properties of Moringa oleifera: An Overview of Promising Healer, J Med Plants Res, 6(27): 4368-4373.

Granmayeh Rad, A., Abbasi, H., Afzali, M.H., 2011. Gold nanoparticles: Sythesising, Characterizing and Reviewing Novel Application in Recent Years. Physics Procedia 22, 203-203. http://doi.org/10.1016/j.phpro.2011.11.032

Harahap, Marwali. 2000. Ilmu Penyakit Kulit. Hipokrates:Jakarta.

Harborne, J. B. 1987. Metode Fitokimia Penuntun Cara modern Menganalisis Tumbuhan. Edisi kedua. Hal 5. 69-76. Diterjemahkan oleh Kosasih padmawinata dan Iwang Soedira. ITB Press. Bandung.

Hariana A., 2008. Tumbuhan Obat dan Khasiatnya Seri 2. Depok : Penebar Swadaya.

Hostettman, K., Hostettman, M., Maerston. 1995. Preparative Chromatography Technique : Application in Natural Product Isolation. (diterjemahkan Oleh Kosasih P) bandung: Penerbit ITB.

Ikalinus, Robertino, Widyastuti, Setiasih Eka. 2015. Skrining Fitokimia Ekstrak Etanol Kulit Batang kelor (Moringa oleifera). Bali: Universitas Udaya.

Kalpana, S., Moorthi, S. and kumara, S. 2013. Antimicrobial activity of difeferent extracts of leaf of Moringa oleifera (Lam) against gram positive and gram negative bacteria, International Journal of Current Microbiology, 12(2).

Khunaifi, M. 2010. Uji Aktivitas Antibakteri ekstrak daun binahong (Anredera Cordifolia (Ten.) Steenis) terhadap Bakteri Staphylococcus aureus dan Pseudomonas aeruginosa. Jurusan biologi fakultas sains dan teknolohi. Skripsi. Malang. Universitas islam Negeri.

Krisnadi, a Dudi. 2015. Kelor Super Nutrisi. Maret 2015. http://kelorina.com/ebook.pdf. Diakses tanggal 29 September 2015.

Kusumaningtyas, E., Astuti, E., Darmono. 2008. Sensitivitas Metode Bioautografi Kontak dan Overlay dalam penentuan Senyawa Antikapang. Jurnal Ilmu Kefarmasian Indonesia, Vol. 6, No. 2. Hal 75-79.

Mitsui, T. 1997. New Cosmetic Science. Amsterdam: Elsevier Science B. V 28-32.

Moyo, b., Masika, P, and Muchenje, V. 2012. Antimicrobial activities of Moringa oleifera Lam leaf extracts. AFRICAN JOURNAL OF BIOTECHNOLOGY. Acamedic journals (Kenya), 11(11), pp. 2797-2802. Doi: 10.5897/AjB10. 686.

Mulyaningsih, S. 2004. Mikrobiologi dasar. Buku FMIPA UII. Yogyakarta.

Ojha, S., Sinha, S., Chaudhuri, S.D., Chadha, H., Jin, S.M., 2019. FORMULATION AND EVALUATION OF FACE SERUM CONTAINING BEE VENOM AND ALOE VERA GEL. World Journal of Pharmaceutical Research 8, 7.

Oluduro, A. O. 2012. Evaluation of Antimicrobial properties and nutritional potentials of Moringa oelifera Lam. Leaf in South-Western Nigeria. Malaysian Journal of Microbiology, 8(2), pp. 59-67.

Pratiwi, Sylvia T. 2008. Mikrobiologi farmasi. Jakarta : Erlangga.

Rocwood, J.L., Anderson, B.G., Casamatta, D.A. 2013. Potential Uses of Moringa oleifera and an Examination of Antibiotic Efficacy Conffered by Moringa oleifera Seed and Leaf Extrac using Crude Extraction Techniques. International Journal Of Phytotherapy Research vol.3.

Rollof, A., H. Weisgerber., U. Lang., B. Stimm. 2009. Moringa oleifera LAM. Weinheim:WILEY-VCH Verlag GmbH & Co. KgaA.

Rowe, R. c. (Ed.). 2009. Handbook of pharmaceutical excipients, 6. ed. Ed. APhA,(PhP) Pharmaceutical Press, London.

Singh, N., et al. 2012. Anti-Ulcer and Antioxidant Activity of Moringa oleifera (Lam) Leaves Against Aspirin and ethanol Induced Gastric ulcer in Rats. In International research Journal of Pharmaceuticals Vol 02. Hal 46-57.

Siti Rofida. 2020. Antibacterial and Characterization of Secondary Metabolite Compound from Ethyl Acetate and Ethanol Fraction of Leaves Moringa oliefera L.

Suriana, Neti., Shobariani Irni. 2013. Ensiklopedia Tanaman Obat. Malang:Rumah Ide.

Thakre, A.D. 2017. Formulation and Development of De Pigment Serum Incorporating Fruits Extract 2, 53.

Toripah. S, S., Abidjulu, J.,dan Wehantouw, F. 2014. Aktivitas Antioksidan dan Kandungan Total Fenolik Ekstrak Daun Kelor (Moringa oleifera Lambk). Manadoe:Program studi Farmasi FMIPA Universitas Samratulangi.

Unegbu, V., et al. 2020. Phytochemical and Antibacterial Properties of Moringa oleifera leaf extracts on Escherichia coli and Staphylococcus aureus.Nigerian Journal of Microbiology, 34(1):-5145-5152.

Utami P, Puspaningtyas DE. 2013. The Miracle of Herbs. Jakarta:Agro Media Pustaka.

Valgas, Cleidson, Simone Machado de Souza, Elza F A Smania, Artusmania Jr. 2006. Screening Methode to Determine Antibacterial Activity of Natural Products. Brazilian Journal Microbiologi.