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Formulation of Anti-Acne Gel of *Moringa Oleifera* Leaves Powder Ethanolic Extract as an Inhibitor of Bacteria

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ABSTRACT

Acne (Acne vulgaris) is the most common disorder which is a chronic inflammatory disease. That cause acne by one of the bacteria is staphylococcus epidermis. Moringa oleifera leaves contain flavonoid, alkaloid and phenolic compound which have inhibit acne growth. The purpose of the study is the compression of bacteriostatic ability of the extracts and gel for formula that can fulfills the physical properties of good gel moringa leaves. Moringa leaves were extracted with maceration method using ethanol 70% in three days. HPMC 4000 as a polymer were used extract was added with variation concentration of 5,10,15% physical evaluation test of gel was organoleptic, homogeneity, PH, viscosity, adhesion, and spread tests for 4 weeks. In-vitro bacteriostatic activity test with 1% Clindamycin gel a positive control and polymer gel negative control. The results that variation concentrations of ethanol extract of moringa leaves affected the physical properties of gel including viscosity PH, adhesion and spread ability.

Key words: Moringa oleifera, Acne vulgaris, clindamycin, HPMC4000.

1. INTRODUCTION

Acne Is the most common disorder, this disease occurs especially in adulthood, young and heal itself and acne can cause by bacterial activity such as staphylococcus epidermidis. Now a day's acne treated by antibacterial therapy which has skin irritation side effects and resistance in long-term use. Moringa is also widely used as a vegetable or animal feed. Moringa leaves are empirically known has an antibacterial activity, because its leaves contain secondary metabolites such as flavonoids, alkaloids, and phenols. Previous research that has been carried out on ointment preparation of moringa leaf extract showed an antibacterial activity against Propionibacterium acne. Ethanolic extract of moringa leaves with concentration 5,10 and 15% in ointment preparation has strong inhibitory activity against staphylococcus aureus.

The 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 ingredients in acne area. Ethanolic extract of moringa leaves gel with HPMC as a polymer.

This study was carried out using a variation concentration 5,10,15% of ethanolic extract of *moringa oleifera* leaves and formulated in to anti-acne gel with HPMC polymer. Ethanolic extract of moringa oleifera leaves and gel preparation were determining 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 epidermis bacteria, as well as physical properties test including, organoleptic test, homogeneity test, PH, viscosity, adhesive, and spread ability test.

2. MATERIALS AND METHODS

2.1 Equipment's

Glassware's, analytical weight scales, rotary evaporator, PH meter, viscometer, moisture analyzer, incubator, autoclaves and other supporting tools.

2.2 Materials

Moringa leaf, HPMC 4000, propylene glycol, methyl paraben, 70% ethanol mucellerhinton agar media, MC farland 0.5, clindamycin 1% gel and staphylococcus bacteria.

2.3 Methods

2.3.1 Extraction of Moringa Leaves

Moringa oleifera powder extracted by using soxhlation method. 70% ethanol used as a solvent in ratio (1:10). extraction was carried out by weighing 300 grams of moringa leaf powder to a soxhlation vessel with 350ml of 70% ethanol, then stirred and close. Solution were left for several hours and occasionally shaken at 3 times.

2.3.2 Phytochemical Detection of Moringa Oleifera

Phytochemical detection was carried out by using thin layer chromatography (TLC) method of ethanolic extract of moringa oleifera L leaves. Sample preparation was carried out by dissolving 300mg ethanolic extract of moringa oleifera L.in 10ml ethanol 70%, and spotted in the stationary phase of silica gel with 5 μ L spot volume of extract, chloroform: ethyl acetate (2:1), used as a mobile phase and eluted 8cm range in a saturated chamber that eluted with filter paper before. It results can be seen by looking at the spots in visible light, like uv 254nm, and uv 366mm then calculated the RF value, the RF value of chemical compounds was identified with standard RF of chemical compounds and can be ascertained using spray reagents.

