

Formulation of Anti-Acne Gel of *Moringa oleifera*, L. Ethanolic Extract and Bacteriostatic Test on *Staphylococcus epidermidis*

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ABSTRACT

Moringa oleifera L. leaves contain flavonoid, alkaloid, and phenolic compounds which have antibacterial activity. *Staphylococcus epidermidis* is one of the bacteria that causes acne. The purpose of this study is to compare bacteriostatic ability of the extracts and gel for *S. epidermidis* bacteria and to get gel formula that can fulfill the physical properties of a good gel. Moringa leaves were extracted with maceration method using ethanol 70% in three days. HPMC 4000 was used as the polymer. Extract was added with variation concentrations of 5, 10, and 15%. Physical evaluation of gel was organoleptic, homogeneity, pH, viscosity, adhesion, and spread tests for 4 weeks. In vitro bacteriostatic activity test with 1% clindamycin gel as positive control and polymer gel as negative control. The result showed that variations concentrations of ethanolic extract of Moringa leaves affected the physical properties of gel including viscosity, pH, adhesion and spreadability. Bacteriostatic activity test of ethanolic extract of Moringa leaf was classified as strong activity, while 15% Moringa leaf ethanol extract gel was classified as moderate activity with average diameter was 9.14 mm according classification of Davis and Stout 1971.

Keywords: moringa leaves,; HPMC 4000; gel; *Staphylococcus epidermidis*

INTRODUCTION

Acne can be caused by bacterial activity such as *Staphylococcus epidermidis* [Djajadisastra, 2009]. Currently acne treatment is antibiotic therapy which has skin irritation side effects and resistance in long-term use [Wasitaatmadja, 2008]. Moringa is a shrub that used widely as a vegetable or animal feed. Moringa leaves are empirically known has an antibacterial activity, because Moringa leaves contain secondary metabolites such as flavonoids, alkaloids, and phenols [Pandev *et al.*, 2012]. Previous research that has been carried out on ointment preparations of Moringa leaf extract showed an antibacterial activity against *Propionibacterium acne* [Choirunisa *et al.*, 2017]. Ethanolic extract of Moringa leaves with concentrations 5, 10, and 15% in ointment preparations has strong inhibitory activity against *Staphylococcus aureus* [Djumaati *et al.*, 2018]. This research was carried out by made a formulation of anti-acne gel using Hydroxy propyl methyl cellulose (HPMC) as polymer and ethanol extract of Moringa leaf for acne treatment. Gel has better potential topical drug facilities than ointments, because gel is not sticky, requires less energy for formulation, more stable, and has good aesthetic value.

Another advantage of gel preparation is quickly absorbed, so it is more effective to help absorption of active ingredient in acne area. Ethanolic extract of Moringa leaves gel with HPMC as a polymers has activity to inhibit *Malassezia furfur* [Yusuf *et al.*, 2017].

This study was carried out using a variation concentration 5, 10, and 15% of ethanolic extract of Moringa oleifera leaves and formulated in to anti-acne gel with HPMC polymers. Ethanolic extract of Moringa oleifera leaves and gel preparation were determine their bacteriostatic activity compare with clindamycin 1% gel. Variation concentration of extract was also carried out to obtain the most effective gel formula against *Staphylococcus epidermidis* bacteria, as well as physical properties test including, organoleptic test, homogeneity test, pH, viscosity, adhesion, and spreadability test.

