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Physical Evaluation of Anti-aging and Anti-acne Andaliman (*Zanthoxylum acanthopodium* DC.) Ethanolic Extract Peel Off Gel Mask

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ABSTRACT

This research reports the formulation and physical evaluation of ethanol extract of andaliman fruit in peel off gel mask as anti-aging and anti-acne dosage form. The extraction process of andaliman fruit was conducted by percolation, then the extract was tested for the anti-oxidant activity by 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method and anti-bacterial effect by disc diffusion method. The optimum antibacterial concentration was formulated in peel off gel mask using Carbomer and HPMC as the gelling agent. The formula was evaluated for the physical properties included organoleptic, pH, homogeneity, viscosity, spreadability, adhesiveness and irritation test. The andaliman ethanolic extract showed strong anti-oxidant activity with IC_{50} of 100,76 mcg/ml. The effective concentration of andaliman ethanolic extract as antibacterial was 300 mg/ml with inhibition diameters for Propionibacterium acnes and Staphylococcus aureus were 15.95 ± 0.46 mm and 14.61 ± 0.53 mm, respectively. The evaluation of peel off gel mask showed that both formulae using HPMC and Carbomer had the same color, odor, and homogeneity. The pH and viscosity evaluation showed that HPMC formula had lower pH and viscosity compared to Carbomer formula. Both HPMC and Carbomer formula had no reaction on irritation test. It is concluded that andaliman fruit ethanolic extract can be formulated in peel off gel mask with acceptable physical properties to be used as anti-aging and anti-acne dosage form.

Keywords: Andaliman fruit, Peel off gel mask, Antioxidant, Antibacterial

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INTRODUCTION

Andaliman (*Zanthoxylum acanthopodium* DC.) is a plant of Rutaceae family which origin from the Toba lakeside. Fruits and seeds of andaliman are used by Batak people as traditional spices and natural food preservatives. Andaliman has antibacterial, antioxidant and immunostimulant effects. Andaliman fruits contain alkaloid, flavonoid and triterpenes/steroids that have antibacterial potentiality. The crude extract of andaliman fruits also has physiological activity as potential antioxidant [1,2] and antimicrobe [3].

Acne vulgaris is a disorder that occurred on the skin of the face. It appears when the oil gland in the skin become very active, therefore the skin pore will be clogged by excessive fat that causes blackheads. If the blackheads have a bacterial infection then the inflammation occurs which is known as acne. Sometimes, it has festered and caused pain feeling. Usually, acne treatment in skin clinic uses antibiotic which can lead to resistance and also cause organ damage and immunohipersensitivity [4].

Various ways had been done to prevent or to cure aging effect. The usage of anti-oxidant is one of the methods that mostly conducted to prevent aging and known as anti-aging [5]. The utilization of anti-oxidant in the dosage form which is purposed to the skin of the face is better if it is formulated in the form of topical cosmetic compared to oral. It is supported by various anti-aging dosage form that had been developed such as in the form of mask [6,7].

Mask dosage form that mostly available in the market is in the form of paste or powder, but in the form of gel is still rarely found. The mask in the form of the gel has several advantages which are easy to apply and easy to clean and wash. Moreover, it can be taken or peeled off like an elastic membrane [8].

The physical quality of peel off mask is influenced by the composition of the ingredients used which mainly by the composition of Polyvinyl Alcohol (PVA) and other polymer used. PVA is used commonly as the base of peel off gel mask, but it has several disadvantages such as the produced film layer tend to be rigid and it has low flexibility. The addition of other polymers like Hydroxypropylmethylcellulose (HPMC) or Carbomer can be used to increase the quality peel off gel mask.

Andaliman has potentiality with the antibacterial and anti-oxidant properties which encouraged researcher to formulate the extract in the form of peel off gel mask that effective, stable, and safe in the usage as anti-acne and anti-aging by comparing HPMC and Carbomer as the gelling agent.

