Utilization of Gaharu Leaf Ethanol Extract as an Anti-Acne Herbal Patch

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Abstract. Acne is a skin disease characterized by the presence of lumps on the surface of the facial skin caused by the presence of bacteria. Gaharu leaves have secondary metabolites, such as flavonoids, which can be used as antibacteria. This research aims to formulate acne patches from gaharu leaves ethanol extract with chitosan polymer and to examine physical characteristics of the acne patch preparation. This acne patch preparation is made by heating at a temperature of 50°C to generate a rigid polymer. Acne patch preparation is made in the form of polymer with variantion of polymer matrix and gaharu leaves extract ratios, namely F1′ (500 mg: 0), F1 (500 mg: 50 mg), F2′ (600 mg: 0), F2 (600 mg: 60 mg), F3′ (700 mg: 0), F3 (700 mg: 70 mg). All formulas generate polymer with good physical properties, including 0.47-0.796 mm thickness, pH, folding resistance >300 times, moisture content <10%, vapor transmission 0.00001-0.0059, moisture uptake 0.981-1.494%, and uniform patch weight uniformity.

Keywords : Anti-Acne, Gaharu Leaves, Herbal Patch

1. Introduction

Acne is a skin disease characterized by the appearance of lumps on the surface of the facial skin due to the presence of bacteria [1]. According to global research, acne resulted in 4.96 million Disability Adjusted Life Years (DALYs) in 2019 [2]. Based on this data, 3.52 million DALYs occurred in the ages of 15-49 years. Acne generally appears during adolescence and may last until adulthood. Apart from that, acne can also cause serious physical and psychological problems that can reduce self-confidence [3]. Several factors that can contribute to the pathophysiology of acne are hyper keratinization of hair follicles, elevated sebum production, inflammation, and the colonization activity of *Propionibacterium acnes (P. acnes)* and *Staphylococcus aureus (S. aureus)* [4]. Currently, acne treatment is starting to use natural ingredients as an alternative treatment method, following the trend of 'Back to Nature'. Gaharu leaves are a natural ingredient that has the potential to have anti-acne effects. The content of gaharu leaves extract, especially flavonoids, can act as an antibacterial by inhibition of DNA and RNA synthesis and disruption of bacterial cell membranes. According to a previous study, gaharu leaf ethanol extract 10% exhibited antibacterial activity against

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S. epidermis, S. aureus and *P. acnes* with inhibition zone of 9.90 ± 0.43 mm, 8.33 ± 1.50 mm and 10.73 ± 0.98 mm [5]. In this study, we optimize the formulation of gaharu leaf extract into a pharmaceutical dosage form in the form of a patch that has anti-acne activity. The research aims to provide alternative choice of anti acne preparation, with minimal side effects by using natural-based active agent originated from Sumatera. Conventional anti acne oral antibiotics, such as clindamycin, may cause several side effect, including stomach ache and diarrhea. Besides, oral and topical antibiotics may induce hypersensitivity reaction.

2. Materials and Method

This study includes optimization of anti-acne patches formulation and evaluation of anti-acne patches physical properties.

2.1 Instrument

The instruments used in this study are digital scales, magnetic stirrers, beaker glasses, measuring glass, erlenmeyer, homogenizer, desiccator, oven, hot plate, spray bottles, micro spatula, labels, stir bars, baskets, containers, heat-resistant mold, test tube, test tube rack, evaporating dish, vortex, UV-Vis spectrophotometry, UV viewing cabinet, measuring flask, jar, micropipette and pH meter.

2.2 Material

The materials used in this study include 70% ethanol extract of gaharu leaves, concentrated HCl, amyl alcohol, Mg ribbon, getalin, $FeCl_3$, 96% Ethanol, ammonia, butanol, chitosan, span 20, glycerol, BHT, 2% acetic acid, distilled water, quercetin, $AlCl_3$, potassium acetate, silica gel, 0.9% physiological NaCl, cotton stopper, chloroform, anhydrous calcium chloride PA, potassium chloride, Dragendorff's reagent, Mayer's reagent, Wagner's reagent, Liberman Burchart's reagent, aluminum chloride, TLC sheets, instrument washer soap, parchment paper, paper filter, plastic wrap, gloves, heat-resistant plastic, and rubber bands.