METHOD

Tools

Glassware (Pyrex), analytical weight scales (PRECISA-XB 620C), rotary evaporator (STUART-RE300DB), pH meter (OHAUS-STARTER300), viskometer (RION-VT-04), moisture analyzer (OHAUS-MB25), incubator

Table I. Gel Formula of Ethanolic Extract of *Moringa oleifera* L. Leaves

Ingredients	Gel Formulae (g)			
	F1	F2	F3	KN
Moringa Leaves Extract	5.0	10.0	15.0	-
HPMC	1.5	1.5	1.50	1.50
Propylen Glycol	12.0	12.0	12.0	12.0
Methyl paraben	0.10	0.10	0.10	0.10
Aquadest	81.40	76.40	71.40	86.40

F1 : Antiacne gel formula with 5% ethanolic extract of moringa leaves; F2 : Antiacne gel formula with 10% ethanolic extract of moringa leaves; F3 : Antiacne gel formula with 15% ethanolic extract of moringa leaves; KN : Gel Formula as negative control

(OH) MEMMERT-UNB 400), autoclaves (HIRAYAMA-HVE-5), and other supporting tools.

Material

Moringa leaf, Hydroxy Propyl Methyl Cellulose 4000 M (Tianpu Chemicals Co. Ltd.), propylene glycol (Dow Chemical Pacific), methyl paraben (Ueno Fine Chemicals Industry Ltd.), 70% ethanol (Medica), Muller Hinton Agar Media (Oxoid), Mc Farland standard 0.5 (Remel), clindamycin 1% gel, and *Staphylococcus epidermidis* bacteria ATCC 12228.

Method

Extraction of *Moringa oleifera*

Moringa oleifera L. extract was made using maceration method. Maceration used 70% ethanol as solvent in ratio (1: 7.5). Extraction was carried out by weighing 600 grams of Moringa leaf powder then transferred to a maceration vessel with 4500 mL of 70% ethanol, then stirred and closed. Solution were left for 24 hours and occasionally shaken at least 3 times.

Phytochemical Detection

Phytochemical detection in ethanolic extract of *Moringa oleifera* L. leaves was carried out by using TLC (Thin Layer Chromatography) method. Sample preparation was carried out by dissolving 300 mg ethanolic extract of *Moringa oleifera* L. in 10.0 mL ethanol 70%, then spotted in the stationary phase of GF-254 silica gel with 5 μ L spot volume of extract, mobile phase is used chloroform: ethyl acetate (2: 1) and eluted 8.0 cm range in a saturated chamber that eluted with filter paper before. TLC results can be seen by looking at the spots in visible light, UV 254

nm, and UV 366 nm and calculate the Rf (Retention factor) value, then Rf value of chemical compounds was identified with standard Rf of chemical components, and can be ascertained using spray reagents.

Formula Design of Antiacne Gel

Design of Antiacne gel formula was carried out by determine HPMC concentration as a polymer that suitable with the characteristics ethanolic extract of Moringa leaves. Formula used to make anti-acne gel preparations can be seen in Table I.

Antiacne gel of ethanolic extract Moringa leaves were made by swelling HPMC into hot aquadest 20 times HPMC weight in 15 minutes. Methyl paraben was dissolved in propylen glycol and stirred, this solution poured into HPMC solution then stirred until homogen. Aquadest was added until form a gel base. Ethanolic extract of Moringa leaves were added to gel base and stirred until homogen.

Physical Evaluation of Antiacne Gel

Physical evaluation of ethanolic extract Moringa leaves antiacne gel includes organoleptic, homogeneity, viscosity, pH, adhesive, spreadability test and stability test. Stability test were obtained in initial week until 4th week each test were replicated in 3 times.

Organoleptic test

Organoleptic test was carried out by describing shape, colour, smell, and texture of the gel. Organoleptic test were evaluated every week in 4 weeks.

Viscosity test

Viscosity test was carried out by using viscometer Rion VT-04. Sample tube was filled

by gel sample, rotor was placed in the middle of the sample tube until the spindle was submerged into the gel, viscometer was turned on and rotor will rotated, rotor pointing needle will automatically move, viscosity were measured by read the 2nd rotor scale.

pH test

pH test was carried out by using pH meter. Sample preparation was carried out by weighing 1.0 g gel then dissolved into 10 mL aquadest. Electrode was dipped to the sample solution, then *read* button was pressed until pH value was constant. pH test was did at room temperature.