MATERIAL AND METHODS

MATERIALS

Andaliman fruits were obtained from Ria-Ria Village, Humbang Hasundutan District, North Sumatera Province, Indonesia. Polyvinyl Alcohol (PVA), carbomer 940, glycerine, HPMC, methyl paraben, propyl paraben, distilled water were purchased from Bratachem. Ethanol, methanol, nutrient agar, and sodium chloride were obtained from Merck. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich. *Propionibacterium acnes* (ATCC 6918) and *Staphylococcus aureus* (ATCC 6358) were obtained from Microbiology Laboratory, Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia. All chemicals were analytical grade and used without further purification.

METHODS

Preparation of extract

The andaliman fruits were dried and ground to obtained dried fine powder. The dried powder of sample was extracted by percolation method using 96% ethanol as the solvent following 4th Edition of Indonesian Pharmacopoeia [9]. The extraction was done in triplicate. The obtained extract later was named andaliman ethanolic extract (AEE).

Antioxidant activity test

The antioxidant activity of AEE was evaluated by Free Radical Scavenger using DPPH as stated in Molyneux [10] with slight modification. Ascorbic acid was used as positive control. The measurement of absorbance was performed by UV-Vis spectrophotometer at 516 nm wavelength.

Antibacterial activity test

The antibacterial activity test was conducted using disc agar method as described in Balouiri et al. [11] on the various concentration of AEE. The AEE was prepared in several concentrations which were 30, 60, 100, 200 and 300 mg/mL. These concentrations were tested on *Propionibacterium acnes* and *Staphylococcus aureus.* The agar was then incubated for 24 h at 35ºC. The inhibition diameters were measured afterward. All of the measurements were done in triplicate.

Preparation of peel off gel mask containing AEE

The effective concentration from the antibacterial test was chosen as the active concentration in the peel off gel mask formula. The peel off gel mask was made with modification of peel off gel standard formula by Rieger [8]. The formula was composed by PVA, glycerine, AEE, methyl paraben, propyl paraben, distilled water and HPMC (F1) or Carbomer 940 (F2). A blank formula was also made with no addition of AEE (F01 and F02).

Evaluation of AEE peel off gel mask

The evaluation of the gel included organoleptic, homogeneity, viscosity, adhesiveness, spreadability, pH, drying time, and irritation tests. All the tests were done by triplicate except for the irritation test.

Organoleptic test

The prepared gel was observed for the color and odor.

Homogeneity test

The homogeneity test was performed by putting the sample on the object glass. The homogeneity was observed under a microscope with 10X magnification as described in Wijayanti [12].

Viscosity test

The viscosity of the gel was measured using Brookfield viscometer as described in Wijayanti [12].

Adhesive ability test

The adhesive ability test was adopted from Wijayanti [12] with slight modification. The sample was put between 2 object glass. Then the upper part of the glass was pressed with 1 kg of the load for 5 min. Afterward, the load was lifted and the object glass was placed in the tester apparatus. The tester apparatus was given 80 g of load and then the time of peel off gel released from the glass was recorded.

Spreadability test

The spreadability of the gel was performed using method stated in Wijayanti [12] with slight modification. The sample was put on the glass sized 20 × 20 cm which placed on the graphical sheet, left for 60 s then the diameter of formed shape was measured. Afterward, the sample was closed with mica plastic and given load until the weight reached 125 g and left for 60 s. Then the diameter of formed shape was measured.

pH test

The gel was diluted into 1% concentration by weighed 1 g of the gel and dissolved in 100 mL of distilled water. The gel pH was measured using pH meter [13].

Drying time test

The drying time test was performed by observing the time needed by the gel to dry, which starting from the gel application on the skin of the face until dry layer was created.

Irritation test

The irritation test was conducted on 12 volunteers by applying the gel on the arm, three times in a day for three days. The symptoms for each gel formula were observed at the third day including redness, itchiness, and inflammation [14].

