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

Cahyarani Intan Ramadhani, Dian Eka Ernawati*
Fakultas MIPA, Universitas Sebelas Maret, Surakarta

Corresponding author: Dian Eka Ernawati; Email: dianekae@staff.uns.ac.id

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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 fulfills 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

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

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gel Formulae (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1</td>
</tr>
<tr>
<td>Moringa Leaves Extract</td>
<td>5.0</td>
</tr>
<tr>
<td>HPMC</td>
<td>1.5</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>12.0</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>0.10</td>
</tr>
<tr>
<td>Aquadest</td>
<td>81.40</td>
</tr>
</tbody>
</table>

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

(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 leaves**
*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 5µL 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 propylene 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 etanolic extract Moringa leaves antiacne gel includes organoleptic, homogeneity, viskosity, 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
aqueast. Electrode was dipped on 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
were 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.

**Ethanolic 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
ethanolic 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. Positive
control was used 1% clindamycin gel, and
negative control was used HPDM 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,
Mathematic and Science Faculty, Universitas
Sebelas Maret, Surakarta. Result of the
determinatton showed that the plant used in this
study was Moringa plant (*Moringa oleifera* L.).

**Extraction and Extract Evaluation**

Moringa leaves were maseder 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
eact. Active compound of Moringa leaves
were fit to the criteria minimum standard of
yield, it was above 10% [Hasanah et al., 2016].
Addhesive test of ethanolic extract of Moringa
leaves was purposed to know consistency level
of ethanolic extract of Moringa leaves. Result
means of addhesive test of ethanolic extract of
Moringa leaves was 1.14±0.03 minute, it means
that addhesive 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 order to
know the differences between formulas and
Table II. Phytochemical Identification ethanolic extract of Moringa leaves spray ingredients

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Spot colour</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeCl₃</td>
<td>Blackish green</td>
<td>Phenolic</td>
</tr>
<tr>
<td>Wagner</td>
<td>Brown</td>
<td>Alkaloid</td>
</tr>
<tr>
<td>Citroboric</td>
<td>Yellow fluorescent at UV 366 nm</td>
<td>Flavonoid</td>
</tr>
<tr>
<td>Lieberman-Burchard</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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

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 [Padmadasastra, 2007]. However all formula still fit to the criteria of normal skin pH (4.5 - 7).

Duration of storage pH value of gel preparation,
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 until 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 decreased 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
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

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

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

<table>
<thead>
<tr>
<th>Observation Parameter</th>
<th>Formula</th>
<th>Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Colour</td>
<td>F1</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>-</td>
</tr>
<tr>
<td>Smell</td>
<td>F2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>-</td>
</tr>
<tr>
<td>Consistency</td>
<td>F2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>-</td>
</tr>
</tbody>
</table>
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,

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

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

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 effectivity 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.

**DAFTAR PUSTAKA**


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