Spreadability test

Amount of 0.5 g gel were placed on petri disc and closed with other petri disc, and wait until 1 minute, spread diameter of the gel were measured from vertical and horizontal side. 50 , 100, and 150 g load were added on the petri disc and left for 1 minute, then diameter of the gel was measured. Load were added until make a constant diameter or gel cannot spread anymore.

Stickiness test

Amount of 0.5 g gel were placed on object glass and were closed with another object glass. Object glass were placed into adhesive test tool, and place 80 g of load, Stickiness test of gel was measured by counting time for object glass to break each other.

Ethanollic Extract of Moringa Leaves Bacteriostatic Activity to *Staphylococcus epidermidis*

Antibacterial activity was carried out by disc diffusion method (Kirby-Bauer test), sterile cotton swab dipped into the *Staphylococcus epidermidis* bacterial suspension, then rotated several times and pressed to the tube wall to remove excessive inoculum in cotton swab. *Staphylococcus epidermidis* were inoculated into agar media. Paper disc (6 mm) were dipped in sample (gel preparations F1, F2, F3, and ethanollic extract of Moringa leaves 5, 10, and 15%) then the paper disc were placed on the surface of the media, position of each paper disc was 2-3 cm from the edge of petri dish. Positional control was used 1% clindamycin gel, and negative control was used HPMC polymer and water. Petri dish were incubated at 37 ° C for 24

hours and then the diameter of inhibition zone were observed.

RESULT AND DISCUSSION

Determination of Plant

Moringa leaves used in this study were from Ngadirojo, Wonogiri. Determination of the plant were observed in Biology Laboratory, Matematic and Science Faculty, Universitas Sebelas Maret, Surakarta. Result of the determination showed that the plant used in this study was Moringa plant (*Moringa oleifera* L.).

Extraction and Extract Evaluation

Moringa leaves were maserated using polar solvent to extract polar molecule (saponin, tannin, and flavonoid). Ethanolic extract of Moringa leaves were brownish-green, smells herbally, and has very viscous consistency. Sample were produced 135.96 g (22.66%) of extract. Active compound of Moringa leaves were fit to the criteria minimum standard of yield, it was above 10% [Hasanah *et al.*, 2016]. Adhesive test of ethanolic extract of Moringa leaves was purposed to know consistency level of ethanolic extract of Moringa leaves. Result means of adhesive test of ethanolic extract of Moringa leaves was 1.14±0.03 minute, it means that adhesive time of this extract was long. and the extract of Moringa leaves has very viscous consistency. Water content of ethanolic extract of Moringa leaves was 2.59%. Water content of extract criteria was <10% [Depkes RI, 1995], so water content of ethanolic extract of Moringa leaves still fit to the criteria.

Phytochemical Detection

Phytochemical detection was carried out to know the group of active compounds into ethanolic extract of Moringa leaves, TLC (Thin Layer Chromatography) method was used in this study. Sample was eluted then sprayed with reagents.

Phytochemical identification of ethanolic extract of Moringa leaves can be seen in Table II. Colour change of the spot showed phenolic, flavonoid, and alkaloid compound in the ethanolic extract of Moringa leaves.

Physical Evaluation of Gel Ethanolic Extract of Moringa Leaves

Physical evaluation was carried in orde to know the differences between formulas and

Table II. Phytochemical Identification ethanolic extract of *Moringa* leaves spray ingredients

Reagents	Spot colour	Compound
FeCl ₃	Blackish green	Phenolic
Wagner	Brown	Alkaloid
Citroboric	Yellow fluorescent at UV 366 nm	Flavonoid
Lieberman-Burchard	-	-

