

PHYSICAL CHARACTERIZATION TEST OF HANDSANITIZER GEL FROM ETHANOL EXTRACT OF Moringa LEAF (*Moringa oleifera* Lamk)

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Abstract

One of the clean living behaviors in preventing the transmission of Covid-19 is to maintain hand hygiene, both by washing hands with soap and by using a hand sanitizer. Moringa (*Moringa oleifera* Lamk) is a potential plant that can be developed as a pharmaceutical preparation in the form of hand sanitizer gel; because it contains various secondary metabolite compounds such as flavonoids, alkaloids, tannins, and saponins that are able to provide antibacterial effects. This study aims to determine the characteristics of Moringa leaf ethanol extract hand sanitizer gel to obtain qualified preparations. The research method used is a quasi-experimental method with completely randomized design with variations in extract concentration; F1 (0.625%), F2 (1.25%), F3 (2.5%), F4 (5%) and F5 (10%), with observations of the characteristics of each formula. The results showed that all five formulas had good organoleptic properties and homogeneity. The colors of the five formulas are different, ranging from brownish-yellow to dark brown. The pH of the F1-F4 formula is in the skin pH range (4.5-7), while the pH of F5 is 4.1 ± 0.047 below the skin pH range. The dispersion of the five formulas is also in the range of 5-7 cm; adhesion not less than 1 second; viscosity is in the range of 2,000 – 4,000 cP; and the average grinding speed of the five formulas when applied to the skin, ranging from 6-8 seconds. Data analysis with the *One Way ANOVA* test ($\alpha=0.05$) showed that there were significant differences ($\text{sig.} > 0.05$) between formulas, both in the tests of dispersion, viscosity, adhesion and grinding speed. Based on the evaluation results, it can be concluded that formula 1 to 4 met the criteria for making gel preparation

Keywords: Gel, Hand sanitizer, *Moringa oleifera* Lamk, characteristics test.

Introduction

One of the applications of clean living behavior is keeping your hands clean, namely washing your hands regularly using soap and running water or using a hand sanitizer (Kemenkes RI, 2020). Hand sanitizer or hand antiseptic products are practical to use because they do not use air and can clean hands from microbes quickly (Mahdi and Setiawan, 2021). Hand sanitizer is available in liquid and gel form. The gel form is preferred because it feels cool on the skin and dries easily. Chemicals contained in hand sanitizers, such as alcohol and triclosan, can cause chronic irritation in the form of dermatitis on the hands (Chernyshov *et.al.*, 2020). Natural ingredients are an alternative active ingredient in hand sanitizer gel preparations (Nurjanah *et al.* 2020). One of them is a Moringa leaf. Moringa leaves are rich in various minerals and vitamins. Secondary metabolites such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids, and reducing sugars. Moringa

leaves have antiasthmatic, hyperglycemic, malaria, pneumonia, diarrhea, syphilis, antimicrobial, anticancer, antioxidant, antidiabetic, and lower blood pressure activities (Gopalakrishnan, *et al.*, 2016). Moringa leaf ethanol extract contains secondary metabolites (every 100 grams): flavonoids (20.76 g), tannins (11.64 g), alkaloids (4.5 g), and saponins (2 g), and has antibacterial activity against the bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella sp.* at a concentration of 25-200 mg/ml (2.5-20%) (Enerijiofi, *et.al.*, 2021). Research conducted by Hasanah *et al.*, (2017) shows that EEDK has antioxidant activity at a concentration of 89,305 ppm, and EEDK gel has antioxidant activity between 97,484 and 129,245 ppm. The antibacterial and antioxidant activity of EEDK can be developed into a hand sanitizer gel preparation. Characteristic and stability tests need to be carried out to obtain a preparation that meets the requirements for a hand sanitizer gel. The characteristic tests carried out are organoleptic, homogeneity, spreadability, pH, stick viscosity, and drying speed.