2.3 Extraction

330 grams of gaharu leaves were weighed and put into a beaker, and 70% ethanol was added with a ratio of ingredient to solvent of 1:10. Ultrasonic Assisted Extraction was used as the extraction method and was carried out for 30 minutes at 45°C. The filtrate was filtered using filter paper and evaporated using a rotary evaporator at 40°C and 700 rpm [6].

2.4 Phytochemical Screening

Phytochemical screening is a qualitative chemical examination of biologically active compounds present within extracts, organic compounds. Therefore, screening is primarily intended for groups of organic compounds such as alkaloids, tannins, flavonoids, saponins and steroids/terpenoids [7].

2.5 Identification with Thin Layer chromatography

Thin layer chromatography was performed using standard methods [8]. Silica Gel 60 F254 (Merck) with a size of 2 cm x 5 cm as a stationary phase. Sample was load was in 0.7 cm from the bottom. Small quantities of extract and quercetin standard were dissolved in 70% ethanol. Varying concentrations of mobile phases was used to select the optimum ratio for separation of flavonoid. The optimum mobile phase ratio is n-butanol: acetic acid: water (3:2:5). Upon the drying of the plates, visualization was performed under UV at 254 nm in UV TLC viewer. Plates was sprayed with $AlCl_3$, and the R_f value of the different spots that were observed was calculated.

2.6 Fabrication of Anti-Acne Herbal Patches

All ingredients listed in Table 1 were weighed according to their respective formulations. Chitosan polymer was dissolved in 2% acetic acid. A mixture of ethanol extract and BHT was made, which was dissolved in Span 20 for 30 minutes [9], [10]. The mixture was added with Chitosan polymer and mixed using a magnetic stirrer at 350 rpm, then glycerol was added, which functions as a plasticizer, and the remaining distilled water was added to the solution until the mixture was homogenous again. The mixture was put into a mold and oven for 12 hours at 50 °C, then put in a desiccator for 20 hours [10]. Once dry, form a circle using sterilized tips.

Table 1. Formulation of Anti-Acne Herbal Patches

Ingredients	F1’	F1	F2’	F2	F3’	F3
Extract of gaharu leaves	-	50 mg	-	60 mg	-	70 mg
Chitosan	500 mg	500 mg	600 mg	600 mg	700 mg	700 mg
Span 20	0,5 ml	0,5 ml	0,5 ml	0,5 ml	0,5 ml	0,5 ml
Glycerol	3 ml	3 ml	3 ml	3 ml	3 ml	3 ml
BHT	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg
Distilled water	ad 30 mL	ad 30 mL	ad 30 mL	ad 30 mL	ad 30 mL	ad 30 mL

2.7 Physical Characterization of Anti-Acne Herbal Patches

Organoleptic. Organoleptic observations carried out included color, odor and patch surface [11].

Thickness. Patch thickness was measured using a micrometer screw gauge at three different places, and the mean value was calculated [12].

Folding Resistance (Elasticity). The folding resistance is the number of creases required to break a polymer patch. This test not only describes the strength of patches prepared using different polymers, but also examines how efficient the polymer provides flexibility. This test involves repeated folding of the patch at the same area of the patch until it breaks. The number of patches that can be folded patch at the same area of the patch without breaking/cracking gives a folding resistance value of the patch [13].

Uniformity of Weight. The patches were subjected to a weight variation test by weighing all the patches on a digital weighing machine. The determinations were carried out in triplicate for each formulation. Average weight and standard deviation values were then calculated [14].

Percentage Moisture Content. All patch formulas were weighed and stored in a desiccator at room temperature (25°C) for 24 hours, and the percentage of moisture content was calculated using the formula:

$$Percentage\ moisture\ content\ = \frac{Initial\ weight\ -\ Final\ weight}{Final\ weight}\ x\ 100\%$$

A good moisture content should be less than 10% [15].