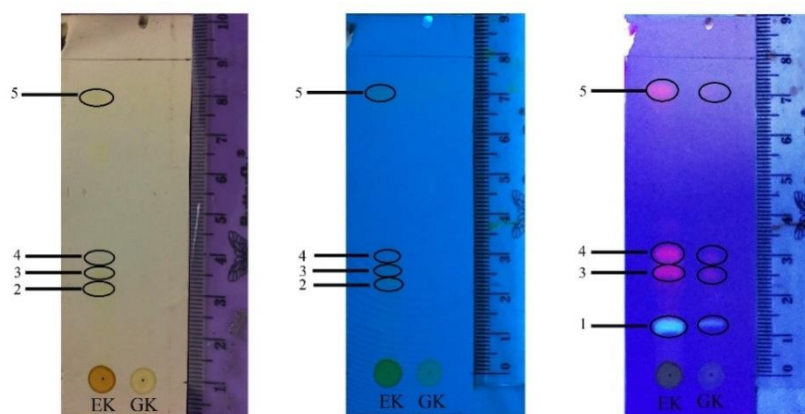


Figure 1. TLC Result of ethanolic extract of *Moringa* leaves (EK) dan Gel of ethanolic extract of *Moringa* leaves (GK) with Stationary phase was Silica Gel GF 254 and eluent was chloroform : Ethyl Asetate (2:1) At Visible Light (a), UV 254 nm (b), dan UV 366 nm (c)

to know suitability between observation with standard criteria. Physical stability test of gel were purposed to know stability of gel formula after 4 week stored in room temperature. Physical evaluation of gel observed organoleptic, pH, viscosity, spreadability, and adhesive test. Result of physical evaluation in initial week can be seen in Table III.

Organoleptic test

Gel organoleptic result in initial week can be seen in Table III. Formula 1 and 2 has brownish colour but formula 3 has darker brown colour, it can caused by higher extract concentration. Gel of *Moringa* Leaves ethanolic extract also produce higher consistency in formula 3, because of higher concentration of *Moringa* leaves ethanolic extract. All formula produce same smell of *Moringa* leaves ethanolic extract. Result study showed various extract concentration can influences its organoleptic properties.

Organoleptic test result after 4 week stored (Table IV), showed there were no difference in formula 1 for all parameter. Consistency parameter for formula 2 and 3 has produce different result, its consistency had changed in week-2 untill week-4. Formula that

can produce better organoleptic stability was formula 1 with *Moringa* leaves ethanolic extract was 5%.

pH Test

pH test was purposed to know the safety of the preparation when used on skin. Topical preparation pH must fit to topical skin pH (4.5-6.5), so it could not make skin irritation [Naibaho *et al.*, 2013]. pH gel in initial week showed in table III, pH value of formula 1 was 5.83; formula 2 was 5.75; and formula 3 was 5.72. All formula has fit to the pH skin criteria, so it is safe to use.

pH gel result in initial week were analyzed with *One Way Anova* and produced *p-value* = 0.00 (<0.05) it means that formula were significantly different caused of variation concentration of extract. *Post Hoc* analysis result produced *p-value* = 0.00, it means that concentration variation of extract has affect pH value of gel preparation.

pH value was decrease after stored, it means gel were more acidic, it can caused by temperature and condition of storage [Padmadisastra, 2007]. However all formula still fit to the criteria of normal skin pH (4.5 - 7). Duration of storage pH value of gel preparation,