Data analysis

All of the parametric data were analyzed using Microsoft Excel program. Data were presented as the mean ± standard deviation.

RESULTS

Yield of extraction

The yield of dried sample was 33.08 ± 1.56% and the yield of AEE was 7.094 ± 0.78% of the wet andaliman fruits.

Antioxidant activity of AEE

The ability of AEE to overcome oxidation process of DPPH as free radical with IC_{50} value was used as the parameter to determine the antioxidant activity of AEE. The IC_{50} value was obtained based on the linear regression equation by plotting the concentration of the sample and the DPPH scavenger percentage. The IC_{50} value of AEE and control can be seen in Table 1.

Table 1: The IC_{50} value of AEE compared to control

Sample	IC_{50} value (µg /ml)
AEE	100.76
Ascorbic acid	1.69

The strength of antioxidant activity which categorized based on the IC_{50} value was referred to Mardawati et. al. [15] which tabulated in Table 2. Based on Table 2, it was found that the antioxidant activity of AEE could be categorized in moderate to strong.

Table 2: The strength category of anti-oxidant activity

Category	Concentration (µg / ml)
Very strong	<50
Strong	50-100
Moderate	101-150
Weak	151-200

Antibacterial activity of AEE

The result of antibacterial activity test showed that AEE could inhibit the growth of P*ropionibacterium acnes* dan *Staphylococcus aureus* which was shown by the clear zone on the bacteria growth media. The inhibition diameter was broadened by the increase of AEE concentration as shown in Table 3.

Based on Indonesian Pharmacopoeia [9], a compound has a satisfied inhibitory ability if the inhibition diameter around 14-16 mm. The inhibition concentration of AEE that fulfilled Indonesian Pharmacopoeia was started from the concentration of 200 mg/mL for *Propionibacterium acnes* with inhibition diameter 14.63 ± 0.52 mm, and 300 mg/mL for *Staphylococcus aureus* with inhibition diameter 14.61 ± 0.53 mm. Therefore the concentration of AEE used in the formulation of peel off gel mask was 300 mg/mL. Afterward, it was stated as a percentage in the formula as much as 30%.

The physical evaluation and irritation results of AEE peel off gel mask

The AEE concentration that used in the peel off gel formula was 30%. The physical evaluation of peel off gel was conducted to investigate the physical properties of the different gelling agent used. The physical properties of the formula can be seen in Table 4.

The irritation test showed that both formula of the peel off gel mask were safe in the term of usage by the result that showed no irritation reaction occurred to the volunteers.

Table 3: Antibacterial activity of andaliman ethanolic extract

Type of bacteria	AEE concentration (mg/ml)	Inhibition diameter ± SD (mm)		
Propionibacterium acnes	300	15.95	±	0.46
	200	14.63	±	0.52
	100	13.04	±	0.42
	60	10.22	±	0.35
	30	6.56	±	0.55
Staphylococcus aureus	300	14.61	±	0.53
	200	13.14	±	0.68
	100	12.85	±	0.41
	60	9.80	±	0.66
	30	6.26	±	0.68

Table 4: The physical properties of peel off gel using different gelling agent

Evaluation	F01	F1	F02	F2
Organoleptic	**Colourless**	**Yellowish brown with specific odor**	**Colourless**	**Yellowish brown with specific odor**
Homogeneity	**Homogen**	**Homogen**	**Homogen**	**Homogen**
Viscosity (cps)	28.3 ± 0.5	6.32 ± 0.207	27.8 ± 1.283	6.26 ± 0.161
Irritation	**Non irritate**	**Non irritate**	**Non irritate**	**Non irritate**
Drying time (minute)	26.3 ± 0.577	25.3 ± 0.577	27.7 ± 0.577	28.7 ± 0.577
Spreadability (cm^2)	25.54 ± 0.46	23.97 ± 0.84	26.55 ± 0.61	25.75 ± 0.793
Adhesive ability (second)	7.33 ± 0.577	8.33 ± 0.577	5.33 ± 0.577	7.33 ± 0.577
pH	6.03 ± 0.208	4.3 ± 0.462	6.7 ± 0.06	4.2 ± 0.115