Water Vapor Transmission Rate (WVTR). Water vapor transmission rate (WVTR) is defined as the quantity of moisture transmitted through unit area of film in unit time. Glass vials of equal volume and diameter were used as transmission cells. The cells were washed properly and dried in the oven. Then, about 1 g of anhydrous fused calcium chloride was placed in each vial, and the patch was fixed over the brim of the vial with the help of an adhesive tape. These vials were then weighed and placed in desiccators containing saturated solution of potassium chloride to maintain 84% relative humidity. These cells were removed from the desiccators and weighed after 24h. The water vapor transmission rate was determined as follows: W: V: T: ¼ W L=S, where W is the weight of water vapors transmitted, L is the thickness of patch and S is the surface area exposed in square centimeters [14].

pH. Patch samples of various formulas were measured using a pH meter. Previously, the patch was dissolved in distilled water. The required pH range that is not skin irritative is 4.5-7 [16]. The patch was taken and dissolved in 5 mL of distilled water, then tested using a digital pH meter [17].

Percentage Moisture Uptake. Patch samples were taken from each formulation and weighed as initial weight then placed in a desiccator at room temperature for 24 hours. After that, it is weighed again as the final weight and the percentage of moisture uptake is calculated. Moisture uptake can be calculated using the equation: [18]

$$Percentage\ Moisture\ Uptake\ = \frac{(final\ weight - initial\ weight)}{initial\ weight}\ x\ 100\%$$

2.8 Statistical analysis

Each experiment was repeated at least three times. The results are expressed as mean±SD. One way ANOVA was used to test the statistical significance of differences among groups. Linear regression is performed to evaluate correlation.

3. Results and Discussion

3.1 Determination of Gaharu Leaves

The gaharu plants used in the study were determined at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung. The results of the determination stated that the plant species was *Aquilaria malaccensis* Lam.

3.2 Extraction

Extraction was carried out using the UAE method with 760 grams of dry powder sample. The extract yield is calculated using the following formula:

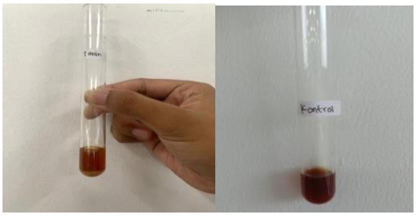
$$\%\, Yield\ of\ gaharu\ leaf\ extract = \frac{Final\ Weight}{Initial\ Weight}\ x\ 100\%$$

The yield of gaharu leaf ethanol extract was 14.1847%. Based on a previous study, 70% ethanol extract of gaharu leaves produced 3.57% yield [19]. This difference in yield can be caused by several factors, such as extraction method, temperature and length of extraction time [20]. In Hasanah’s (2020) study, The use of UAE produces a greater yield and a faster extraction process compared to the maceration method [21].

3.3 Phytochemical Screening

The result of phytochemical screening of a 70% ethanol extract of gaharu leaves in table 2 showed the presence of metabolite compounds such as tannins, flavonoids, and saponins. Based on a previous study, 70% ethanol extract contains flavonoids, tannins and triterpenoids [5]. Differences in metabolite compounds are influenced by several factors such as planting location, soil conditions, temperature, rainfall, humidity, altitude location of growth, processing method and use of different tools [22], [23].

Table 2. Result of Phytochemical Screening

Secondary metabolites	Interpretation of Results	Extract Results
Alkaloids	-	
Tannins	+	
Flavonoids	+	
Saponins	+	
Steroid and Terpenoids	-	

Explanation:
(+) = there are compounds
(-) = no compounds

3.4 Identification with Thin Layer Chromatography

The TLC results show spots with both the extract and quercetin have the same color change to yellow, which indicates flavonoids [24], and an R_f value of 0.8 that meets the good TLC requirements. R_f value meets the TLC requirements, which is within the range of 0.2 - 0.8 [25]. TLC results can be seen in figure 1.

