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FORMULATION AND ANTIBACTERIAL ACTIVITY TEST OF ANTI-ACNE GEL FROM ETHANOL EXTRACT OF SENDUDUK LEAVES (*Melastoma malabathricum. L*)

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ABSTRACT

Treatment of inflamed acne is done by administering antibiotics and chemicals, which can usually cause side effects such as skin irritation and resistance. To avoid this, it can be replaced by doing traditional treatment using natural ingredients. Senduduk plant (Melastoma malabathricum L.) is one of the natural ingredients that can be used to treat acne because it contains secondary metabolites of flavonoids, phenolics, tannins and terpenoids that are very potential as antibacterials. This study aims to determine the ethanol extract of senduduk leaves can be formulated in a physically stable gel dosage form and determine the antibacterial activity against Propionibacterium acne and Staphylococcus aureus bacteria. This study used an experimental method by making 3 types of formulas containing ethanol extract of senduduk leaves (Melastoma malabathricum L.) 1.5%, 2.5% and 3.5%. Furthermore, the evaluation of the gel preparation included organoleptic test, homogeneity test, pH test, spreadability test and adhesion test. Antibacterial activity testing was carried out by the

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pitting diffusion method using Klindamycin as a positive control. Based on the results of the gel preparation evaluation, it shows that the anti-acne gel preparation has physical quality that meets the standards. Antibacterial activity test of senduduk leaf ethanol extract gel against S.aureus bacteria at concentrations of 1.5%, 2.5% and 3.5% had inhibition zone diameters of 20.3 mm, 21.4 mm, and 24.2 mm, respectively, while against P. acnes bacteria it was 21.6 mm, 25.3 mm, and 26.5 mm. It can be concluded that the anti-acne gel of senduduk leaf ethanol extract has antibacterial activity against S. aureus and P. acnes bacteria and can be made into an anti-acne gel dosage form.

INTRODUCTION

A common disorder on facial skin is acne. The prevalence of acne patients in Indonesia ranges from 80 - 85% in adolescents with a peak incidence of 15 - 18 years of age, 12% in women aged > 25 years and 3% in 35 - 44 years of age. Acne or Acne vulgaris is a chronic inflammatory skin disease with a complex pathogenesis involving several components. The main factors involved in acne formation are increased sebum production, keratinocyte decay, bacterial growth and inflammation. Microorganisms such as *Propionibacterium acne*, *Staphylococcus epidermidis* and *Staphylococcus aureus* play a role in the pathogenesis of this disease (Madelina & Sulistiyaningsih, 2018).

Treatment of inflamed acne is usually done by administering antibiotics such as tetracycline, erythromycin, doxycycline and clindamycin. In addition, acne treatment can also use benzoyl peroxide, azelic acid and retinoids. However, these drugs have side effects such as irritation, while long-term use of antibiotics can cause resistance and organ damage. The incidence of antibiotic resistance in acne treatment has recently increased in various countries, resulting in the ineffectiveness of antibiotics commonly used for acne treatment. Therefore, other alternatives are sought in treating acne, namely by using ingredients from nature, with the hope of minimizing side effects on patients (Madelina & Sulistiyaningsih, 2018). This condition encourages the development of natural antibacterial research from plants in Indonesia including senduduk leaves (*Melastoma malabathricum* L.).

Empirically, senduduk leaves are used for the treatment of boils and external wounds by mashing until smooth then the results of the mash are attached to the boils or external wounds. Based on research by Kusumowati et al. (2014) Senduduk leaves contain secondary metabolite compounds namely flavonoids, saponins and tannins that have antibacterial activity. The antibacterial activity of ethanol extract of senduduk leaves at a concentration of 40 mg/ml with the agar diffusion method has a diameter of inhibition against the growth of *Staphylococcus aureus* bacteria of 10.40mm (Rotua, 2014). In addition, according to research by Marlina (2020) ethanol extract of senduduk leaves with a concentration of 4% can inhibit the growth of *Staphylococcus aureus* bacteria by 16.5 mm which is categorized as strong. Based on this, the researcher is interested in further research and proving the antibacterial inhibition activity of senduduk leaves (*Melastoma malabathricum* L.) on *S. aureus* and *P. acnes* bacteria using variations in extract concentrations of 1.5%, 2.5% and 3.5%.