Materials and Methods

Type of laboratory experimental research and research design: RAL. Research at the Kupang Ministry of Health Polytechnic Pharmacy Study Laboratory. The tools used are glassware, No. 60 sieve, an analytical balance (TypeEW-220-3NM), a blender (Philips), evaporator (EyelatypeN-1000), hotplate, water bath Memmert), 45 mesh sieve, pH meter, UV-Vis Spectrophotometer (Shimadzu Type UV-1700). Ingredients are Moringa leaves, 95% ethanol, n-hexane, HPMC, methyl paraben, propylene glycol, DPPH, *Escheria coli* bacteria, *Pseudomonas aeruginosa*, and *Stapylococcus aureus*. Media: MHA, PDF, PDA, NA, BHIB.

A. Moringa Leaf Extraction

Put 50 g of Moringa leaf powder in a 500 ml measuring flask and, add 200 mL of ethanol. Stored for 12 hours with constant stirring at 30 minute intervals. The extract was filtered using Whatman filter paper no. 1. The filtrate was evaporated using an evaporator, and the extract was thickened over a water bath at a temperature of 40°C (Singh and Tafida, 2014). Moringa leaf ethanol extract was dechlorophyllated by: five grams of EEDK gel ware dissolved in 50 mL of a mixture of water and 90% ethanol (1:1), put in a separating funnel, and 50 mL of n-hexane, separated by the n-hexane phase. Add 50 mL of n-hexane repeatedly until the n-hexane phase in the separating funnel looks clear (Pebriana, *et.al.*, 2017).

B. Phytochemical Test

Flavonoid Test: 3 g of extract to 10 mL of water, shake the mixture, and add 1 ml of a 10% NaOH solution. The result is positive if it produces a yellow color. Test the saponin using a foam test by adding 3 g of extract to 2 ml of water in a test tube and then shaking vigorously. Positive result if bubbles form. In the steroid test (Salkowski test) 5 drops of H₂SO₄p plus 1 g of extract produce, a reddish brown color is formed (Harbone, 1987).

C. Handsanitizer Gel Formulation

Ethanol Extract of Moringa Leaf is formulated into a hand sanitizer gel preparation by mixing HPMC as a gelling agent. The formulation process begins with weighing the ingredients according to the amounts in the formula in Table 1. Next, the gelling agent (HPMC) is dissolved in a portion of warm distilled water (70°C) and stirred constantly until it swells (Mixture 1). Methyl paraben is dissolved in a portion of propylenglycol, then add the ethanol extract of Moringa oleifera Lamk leaves. While stirring, add the remaining propylene glycol then stir until homogeneous (Mixture 2). Add mixture 2 to mixture 1, then add the remaining 100 grams of distilled water, then stir again constantly until a homogeneous gel mass is formed (Yusuf, *et.al.*, 2017).

Table 1. Moringa Leaf Ethanol Extract Hand Sanitizer Gel Formula

Material	FORMULA (%)					FUNCTION
	1	2	3	4	5	
Ethanol Extract of Moringa Leaves	0,625	1,25	2,5		10	Active ingredients
HPMC	1	1	1	1	1	Gelling agent
Metil Paraben	0,2	0,2	0,2	0,2	0,2	preservative
Propilenglikol	12	12	12	12	12	Humektan
Aquades hingga	100 g	100 g	100 g		100 g	Base

D. Characteristic Test

Organoleptic tests observe the shape, color and odor of the preparation. The visual observation homogeneity test looks at the appearance and presence or absence of coarse grains (Rathod and Mehta, 2015). The pH requirement must be a pH close to the skin. Skin pH 4-6 (RPS 23th, 2020). Measurement of pH with a pH meter. Viscosity test using a Brookfield viscometer. Test the spreadability by applying a load to a piece of glass that has been smeared with the preparation. The gel is smeared on a glass plate, the two plates are attached until they merge. Then it is given a weight and attached to the adhesive force device, released with a load of 80 g, the time the two plates are released is recorded (Pratasik, *et.al.*, 2019). Test the speed of movement by applying the preparation to the inner side of the forearm and calculating the time it dries from the skin (Fitriansyah *et.al.*, 2016).