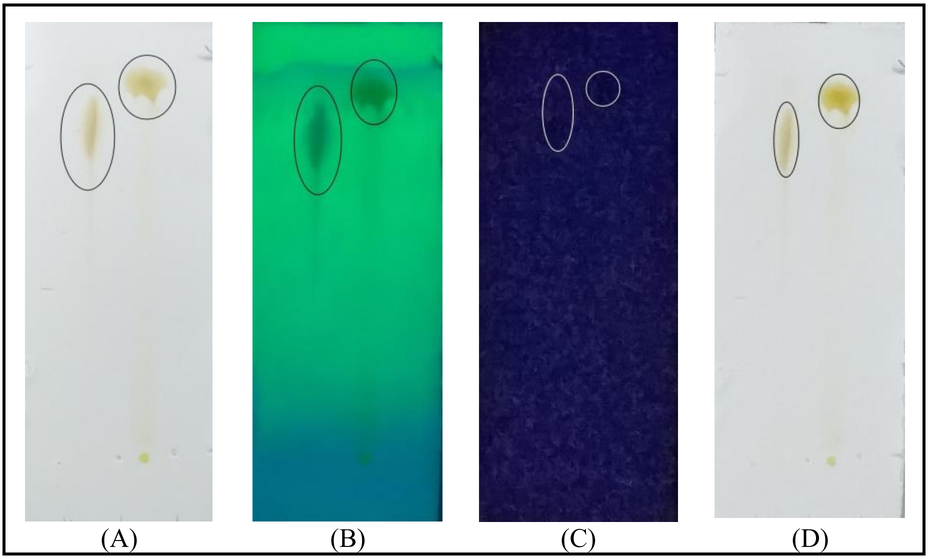


Figure 1. The Result of Thin Layer Chromatography of Gaharu Leaves Extract
Explanation:
Left : Extract of gaharu leaves
Right : Quercetin
A: TLC of gaharu leaves extract (spot in black circle)
B: TLC of gaharu leaves extract under UV 254 light (spot in black circle)
C: TLC of gaharu leaves extract under UV 366 light (spot in white circle)
D: TLC of gaharu leaves extract after sprayed $AlCl_3$

3.5 Formulation of Anti-Acne Herbal Patches

The anti-acne patch formulation contains ethanol extract of gaharu leaves as the active substance with chitosan polymer as polymer matrix, glycerol as a plasticizer, Span 20 as a penetration enhancer, and BHT as an antioxidant because of the use of Span 20 and distilled water as a solvent.

3.6 Physical Characteristics of Anti-Acne Herbal Patches

Organoleptic Test. The organoleptic test results in Table 2 includes color, odor and surface condition of the patch, indicate color differences between the base and the extract. F1’, F2’ and F3’ were clear because the polymer can be mixed evenly in the solvent. Chitosan polymer as a matrix can produce a smooth, wrinkle-free physical appearance. F1, F2 and F3 were brown because it contains a 70% ethanol extract of gaharu leaves.

Table 2. The Result of Organoleptic Test

Formulation Codes	Organoleptic		
	Color	Odor	Patch Surface
F1’ (500 mg base)	transparent	odorless	flat, smooth sticky surface
F1	brown	odorless	flat, smooth sticky surface
F2’ (600 mg base)	transparent	odorless	flat, smooth sticky surface
F2	brown	odorless	flat, smooth sticky surface
F3’ (700 mg base)	transparent	odorless	flat, smooth sticky surface
Distilled water	brown	odorless	flat, smooth sticky surface

Thickness. Thickness measuring aims to determine the uniformity of the thickness of the patch produced, and the obtained thickness shows the uniformity of the patch solution poured into the mold [26]. The patch thickness ranged between 0.4700 and 0.7960 mm (Table 3). The measured thickness of this patch meets the requirements, which is less than 1 mm [26]. The thickness of the patch will affect the release of active substances from the matrix [27]. Based on the statistical analysis, there is a significant difference in patch thickness formula, indicated by p-value < 0.05. A positive correlation value indicates that the greater the polymer amount, the thicker the patch size. This result is in line with the theory that polymer concentrations are related to patch thickness [26].

Folding Endurance (Elasticity). The folding endurance test aims to determine the folding capacity of the patch to withstand adverse conditions during manufacture, packaging and transportation [28]. The results of the folding endurance test ranged between 325 and 541 folds (Table 3). This result meets the requirements, which are more than 300 folds [29]. The factor that influences the elasticity of the patch is the plasticizer. In this study, glycerin was used as the plasticizer so that the patch was elastic and didn't break easily [30].

The results of the statistical analysis showed $p < 0.05$, which indicates a significant difference in folding endurance between the base and extract formulation. A positive calculated r value indicates that the higher the polymer concentration, the higher the foldability produced. According to Wardani (2021), differences in polymer concentration affect the foldability of the patch.