2.3.3 Formulation Design of Anti-Acne Gel of Moringa Oleifera Leaves

Formulation that used to make anti-acne gel of preparation can be seen in table 1. Ethanolic extract of anti-acne gel of moringa leaves were made by swelling HPMC into not aquadest 20 times. HPMC weight in 15nminutes methyl paraben were dissolved in propylen glycol and stirred, this solution poured into HPMC solution then stirred until homogen aquadest were added until form a gel base. Ethanolic extract of moringa leaves were added to gel base and stirred until homogen.

Table 1: Gel Formula of Extract of *Moringa oleifera* L. Leaves

Ingredient	Gel Formula(g)		
	F 1	F 2	F 3
Moringa leaves extract	5.0	10.0	15.0
HPLC	1.5	1.5	1.50
Propylen glycol	12.0	12.0	12.0
Methyl paraben	0.10	0.10	0.10
Aquadest	81.40	76.40	71.40

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

2.4 Physical Evaluation of Anti-Acne Gel

Physical test for ethanolic extract moringa leaves anti-acne gel such as organoleptic, homogeneity, viscosity, PH, adhesive, spread ability test and stability test.

A. Organoleptic Test

In this test was carried out by describing shape, color, smell and texture of the gel and it is evaluated every week in 4 weeks.

B. Viscosity

This test is carried out by using viscometer RIONYT04 rotar pointing needle will automatically move, viscosity were measured by read the 2nd rotor scale.

C. pH Test

pH test carried out by using pH meter. Weighing 1gm gel then dissolved into 10ml 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.

D. Spreadability Test

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

E. Adhesive

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

2.5 Ethanolic Extract of Moringa Leaves Bacteriostatic Activity to Staphylococcus Epidermis

Anti-bacterial activity was carried out by disc diffusion method, sterile cotton swab dipped into the staphylococcus epidermis bacterial suspension, then rotated several times and pressed to the tube wall to remove excessive inoculum in cotton swab, staphylococcus epidermis were inoculated into agar media.

Paper disc (6mm) was dipped in sample (gel preparation F1, F2, F3 and ethanolic extract of moringa leaves 5,10,15%) then the paper disc were placed on the surface of the media, position of each paper disc was 2-3cm from the edge of Petridis. Positive control was used 1% clindamycin gel, and negative control was used HPMC polymer and water. Petridis were incubated at 37°C for 24 hours and then the diameter of inhibition zone were observed.

3. RESULTS AND DISCUSSION

3.1 Extract Evaluation

soxhlation were used extract saponin, tannin and flavonoid. Ethanolic extract of moringa leaves were brownish-green smells herbally and has viscous consistency. Active compound of moringa leaves were fit to the criteria minimum standard of yield, it was above 10%.

Adhesive test ethanolic extract of moringa leaves was purposed to know consistency level and results means of adhesive test of ethanolic extract of moringa leaves was 1.14 0.03 minute, it means that adhesive time of the extract was long and extract of moringa leaves has very viscous consistency.

3.2 Phytochemical Detection

Phytochemical detection was did to know group of contained compounds in ethanolic extract of moringa leaves, TLC method was used in this study. Sample was eluted then sprayed with reagent color change of the spot showed phenolic, flavonoid and alkaloid compound in the ethanolic extract of moringa leaves and it show in table 2.

Table 2: Phytochemical identification ethanolic extract of moringa leaves spraying reagents

Reagents	Spot	Compound
Fecl3	Blackish green	phenolic
wagner	Brown	Alkaloid
citroboric	Yellow fluorescent at UV 366nm	Flavonoid

3.3 Physical Evaluation of Gel Ethanolic of Moringa Leaves

Physical evaluation of gel observed given in table 3. Organoleptic, PH, viscosity, spreadibility, and adhesivetest result of physical evolution in initial week can be seen.