F01 = Blank of Carbomer 940 formula; F1 = Carbomer 940 formula with 30% of AEE; F02 = Blank of HPMC formula; F2 = HPMC formula with 30% of AEE

DISCUSSION

Antioxidant activity is a parameter of the extract if we want to use the extract to be formulated in a dosage form with anti-aging effect. Therefore, it is important to know the strength of the antioxidant activity before we further do the formulation. In this study, it was found that andaliman ethanolic extract had moderate to strong antioxidant activity. With this category, AEE can be suggested to be formulated as an anti-aging dosage form. The antioxidant activity of AEE was not equivalent or below than the positive control which was the ascorbic acid with very strong antioxidant activity. It could be caused that AEE was not a pure compound like ascorbic acid, but it still contained other compounds which probably had no antioxidant activity. Andaliman fruits have antioxidant activity due to the flavonoid compound. Flavonoid compound which contains hydroxyl group donates hydrogen to the free radical. The compound can neutralize the free radical by giving electrons, therefore atom with the unpaired electron can get electron and no longer become free radical.

The antibacterial activity of andaliman ethanolic extract at the concentration of 300 mg/mL gave inhibition zone diameter greater on *Propionibacterium acne* bacteria compared to *Staphylococcus aureus* bacteria. Moreover, AEE at the concentration of 200 mg/mL had inhibited the growth of *Propionibacterium acne* with satisfied inhibition zone diameter. It showed that the *Propionibacterium acne* was more susceptible to AEE compared to *Staphylococcus aureus*.

The physical evaluation of the peel off gel showed that both formula which using HPMC and Carbomer 940 gave the same color and specific odor of andaliman, also demonstrated a homogeny composition of formula. At the pH evaluation, it is shown that blank formula contained HPMC had lower pH compared to the blank formula that using Carbomer 940. Furthermore with the addition of andaliman ethanolic extract to the blank formula caused the pH of the gel decreased due to the acidic pH of andaliman ethanolic extract. However, the peel off gel still can be safely used because it was in the range of 4.5-7 of physiologic skin pH [16]. The formula that using HPMC showed lower viscosity value compared to the formula using Carbomer 940. Viscosity affects the spreadability parameter and the release of the active compound from the peel off mask. The peel off gel mask that has optimum viscosity can retain the dispersion of active compound in the mask base and increase the concentration of the peel off mask.

Spreadability test is an evaluation to determine the gel ability to spread. The spread area of 25-49 cm^2 showing semisolid texture

that convenience in use [17]. Spreadability of the peel off gel that using Carbomer 940 is lower compared to the formula that using HPMC. The low spreadability was related to the high viscosity of the of the gel if the given pressure was applied equally in each gel formula.

The adhesiveness test was conducted to investigate the gel ability to attach on the skin. If the formula attached extensively, then the therapeutic effect given by the product will be prolonged due to the long contact time on the skin [18]. There is no specific requirement regarding the adhesiveness of the semisolid product. However, the adhesiveness of the semisolid product is better more than 1 s [19]. From the evaluation test, it was gained that the adhesive ability of mask formula using Carbomer 940 is longer compared to the mask formula using HPMC.

The drying time for both formulae is tended to be slow due to the high amount of water and the small amount of ethanol used in the formula. The usage of ethanol in the peel off gel mask formula acts to increase the drying time of the product. When the ethanol evaporated, it will give influence on the drying time of the product [20].

CONCLUSION

Andaliman fruit ethanolic extract can be formulated in peel off gel mask with acceptable physical properties to be used as anti-aging and anti-acne dosage form.

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