E. Data analysis

Characteristic test data were analyzed using qualitative descriptive methods and Anova. If the data is not homogeneously distributed, the Kruskal-Wallis test is carried out using SPSS. A gel is said to meet organoleptic requirements if there is no phase separation in the gel. The gel meets the homogeneity test, if when applied to the glass there are no visible fine grains (Rompis, *et.al.*, 2019). The gel is said to meet the pH test requirements if the pH is in the skin pH range. The pH required is 4.5 – 7 (Pratasik, *et.al.*, 2019). The gel meets the spreadability requirements if the spreadability is in the range of 5-7 cm. Meets the viscosity test if the viscosity is in the range of 2.000-50.000 cPs (Badan Standardisasi Nasional, 1996). There are no spesific provisions for adhesion strength but god adhesion strength for semi-solid preparation is not less than 1 second (Suhesti, *et. al.*, 2022). Adhesion time exceeds the minimum test requirements (≥ 1 second) (Suyudi, 2014)

Results and Discussion

A. Extraction of Moringa Leaf Ethanol Extract (*Moringa oleifera* Lamk)

The extraction process begins with making simplicia. The sample used was the leaves of the Moringa plant (*Moringa oleifera* Lamk). Samples were collected and sorted wet, then washed with running water and then drained. Next, the samples were chopped by separating the leaves from the stems. The samples were then dried in an oven at a temperature of $\pm 60^{\circ}\text{C}$ until dry. The dried simplicia is then ground with a herb grinder and sieved using a sieve with a mesh number of 45. The aim of sieving is to produce uniform simplicia particles and expand the particle size so as to maximize contact between the active substance and the solvent. Extraction was carried out using the maceration method using 95% ethanol solvent. A total of 5,000 grams of moringa leaf simplicia powder (*Moringa oleifera* Lamk) was macerated with 95% ethanol (1: 4). The amount of thick extract obtained from the extraction process in this study was 361.61 grams. The extract yield obtained was 7.252% (Figure 1 a-c).

Chlorophyll is a green component found in plant leaves and stems (Steer, 1999). Chlorophyll influences the color and panelists' acceptance of the gel. Dechlorophyllation is carried out to reduce chlorophyll levels using liquid-liquid separation techniques using a separating funnel and n-Hexane solvent (Susanty, 2019). The clear n-hexane fraction indicates that the chlorophyll in the ethanol extract of *Moringa oleifera* Lamk leaves has been reduced or lost. This is because n-hexane is a non-polar solvent so it is able to attract chlorophyll from the extract. The amount of dechlorophyllated ethanol extract of *Moringa oleifera* Lamk leaves (Figure 1d) obtained after the reconcentration

process was 171.19 grams with a soakage percentage of 3.56%. The extract obtained is a thick extract, has a distinctive extract odor, brownish color.

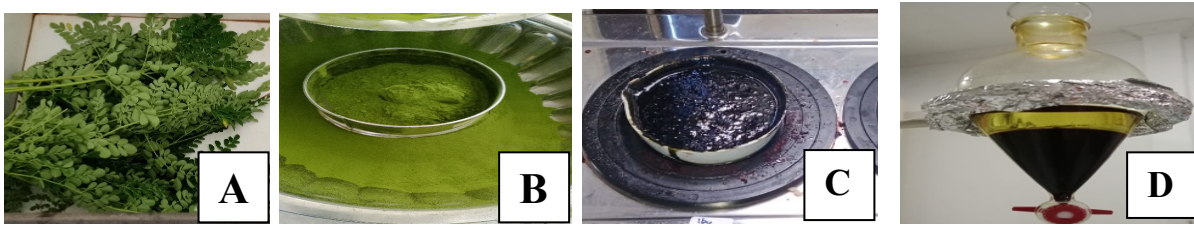


Figure 1. Extraction of Moringa Leaf Ethanol Extract (*Moringa oleifera* Lamk). from left to right A. Moringa Leaf, B. Simplicia powder of Moringa Leaf, C. Ethanol Ectract of Moringa leaf. D. Deklorofilasi Ethanol Ectract of Moringa leaf.