Based on the explanation in the previous paragraph, it shows that the ethanol extract of senduduk leaves has antibacterial activity. To maximize the potential of senduduk leaves, the

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researchers formulated senduduk leaves into an anti-acne gel preparation. Gel is a topical preparation with a semisolid form made from small organic particles or large molecules consisting of a suspension and can penetrate by a liquid ((Depkes) Departemen Kesehatan Indonesia, 1995). Gel with good consistency will increase therapeutic effectiveness and be able to provide a sense of comfort when applied to the skin. Gels are composed of several components consisting of active substances, humectants, preservatives, and gelling agents (Lachman et al., 1994).

RESEARCH METHOD

The type of research carried out was experimental research by making 3 types of formula containing ethanol extract of senduduk leaves (*Melastoma malabatricum* L.) with concentrations of 1.5%, 2.5% and 3.5% with carbopol 940 as the gel base.

Tools

The tools used in this research are mortar, stamfer, measuring cup, vaporizer cup, test tube, stirring rod, dropper pipette, erlemeyer (pyrex®), test tube rack, spray bottle, beaker glass (pyrex®), spatel, parchment paper, perti cup, ose needle, porcelain crucible, filter paper, waterbath, paper disc, incubation, autoclave, gas pack, maceration bottle, rotary evaporator (sigma®), funnel, analytical balance (adam®), oven (memmert®), watch glass, tweezers.

Material

The materials used in this study were senduduk leaves, 96% ethanol, H2SO4 p.a, FeCl2 5%, chloroform, anhydrous acetic acid, sulfuric acid, ammonia, mayer reagent, distilled water, sodium agar (NA), *Propionibacterium acne*, *Staphylococcus aureus*, NaCl 0.9%, Mc Farland, thioglycollate media.

Preparation of Senduduk Leaves Samples

Senduduk Leaves (*Melastoma malabathricum* L.) will be taken in the Lubuk Buaya district, Koto Tangah Subdistrict, Padang city, and then identified samples from the plant senduduk at the Andalas University Herbarium (UNAND). Furthermore, the manufacture of simplicia is carried out by cleaning the senduduk leaf sample with running water, then dried without direct sunlight for 5 days and then sample is mashed.

Preparation of Ethanol Extract of Senduduk Leaf (*Melastoma malabathricum* L.)

Extraction begins with collecting 2 grams of fresh senduduk leaves which are then sorted and air dried, then powdered using a blender. The powdered simplisia was macerated using 96% ethanol solvent which was then covered and left for 72 hours at room temperature protected from light while occasionally stirring, then filtered using filter paper which produced filtrate and dregs. The dregs were then added again with enough 96% ethanol,

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closed and left. The extract obtained is collected and then evaporated with a rotary evaporator until a thick extract is obtained. Furthermore, the evaluation of extracts includes, examination of yield and phytochemical tests. Phytochemical examination of extracts includes examination of phenolics, flavonoids, saponins, steroids, terpenoids, and alkaloids.

Gel Formulation of Ethanol Extract of Senduduk Leaf (*Melastoma malabathricum* L.)

Anti-acne gel was prepared in 3 types of formulas containing ethanol extract of senduduk leaves (Melastoma malabathricum L.) 1.5%, 2.5% and 3.5% with carbopol 940 as gel base. Then continued with the evaluation of anti-acne gel preparations including organoleptic testing, homogeneity, pH, adhesion and spreadability of anti-acne gel preparations of senduduk leaf ethanol extract. (*Melastoma malabathricum L*.).

Table 1. Gel Formula of Ethanol Extract Senduduk (*Melastoma malabathricum L*.) Leaves

Ingredients	**Formula (%)**			
	FI	**F2**	**F3**	**Control**
Ethanol Extract Senduduk Leaves	1,5	2,5	3,5	-
Carbopol 940	1,5	1,5	1,5	1,5
Propylene glycol	5	5	5	5
Metyl paraben	0,2	0,2	0,2	0,2
Trietanolamine	2	2	2	2
Aquadest ad	100	100	100	100

Gel Preparation Evaluation

Organoleptical Test

Organoleptic test was conducted to see the physical appearance of the gel preparation of ethanol extract of senduduk leaves by visually observing the shape, color, and smell that had been formulated from the gel preparation using the five senses.

Homogeneity Test

Homogeneity test of senduduk leaf ethanol extract gel was conducted by applying 0.5 g of gel each on a glass slide to observe its homogeneity. The gel serum is declared homogeneous if there are no coarse grains on the glass surface (Direktorat Jenderal Kefarmasian dan Alat Kesehatan, 2020).

pH test

The pH test was conducted using a pH meter. Previously, the electrode was calibrated first with pH 4 and ph 7 buffer, then rinsed using distilled water. Then the electrode was dipped 3 cm deep into each formulation (F0, F1, F2, F3). Each formula was weighed 1g and

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dissolved in 10 ml of distilled water. View and record the pH value listed on the portable pH meter. The pH of the gel preparation must be in accordance with the ph of the skin, which is in the range of 4.5-6.5.