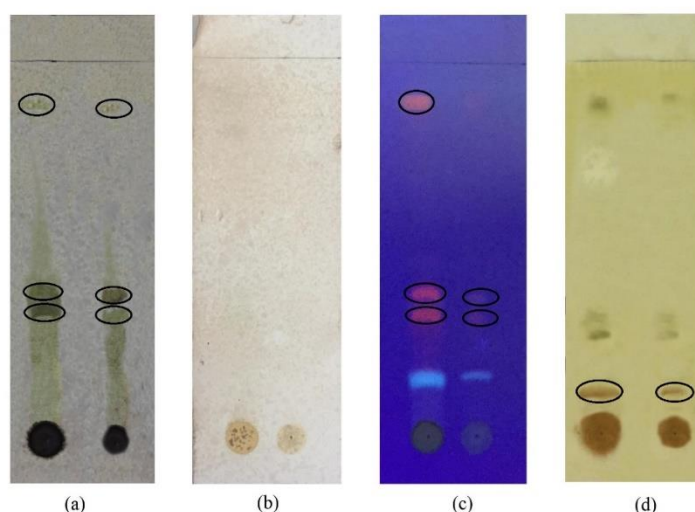


Figure 2. Compound Identification Result Using Spray Reagent to Make Colour Change to Spot in TLC Ethanolic Extract of Moringa Leaves (EK) and Gel of Ethanolic extract of Moringa Leaves (GK) Using FeCl₃ (a), Lieberman-Burchard (b), Citroboric (c), Wagner (d)

Table III. Physical Evaluation of Gel Ethanolic Extract of Moringa Leaves Initial Week

Parameter	Observation			
	F1	F2	F3	
Organoleptic	Colour	+	+	++
	Smell	+	+	+
	Consistency	+	+	++
Homogeneity	Homogen	Homogen	Homogen	
pH	5.83±0.01	5.75±0.01	5.72±0.01	
Viscosity (dPa.s)	900±0.00	1000±0.00	1100±0.00	
Addhesive (sec)	3.78±1.62	4.43±0.22	5.36±0.01	
Spreadability (cm)	5.00±0.04	4.70±0.03	4.50±0.03	

F1 : Antiacne gel formula with 5% etanolic extract of moringa leaves; F2 : Antiacne gel formula with 10% etanolic extract of moringa leaves; F3 : Antiacne gel formula with 15% etanolic extract of moringa leaves; KN : Gel Formula as negative control

Explanation: Colour + : Brown; Colour ++ : Dark Brown; Smell + : Typically Moringa Leaves Extract Smells; Consistency + : Viscous; Consistency ++ : Very viscous

it means that all formulas are unstable in storage.

Viscosity Test

Viscosity expressed resistance of liquid to flow. Viscosity value in initial week can be seen in table III. Viscosity value of formula 1 was 900 dPa.s (90 Pa.s), formula 2 was 1000 dPa.s (100 Pa.s) and formula 3 was 1100 dPa.s (110 Pa.s). it means that variation concentration of extract affected its viscosity. Viscosity criteria standard for gel was 6000-50000 cP (6-50 Pa.S) based on SNI 16-4399-1996 [Hidayanti *et al.*, 2015], it

means all viscosity value still not fit to the criteria.

Viscosity of gel evaluation result in 4 week stored can be seen in figure 2. Viscosity level of formula 1 in initial week untill week-4 were in range of 850-900 dPa.s it means that there were no significant change in viscosity, however there were significant change in formula 2 and 3. Viscosity were decrease because of Moringa leaves ethanolic extract had acidic pH. HPMC polymer were basic polymer, so HPMC polymer were hydrolyzed in acidic pH, it caused change of gel viscosity to a more