B. Phytochemical Screening

Phytochemical screening was carried out to identify the presence of alkaloids, flavonoids, saponins, steroids and tannins in the dechlorophyllated ethanol extract of *Moringa oleifera* Lamk leaves (Table 2). Phytochemical screening of the ethanol extract of *Moringa oleifera* Lamk leaves showed positive results for alkaloid compounds, flavonoids, saponins and tannins. Meanwhile, the results were negative for steroids because there was no green color change in the sample. This is because steroid compounds are non-polar compounds so they are attracted by the n-hexane solvent during the extract dechlorophyllation process (Figure 2) (Pebriana, *et.al.*, 2017).

Table 2. Phytochemical Screening of Ethanol Extract of Moringa Leaves (*Moringa oleifera*)

Compound	Reagent	Test Requirements	Test Result
Alkaloids	Mayer	White precipitate	+
	Wagner	Brown precipitate	+
	Bourchadat	Blackidh brown precipitate	+
Flavonoids	NaOH 10 %	Yellow colour is formed	+
Saponins	Aquades + HCl 2N	Froth	+
Steroid	Etil asetat + Asam asetat anhidrat; H ₂ SO ₄ p	Grren colour appears	-
Tannin	FeCl ₃ 1%	Dark green colour appears	+



Figure 2. Phytochemical Screening from left to right A. alkaloids test, B. flavonoids test, C. saponin test, D. steroid test and E. tanin test

C. Gel Handsanitizer Formula

The formulation of the hand sanitizer gel preparation for the ethanol extract of Moringa leaves (*Moringa oleifera* Lamk) was 5 formulas and 3 replications were made for each formula. The difference in the 5 formulas was the concentration of the Moringa leaf ethanol extract, formula 1 had the smallest concentration and formula 5 had the largest concentration. The difference in concentration of Moringa leaf ethanol extract is to obtain a formula that meets the requirements for gel characteristics. Initially using a 2% HPMC concentration, but after orienting it produced a very viscous preparation that did not meet the viscosity and spreadability requirements.

D. Characteristic Tes

Characterization tests on samples of *Moringa oleifera* Lamk (*Moringa oleifera* Lamk) ethanol extract hand sanitizer gel include organoleptic tests, pH test, homogeneity tests, spreadability tests, adhesiveness test, viscosity tests and drying speed tests.

The results of the organoleptic test (Table 3) showed that the concentration of the ethanol extract of *Moringa oleifera* Lamk leaves effected the color of the preparation. The addition of different amounts of extract to each formula resulted in differences in the color, of the preparation between formulas. Color different amounts of extract to each formula resulting in differences in the color of the preparation between formulas (Figure 3a). The greates of amount of extract, the darker the color of the preparation.

Table 3. Handsanitizer Gel Formula Extract Ethanol Moringa Leaf

Formula	Organoleptic Test		
	Form	Color	Smell
F1 (0,625%)	Semisolid	Brownish Yellow	Typica
F2 (1,25%)	Semisolid	Brownish Yellow	Typica
F3 (2,5%)	Semisolid	Brown	Typica
F4 (5%)	Semisolid	Brown	Typica
F5 (10%)	Semisolid	Dark Brown	Typica

A pH test is carried out to observe safety when used on the skin. The pH test showed that the formulas F1, F2, F3, and F4 had average pH values that met the requirements because they were in the pH range of 4.5 – 7 (Table 4) (Figure 3b) (Nadhifah, *et.al.* 2022); Meanwhile, the average pH value of the F5 formula (10%) does not meet the requirements because the pH value is below 4.5. From the test results data, it can be seen that the greater the extract content in the preparation, the greater the decrease in the pH value of the preparation. This can happen because the ethanol extract of *Moringa oleifera* Lamk leaves contains phenolic compounds such as tannins and flavonids, thereby allowing a decrease in the pH value of the preparation. In the Kruskal-Wallis test, there was a

significant difference ($0.009 < 0.05$) between Formulas 1-5, meaning that the difference in the concentration of Moringa leaf ethanol extract affected the pH of the gel.