Spreadability Test

Weighed 0.5 g of senduduk leaf ethanol extract gel on a watch glass and overwritten with another watch glass and left for 1 minute, then given a load (50 grams, 100 grams, 150 grams, 200 grams and 250 grams) each load was allowed to stand for 1 minute, then measured the diameter.

Adhesiveness Test

Weighed 0.5 grams of senduduk leaf gel placed on a glass object covered by another glass object, then given a load of 250 grams and allowed to stand for 5 minutes, and count the time until the two glass objects are released.

Antibacterial Activity Test

Testing the antibacterial activity of the gel preparation of ethanol extract of senduduk leaves was carried out using the pitting diffusion method. By making wells on NA media, then each preparation is inserted into the wells. In testing the antibacterial activity of this preparation, a positive control was used, namely clindamycin (medi-clin). After each formula is inserted into the wells, then incubate for 1x24 hours. Next, the inhibition zone was observed by measuring the clear zone around the disc paper using a caliper.

Processing and Analysis Data

Data collection for the gel formulation of the ethanol extract of seduduk leaves (Melastoma malabathricum) is carried out by directly observing and measuring the organoleptic, homogeneity, pH, adhesive and spreadability resulting from the gel preparation during storage. The data obtained was recapitulated using analytical descriptive methods. Meanwhile, for the antibacterial activity test, the data processing and analysis method is by measuring the diameter of the bacterial inhibition zone Staphylococcus aureus and Propionibacterium acne which was compared with the results of the positive control.

RESULT AND DISCUSSION

Sample identification

Senduduk plants were taken in the Lubuk Buaya area, Koto Tangah District, Padang City, then identified at the Herbarium of Andalas University Padang (UNAND). Based on the results of plant identification conducted at the herbarium of Andalas University Padang (UNAND) stated that the plants used in this study are true senduduk plants (Melastoma malabathricum L.) Melastomacea family.

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Yield Determination

The yield of ethanol extract of senduduk leaves will be calculated by comparing the weight of the thick extract obtained with the weight of the initial sample. Then the yield is calculated based on weight, using the following equation: (Departemen Kesehatan RI, 2008).

$$\%\ Yield = \frac{Weight\ of\ extract\ obtained}{Weight\ of\ initial\ sample} \times 100\%$$

Based on the results of determining the yield of senduduk leaf extract, it was found that from 250g of senduduk leaf simplisia, 36.0261g of extract was obtained with a percentage yield of 14.42%, determining the yield value aims to determine the weight of the sample that has been extracted. The higher the yield value produced indicates a better process. Particle size will also have an impact on good solvent concentration on cell material which in turn will increase the rate of mass transfer in tissues and facilitate the transfer of active compounds from cells to solvents. This is what causes the substance to be dissolved in the solvent to be higher so that the yield value will be greater.

Phytochemical Test

Phytochemical testing of extracts includes examination of phenolics, flavonoids, saponins, steroids, terpenoids, and alkaloids. The purpose of this phytochemical examination is to see the content of secondary metabolites present in the extract using color reagents.

Table 2. Phytochemical Screening Test Results

Testing	Result	Reactions that occur
Flavonoid	+	Formed red color
Fenolik	+	Formed blue color
Terpenoid	+	Formed red color
Saponin	-	No permanent foam build-up
Alkaloid	-	No change occurred
Steroid	-	No change occurred

Based on the results of the phytochemical test that has been carried out, it shows that the ethanol extract of senduduk leaves contains flavonoid, phenolic, and terpenoid compounds that are efficacious as antibacterial and anti-inflammatory. It means that in this study the ethanol extract of senduduk leaves can be made into an anti-acne gel preparation.

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Gel Preparation Evaluation of Ethanol Extract of Senduduk Leaf (*Melastoma malabathricum* L.)

Organoleptical Test

Organoleptic observations were made by observing the gel produced to see the physical appearance of the ethanol extract of senduduk leaves by observing the shape, color and smell of the extract. Organoleptic examination of the gel is intended for simple and objective initial recognition using the five senses. The results of the organoleptic examination showed that the gel containing ethanol extract of senduduk leaves was thick, brown in color, and smelled distinctive.