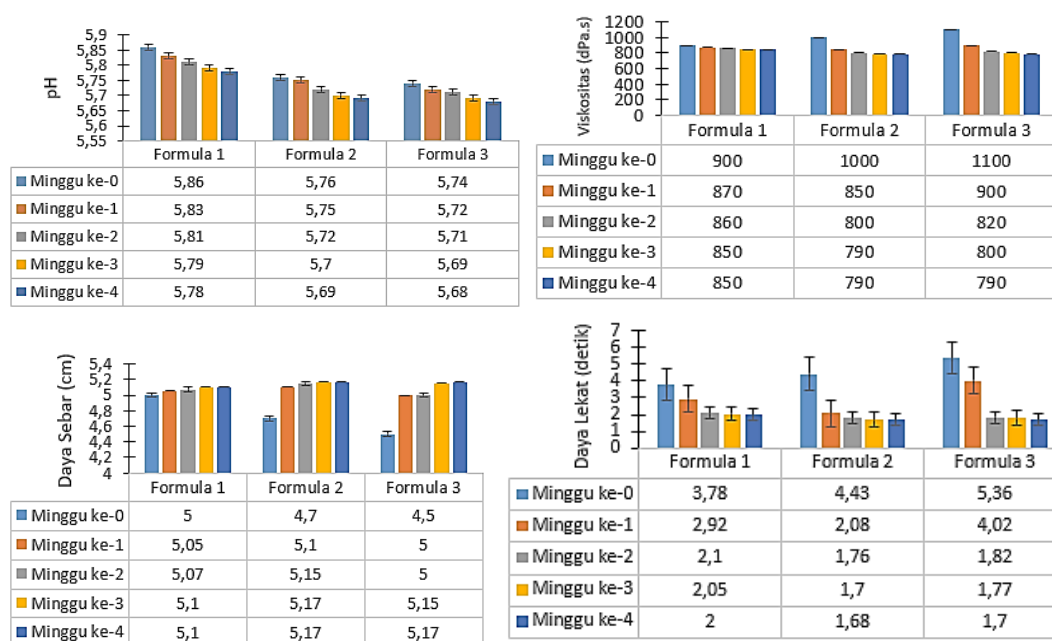


Figure 3. The chart result of Physical Evaluation of Gel of Moringa Leaves Ethanolic Extract in 4 week, involved pH, Viscosity, Spreadability and Adhesive test

Table IV. Organoleptic Study of Gel of Moringa Leaves Ethanolic Extract in 4 Weeks

Observation Parameter	Formula	Week				
		0	1	2	3	4
Colour	F1	-	-	-	-	-
	F2	-	-	-	-	-
	F3	-	-	-	-	-
Smell	F1	-	-	-	-	-
	F2	-	-	-	-	-
	F3	-	-	-	-	-
Consistency	F1	-	-	-	-	-
	F2	-	-	+	+	+
	F3	-	-	+	+	+

aqueous form (Astuti *et al.*, 2012). Stored duration can affect gel viscosity in formula 2 and 3, but non-significantly affect viscosity in formula 1.

Adhesion Test

Adhesion gel more greater, absorption of active substance also can be greater, due to longer interaction of gel with skin, so gel base will release more active substance. Results of adhesive test for gel of Moringa leaves ethanolic extract in initial week can be seen in table III. Adhesive value of formula 3 was 5.36 seconds, it was the highest adhesive value, adhesive value of formula 2 was 4.43 seconds and formula 1

was 3.78 seconds. Adhesion value criteria for topical preparations is not less than 4 seconds [Ulaen *et al.*, 2012]. Formula 2 and 3 were fit to the criteria of good adhesion value. Variation concentration of extract were affected adhesion value of anti-acne gel. *One Way Anova* analysis of adhesion test in initial week produced p -value = 0.53 (>0.05) it means that variation concentration of extract has non-significantly different adhesion value. Variation concentration of extract was non-significantly affect the adhesion value.

Adhesion value was decrease in 4 week stored, it can caused by unstable temperature and acidic effect of extract that caused

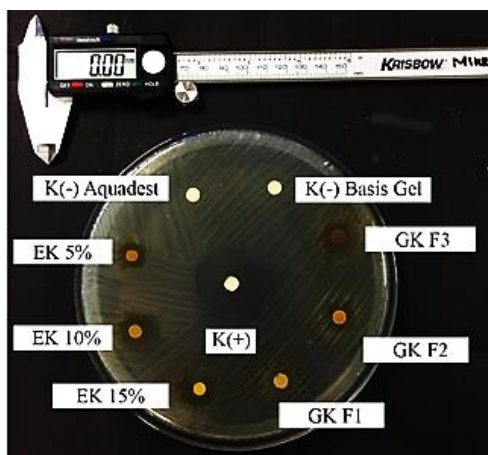


Figure 4. Inhibition Zone of Antiacne Gel (GK), Ethanolic Extract of Moringa Leaves (EK), Negative Control (K(-)), and Positive Control (K(+)) to *Staphylococcus epidermidis* in MHA Medium