3.3.1 Organoleptic Test

Gel organoleptic result in initial week can be seen in table formula 1 and 2 has brownish color but formula 3 has darker brown color. It can cause by higher extract concentration. Gel also produce higher consistency in formula 3, because of higher ethanolic extract. Result study showed various extract concentration can influence its organoleptic properties. Organoleptic test result given in table 4.

Organoleptic test result after 4 week stored, showed there were no difference in formula 1 for all parameter consistency parameter for formula 2 and 3 has produced different result, it consistency has changed in week 2 untill week 4 formula that can produced better organoleptic stability was formula 1 with moringa leaves ethanolic extract was 5%.

3.3.2 pH Test

pH test was purposed to know the safety of the preparation when used on skin. Topical skin pH(4.5-6.5) so it could not make irritation.

In initial week showed, 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 value was decrease after stored it means gel were more acidic, it can cause by temperature and condition of storage. However all formula still fit to the criteria of normal skin pH(4.5-7) duration of store pH value of gel preparation; It means that all formula are unstable in storage.

3.3.3 Viscosity Test

Viscosity expressed resistance of liquid to flow. Viscosity value in initial week are of formula 1 was 900dpa.s.(90pas) formula 2 was 1000dpa.s(100pas) and formula 3 was 1100dpa.s(110dps). it mean that variation concentration of extract affected its viscosity. Standard of viscosity of gel was 6000-50000cp(6-50pa.S). it means all viscosity value still not fit to the criteria. Viscosity of gel result in 4 week stored can show viscosity level of formula 1 in initial week until week 4 were in range of 805-900da.S. it means no changes in viscosity, however there were change in formula 2 and 3 viscosity were decreases 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.

3.3.4 Adhesion Test

Due to longer interaction of gel with skin, so gel base will release more active substance. Result of adhesive test for gel of initial week can be seen ; adhesive value of formula 3 was 5.36 seconds, it was the highest adhesive value, adhesive value of formula 2 was 4.43 and 1 was 3.78 seconds. Adhesive value criteria for topical preparation is not less than 4 seconds Formula 2 and 3 were fit to the criteria good adhesive value, adhesion value was decrease in 4 week stored, it can caused by unstable temperature and acidic effect of extract that caused instability of HPMC as polymer. Adhesive time was directly proportional with viscosity, lower viscosity, will also produce lower adhesive time. Adhesion value result of his study showed that gel formulation were unstable while stored.

3.3.5 Spreadability Test

Gel are expected to be easily spread on the skin without significant pressure, gel can distributed equally on skin, will be more easy for gel to be applied good gel dispersion between 5 to

7cm. Spreadability test in initial week , showed that formula 1 fit to criteria of good spreadability value, it was 5.00cm, formula 2 and 3 did not fit the criteria of good spreadability value it was 4.70cm and 4.50cm. Spreadability value in 4 week stored showed, 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 then formula 2 and 3 after 4 week stored in room temperature.

3.3.6 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 [4]. 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 can caused by excipient in gel, it affect effectivity of *Moringa* leaves ethanolic extract. Gel formulation were not fit for these active compound to inhibit *Staphylococcus epidermidis*.

Table 3: Physical evaluation of gel of moringa leaves Initial week

parameter	observation			
		F1	F2	F3
organoleptic	Color	+	+	++
	Smell consistency	+	+	+
		+	+	++
Homogeneity		Homogen	Homogen	Homogen
PH		5,83±0,01	5,75±0,01	5,72±0,01
Viscosity (dPa.S)		900±0	1000±0	1100±0
Adhesive(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

Table 4: Organoleptic study of gel of moringa leaves Ethanolic Extract in 4 weeks

Observation parameter	Formula	Week				
		0	1	2	3	4
Color	F1	-	-	-	-	-
	F2	-	-	-	-	-
	F3	-	-	-	-	-
Smell	F1	-	-	-	-	-
	F2	-	-	-	-	-
	F3	-	-	-	-	-
Consistency	F1	-	-	-	-	-
	F2	-	-	+	+	+
	F3	-	-	+	+	+

4. CONCLUSION

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%.

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