Table 3. Results of Organoleptical Testing of Gel

The Formula	Organoleptics	Before Storage	After Storage
F0	Shape	Gel	Gel
	Color	Transparent	Transparent
	Smell	Odorless	Odorless
	Taste	Cold	Cold
F1	Shape	Gel	Gel
	Color	Brown	Brown
	Smell	Distinctive extract	Distinctive extract
	Taste	Cold	Cold
F2	Shape	Gel	Gel
	Color	Brown	Brown
	Smell	Distinctive extract	Distinctive extract
	Taste	Cold	Cold
F3	Shape	Gel	Gel
	Color	Brown	Brown
	Smell	Distinctive extract	Distinctive extract
	Taste	Cold	Cold

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pH Test

The pH test aims to determine the pH of the preparation whether it is in accordance with the pH of the skin, which ranges from 4.5-6.5. The pH results of each formula still meet the skin pH requirements of 4.5-6.5. The degree of acidity or pH is an important parameter in cosmetic products because if the pH is very high it can cause scaly skin and if the pH is too low it can irritate the skin (Tranggono, R.I. & Latifah, 2007). Ideally the pH of topical preparations is in accordance with the pH of the skin which is 4.5 - 6.5, so the pH produced in this study meets the requirements of both the pH in the control formula and the pH in the formula containing senduduk leaf extract.

Table 4. Results of pH Test

The Formula	The Treatments		Average
	Before Storage	Before Storage	
F0	6,50	6,46	6,48
F1	6,43	6,43	6,43
F2	6,38	6,39	6,39
F3	6,34	6,38	6,34

Homogeneity Test

The homogeneity check aims to see the presence or absence of coarse particles in the preparation and to see the mixing of active ingredients and formulated additives. The results of the homogeneity check showed that F0, F1, F2 and F3 were homogeneous which was characterized by the absence of coarse particles. In the research of (Rahmawati, A.D. et al., 2018) on the test of gel properties of ethanol extract of brotowali stem (Tinospora crispa L.) obtained homogeneous preparation results characterized by evenly distributed preparation color and the absence of particles or coarse grains when observed both using a microscope and visually. This is in accordance with what (Idson & Lazarus, 1994) said that stable gel preparations show good homogeneity during storage. So it can be concluded that anti-acne gel preparations containing ethanol extract of senduduk leaves have homogeneity preparations that meet the requirements of being stable during storage tests at room temperature.

Table 5. Result of Homogeneity Test

The Formula	The Treatment	
	Before Storage	After Storage
F0	Homogen	Homogen
F1	Homogen	Homogen
F2	Homogen	Homogen
F3	Homogen	Homogen

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Spreadability Test

Spreadability testing aims to determine the ability of the gel to spread when given a certain amount of force, so that the ability of the preparation to spread on the skin is known. Spreadability is a characteristic used to take into account ease of use, dispensing from containers and affects consumer acceptance. A preparation is good and preferred if it can spread easily on the skin and is comfortable to use. The results on the observation of each formula gave results ranging from 3-5 cm. So it has a spreadability that is in accordance with the range of good spreadability, which is between 3-5 cm for semi-solid topical preparations, if the diameter of the spreadability is less than 5 cm then the gel is classified as a semi-rigid preparation (semistiff), but if the diameter of the spreadability is between 5-7 cm then the gel is classified as a semi-liquid preparation (semifluid) (Garg et al., 2002).

Tabel 6. Result os Spreadability Test

The Formula	The Treatment		Average
	Before Storage	After Storage	
F0	3,9	4,6	4,25
F1	4,2	5,3	4,75
F2	4,5	5,4	4,95
F3	4,8	5,8	5,3

Adhesiveness Test

Adhesion testing is done to determine the ability of the gel to adhere to the skin when used. A good gel has high adhesion, the higher the adhesion the better for the gel preparation. The results on the observation of each formula have an adhesion that is not too high but is in the range of adhesion that meets the requirements of cosmetic preparations, which is more than 1 second (Irianto et al., 2020).