Table IV. Inhibition zone of Antiacne Gel to *Staphylococcus epidermidis*

	Inhibition Zone Diameter (mm)				Antibacterial Activity
	Replication 1	Replication 2	Replication 3	Mean	
K1 (-)	-	-	-	-	-
K2 (-)	-	-	-	-	-
K (+)	31.35	32.29	32.82	32.15±0.74	Very Strong
EK 5%	6.89	5.23	7.54	6.55±1.19	Moderate
EK 10 %	11.62	10.87	9.54	10.68±1.05	Strong
EK 15 %	19.18	19.53	19.70	19.47±0.27	Strong
GK F1	4.89	4.98	5.38	5.08±0.26	Moderate
GK F2	5.87	6.47	5.72	6.02±0.40	Moderate
GK F3	8.82	9.44	9.15	9.14±0.31	Moderate

unstability of HPMC as polymer. Adhesion time was directly proportional with viscosity, lower viscosity, will also produce lower adhesion time [Astuti *et al.*, 2012]. Adhesion value result of his study showed that gel formulation were unstable while stored.

Spreadability Test

Gel preparation are expected to be easily spread on the skin without significant pressure, more great contact of gel with the skin surface area, will be more easy for gel to be applied, it means gel can distributed equally on skin [Windriyatri *et al.*, 2007]. Good gel dispersion is between 5-7 cm [Grag, 2002]. Spreadability test in initial week (Table III), showed that formula 1 fit to the criteria of good spreadability value, it was 5.00 cm, formula 2 and 3 did not fit the criteria of good spreadability value it was 4.70 cm and 4.50 cm.

One Way Anova test of spreadability test in initial week produced a significant p-value =

0.00 (<0.05) it means that in there were a significant difference in the spreadability value due to variation concentration of extract. *Post Hoc* test results showed a significant differences of spreadability value in all formula (*p-value* = 0.00). It can be concluded that variation concentration of ethanolic extract affected the spreadability value. Spreadability value in 4 week stored result showed in figure 4. All formula were increase its spreadability value every week, it can caused by consistency change. Lower viscosity of gel after stored caused higher fluid flow. Formula 1 produce better stability of spreadability value than formula 2 and 3 after 4 weeks stored in room temperature.

Antibacterial Activity

Antibacterial activity of gel antiacne gel has inhibition zone diameter mean 5.08 mm for formula 1, formula 2 was 6.02 mm, and formula 3 was 9.14 mm, all formula were included moderate inhibition category [Davis and Stout,

1971]. Positive control (Clindamycin gel 1%) showed inhibition zone diameter was 32.15 mm, it was included very strong inhibition category and there was no inhibition zone in negative control. Higher inhibition zone was formula 3, it caused by ethanolic extract concentration was higher than other formula (15%).

Concentration variation of *Moringa* leaves ethanolic extract (5, 10, and 15%) affected *Staphylococcus epidermidis* inhibition, higher concentration of *Moringa* leaves ethanolic extract will also higher antibacterial activity, because of higher ethanolic extract also has higher chemical compound that inhibit bacterial growth. Inhibition zone diameter gel contain variation concentration of extract (5, 10, dan 15%) showed that was not aligned with inhibition zone diameter in ethanolic extract of *Moringa* leaves. It may caused by gel polymer holding the active substance released, because gel polymer was usually used to extend the effect of active components, so it was affect in efectivity of *Moringa* leaves ethanolic extract.

KESIMPULAN

Higher concentration of *Moringa* leaves ethanolic extract produce higher activity antibacterial with higher inhibition zone diameter to *Staphylococcus epidermidis*. Antiacne gel of *Moringa* leaves ethanolic extract has antibacterial activity to *Staphylococcus epidermidis* with moderate inhibition category, better formula for antibacterial activity was formula 3 with concentration of *Moringa* leaves ethanolic extract was 15% and fulfill the best gel physical properties.

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