Uniformity of Weight. The uniformity of weight test aims to determine the reproducibility of patch preparation process [26]. Based on the average value and standard deviation, the coefficient of variance can be calculated. The coefficient of variance for all formulations is < 5% (Table 3) which indicates that the uniformity of weight meets the requirements.

The statistical results of the uniformity of weight test show that there is a significant difference in between the extract and base formulations with patch weight. Further, the calculated r value is determine. The positive value of r indicates that the higher the base and extract formulation, the higher the content uniformity.

Percentage Moisture Content. The moisture content test aims to determine the moisture content of the patch, which can affect its stability [31]. The moisture content in this study ranged between 0.7401 and 8.9642% (Table 3). Low moisture content will help the formulation remain stable and prevent the film from becoming completely dry and brittle [32].

The results of statistical test shows p-value < 0.05, indicating significant difference in moisture content between the base and extract formulation. The base formula has a low moisture content due to the hydrophobic chitosan factor. Higher concentration of chitosan generates lower moisture content of the patch [33].

Water Vapor Transmission Rate (WVTR). The vapor transmission rate test aims to measure the permeability of the patch film against water evaporation [34]. From the data in Table 3, it can be seen that the higher concentration of the polymer resulted in the smaller vapor transmission rate. The smaller the vapor transmission rate produces better film's resistance to water vapor [35].

The statistical test shows significant results with p value <0.05, indicating that the greater the base and extract formulation, the greater the vapor transmission in the preparations.

pH. pH measurements were carried out to ensure the pH of the patch matches the skin condition. The pH of the patch tend to be acidic due to the addition of acetic acid to dissolve the chitosan polymer. The pH measurement results (Table 3) show that the patch is safe and does not irritate the skin because it is still within the standard range 4.5 and 7 [16].

Statistical analysis shows that there is a significant difference in pH between the patch formulas. A negative result on the calculated r indicates that the larger the extract formulation, the lower the pH of the patch. Based on the literature, polyphenol compounds are not resistant to heating and can be damaged by oxygen reactions [36], [37]. This research shows that the addition of extract will reduce the pH of the preparation and the patch printing process in the oven can also affect the degradation of phenolic compounds.

Percentage Moisture Uptake. The moisture uptake test aims to determine the moisture absorption capacity of the skin. The absorption capacity (Table 3) are within the range of 0.981 to 6.26%. Low moisture absorption capacity can protect the patch from microbial contamination and stiffness of the patch [32].

The p-value < 0.05 resulted from the statistical analysis showed that there is a significant difference in the moisture uptake between the base and extract formulations. The percentage of moisture absorption is influenced by the hydrophilicity of the polymer or plasticizer used [38].

Table 3. The Result of Physical Characteristics of Anti-Acne Herbal Patches

Ingredients	Formula					
	F1’	F1	F2’	F2	F3’	F3
Thickness (mm)	0.4700 ± 0.0216	0.6566 ± 0.0531	0.526 ± 0.01247	0.7166 ± 0.0849	0.7066 ± 0.0329	0,7966 ± 0.0249
Folding resistance (elasticity)	397.3333 ± 2.4944	431.3333 ± 2.6246	470 ± 3.7416	548 ± 3.7416	475.3333 ± 4.6427	564.6666± 4.4969
Uniformity of Weight	8.5875 ± 0.0009	8.6144 ± 0.0033	8.7410 ± 0.0015	9.3995 ± 0.0101	8.9914 ± 0.0052	9.5499 ± 0.0280
Percentage Moisture Content (%)	4.69 ± 0.13	3.02 ± 0.21	4.19 ± 0.37	3.99 ± 0.21	4.02 ± 0.18	6.15 ± 0.08
Water Vapor Transmission Rate	0.0059 ± 0.0025	0.0031 ± 0.0018	0.0003 ± 0.0008	0.0013 ± 0.0009	0.00009 ± 0.00004	0.00018 ± 4.1919×10^{-5}
pH	6.49 ± 0.0124	6.42 ± 0.0169	6.33 ± 0.0399	5.84 ± 0.1744	6.20 ± 0.0355	5.48 ± 0.0216

4. Conclusion

All formulas have good physical properties and characteristics, 0.47-0.796 mm thickness, pH, folding resistance >300 times, moisture content <10%, vapor transmission 0.00001-0.0059, moisture uptake 0.0981-1.494%, and uniform patch weight uniformity.

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