The homogeneity test showed that the five preparation formulas had good homogeneity (Figure 3c), indicated by the absence of coarse grains on the glass plate smeared with the preparations. The homogeneity test results show that formulas 1-5 meet the requirements, namely homogeneity. The homogeneity of the preparation is caused by the constant stirring process, so that the gel mass expands evenly / does not clump. In the preparation process to help form a homogeneous gel, a hand mixer is used.

Table 4. Characteristic test Result Handsanitizer Gel Ethanol Extract of Moringa Leaves (Moringa oleifera)

Formula	Characteristic test Result Handsanitizer Gel				
	pH	Spereas-ability (cm)	Viscosoty	adhesive (Second)	Drying Speed (Second)
F1 (0,625%)	$6,2 \pm 0,047$	$6,67 \pm 0,22$	$2.649,7 \pm 181,16$	$1,88 \pm 0,33$	$6,77 \pm 0,13$
F2 (1,25%)	$5,9 \pm 0,17$	$6,41 \pm 0,19$	$2.434,3 \pm 995,3$	$1,93 \pm 0,48$	$7,08 \pm 0,24$
F3 (2,5%)	$5,1 \pm 0,047$	$6,33 \pm 0,29$	$2.850,3 \pm 782,57$	$1,81 \pm 0,34$	$6,56 \pm 0,15$
F4 (5%)	$4,8 \pm 0,047$	$6,44 \pm 0,23$	$2.867,3 \pm 215,96$	$2,01 \pm 0,36$	$7,23 \pm 0,26$
F5 (10%)	$4,1 \pm 0,047^*$	$6,42 \pm 0,19$	$2.964,3 \pm 233,26$	$1,86 \pm 0,17$	$7,03 \pm 0,20$

*Not eligible

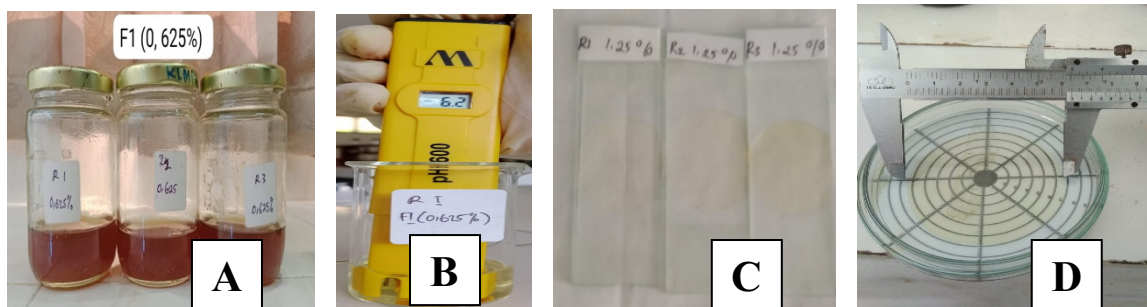


Figure 3. Characterization Test from left to right A. organoleptic test, B. pH test, C. homogeneity test, D. spreadability test

The spreadability test aims to determine the spreadability of the gel preparation. Gel preparations are said to have good dispersal power and will absorb the drug quickly because the contact area between the skin and the preparation is large. The spreadability test results (Figure 3d) of the five dosage formulas (Table 4) met the test requirements because the average spreadability was in the range of 5-7 cm (Garg *et al.* 2002). Analysis of spreadability data can be carried out using the One Way ANOVA test because the test data is normally distributed and homogeneous. The results of data

analysis show that the five formulas have significant differences, as shown by the significant value in the ANOVA table ($\text{sig} = 0.652 > 0.05$); and continued with Bonferroni correction in the Multiple Comparisons table also shows that the formulas have significant differences with a sig value > 0.05 . This means that differences in the concentration of Moringa leaf ethanol extract affect the spreadability of the gel.