Table 7. Result of Adhesiveness Test

The Formula	The Treatmen (Second)		Average
	Before Storage	After Storage	
F0	1,15	1,04	1,10
F1	1,12	0,97	1,05
F2	1,11	0,99	1,05
F3	1,12	0,99	1,06

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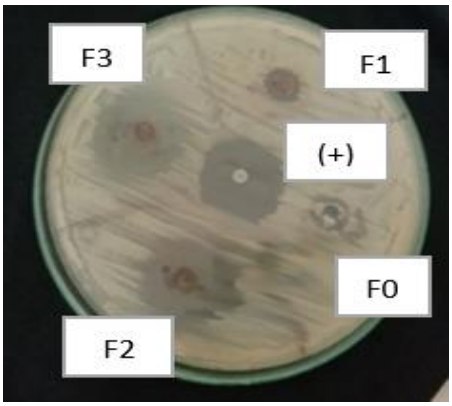
Antibacterial Actitivity Test

This antibacterial activity test is to determine the amount of release of active substances by measuring the diameter of the inhibition zone of bacterial growth.



Picture 1. Antibacterial activity test of senduduk leaf ethanol extract gel against *Staphylococcus aureus* bacteria

Picture 1 shows that senduduk leaf ethanol extract gel with concentrations of 1.5%, 2.5%, and 3.5% has activity against *Staphylococcus aureus* bacteria. Based on the table below, it can be seen from the results of measuring the average diameter of the clear zone obtained, the antibacterial power against *S. aureus* test bacteria from F0 is 0 mm, F1 20.27 mm, F2 21.40 mm, F3 24.23 mm, and positive control 40.27 mm. So it is included in the very strong category. According to the Clinical and Laboratory Standart Institute (CLSI) (Clinical Laboratory Standart Institute, n.d.), the inhibition response is weak when the diameter of the antibacterial inhibition zone is ≤ 14 mm, the inhibition response is moderate when the diameter of the antibacterial inhibition zone is 15-18 mm, the inhibition response is strong when the diameter of the antibacterial inhibition zone is ≥ 19 mm.

Table 8. Antibacterial Activity Check Result of Gel (Staphylococcus aureus)

The Formula	Diameter of the clear zone in repetition -			Average	Categories
	1	2	3		
F0 (0%)	0	0	0	0	There is no activity
F1 (1,5%)	20,3	20,4	20,1	20,27	Highly potent
F2 (2,5%)	21,4	21,3	21,5	21,40	Highly potent
F3 (3,5%)	24,2	24,1	24,4	24,23	Highly potent
Kontrol +	40,1	40,2	40,5	40,27	Highly potent

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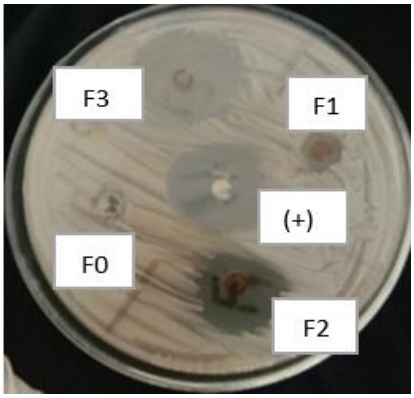

Picture 2. Antibacterial activity test of ethanol extract of senduduk leaves against *Propionibacterium acne* bacteria

The picture above shows that the gel of ethanol extract of senduduk leaves with a concentration of 1.5%, 2.5%, and 3.5% has activity against Propionibacterium acne bacteria. Based on the table below, it can be seen from the results of measuring the average diameter of the clear zone obtained, the antibacterial power against Propionibacterium acnes test bacteria from F0 is 0 mm, F1 21.6 mm, F2 25.3 mm, F3 26.53 mm, and positive control 40.3 mm. So it is included in the very strong category. According to the Clinical and Laboratory Standart Institute (CLSI) (Clinical Laboratory Standart Institute, n.d.), weak inhibition response when the diameter of the antibacterial inhibition zone ≤ 14 mm, moderate inhibition response when the diameter of the antibacterial inhibition zone is 15-18 mm, strong inhibition response when the diameter of the antibacterial inhibition zone ≥ 19 mm.

Table 9. Antibacterial Activity Check Result of Gel (Propionibacterium acnes)

The Formula	Diameter of the clear zone in repetition -			Average	Categories
	1	2	3		
F0 (0%)	0	0	0	0	There is activity
F1 (1,5%)	21,5	21,6	21,8	21,6	Highly potent
F2 (2,5%)	25,4	25,3	25,1	25,3	Highly potent
F3 (3,5%)	26,3	26,4	26,7	26,5	Highly potent
Kontrol +	40,1	40,2	40,5	40,3	Highly potent

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CONCLUSION

The anti-acne gel of ethanol extract of senduduk leaves has antibacterial activity against *Staphylococcus aureus* and *Propionibacterium acnes* bacteria with a very strong inhibition category and can be made into an anti-acne gel dosage form.

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