The viscosity test shows that the five formulations have good viscosity (Figure 4a) (Table 4); The average viscosity value is in the range of 2,000-4,000 cP (Sujono, *et.al.*, 2014). The spreadability and viscosity of the preparation are influenced by the use of gelling agent HMPC 1% as a mass former in the gel formula. The normality test results in the Tests of Normality table in the Shapiro-Wilk column show that the dispersion characteristic test data is normally distributed ($\text{sig} 0.228 > 0.05$). Because the data to be analyzed is normally distributed and homogeneous, analysis can be carried out using the One Way ANOVA method. The results of the analysis show that there is a significant difference between the average viscosity test results in the ANOVA table ($\text{sig} = 0.898 > 0.05$); and the test was continued with Bonferroni correction in the Multiple Comparisons table, showing that the formulas had significant differences with a sig value > 0.05 . This means that differences in the concentration of Moringa leaf ethanol extract affect the viscosity of the gel.

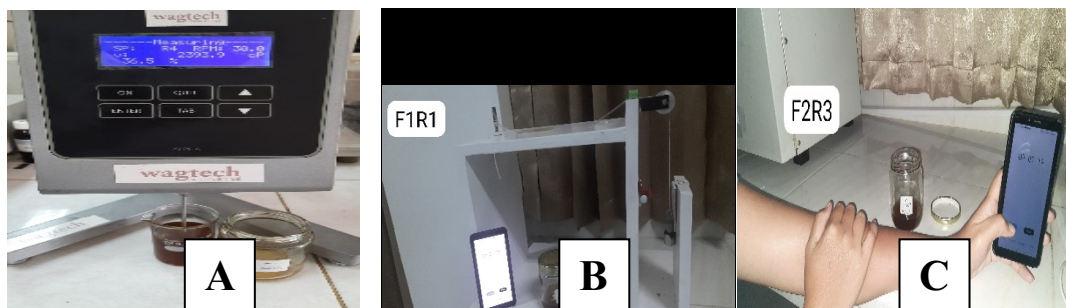


Figure 4. Characterization Test from left to right A. viscosity test, B. adhesive test, C. Drying speed test

The adhesive test aims to determine the ability of the gel to stick to the skin. The average adhesion test results also show that the five gel preparation formulas have good adhesion (Figure 4b and Table 4) because the average adhesion time exceeds the minimum test requirements (≥ 1 second) (Suyudi, 2014). The normality test results in the Tests of Normality table in the Shapiro-Wilk column show that the adhesion characteristic test data is normally distributed ($\text{sig} 0.653 > 0.05$), so analysis was carried out using the One Way ANOVA method. The results of the analysis show that there is a significant difference between the means of the kece test results in the ANOVA table ($\text{sig} = 0.896 > 0.05$); and the test continued with Bonferroni correction in the Multiple Comparisons table also shows

that the formulas have a significant difference with a sig value > 0.05 . This means that differences in the concentration of Moringa leaf ethanol extract affect the adhesive power of the gel.

The drying speed test was carried out to find out how long it took the gel to dry. The drying speed test results (Figure 4c and Table 4) of the preparation when applied to the skin of the palms ranged from 6-8 seconds. The normality test results in the Tests of Normality table in the Shapiro-Wilk column show that the dispersion characteristic test data is normally distributed (sig 0.565 > 0.05). Because the data to be analyzed is normally distributed and homogeneous, analysis can be carried out using the One Way ANOVA method. The results of the analysis showed that there was a significant difference between the mean results of the drying speed test and ANOVA (sig = 0.384 > 0.05); and the follow-up test with Bonferroni correction in the Multiple Comparisons table also shows that the formulas have a significant difference with a sig value > 0.05 . This means that differences in the concentration of Moringa leaf ethanol extract affect the speed at which the gel dries.

Conclusion

Based on the research results, it can be concluded that formulas 1 to 4 are formulas that meet the characteristic tests, namely organoleptic, homogeneity, spreadability, pH, adhesive viscosity and drying speed.

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