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Development of transdermal patch preparations binahong leaf extract (*Anredera cordifolia* (Ten.) Steenis) and antihyperglycemia test in rats

Galang Pergiawan Ramadhan, Iis Wahyuningsih*, Wahyu Widyaningsih
Faculty of Pharmacy, Universitas Ahmad Dahlan,
Jl. Prof. Dr. Soepomo, SH, Warungboto, Umbulharjo, Yogyakarta, Indonesia

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ABSTRACT

Binahong (*Anredera cordifolia)* leaves are plants that have great potential as antihyperglycemics, and have chemical content including triterpenoids, flavonoids, and saponins. However, the development of binahong leaves in pharmaceutical preparations is still very lacking. Objectives of this study are knowing the transdermal patch formulation of binahong leaf extract (BLE) produces good physical properties and the development of BLE transdermal patches with different extract concentrations may blood glucose lower (BGL) levels in glucose-induced mice. This study uses a single-layer patch because it has the advantage of being able to penetrate faster and is suitable for compounds that have solubility in polar solvents. The research began with the extraction of binahong leaves, then formulated a transdermal BLE patch using a formula that includes HPMC (polymer), glycerol (plasticizer), oleic acid (enhancer) and tween 80 (enhancer). Diabetic rats were divided into 4 groups of 6 rats, negative control group (glucose + placebo), positive control group (glucose + insulin), FI group (glucose+ transdermal patch BLE 30%), and FII group (glucose + transdermal patch BLE 47%). The BGL measurements are carried out on the 0th, 3rd, 5th, and 7th days. The physical characterization carried out includes organoleptic, weight uniformity, thickness, pH, folding resistance, and moisture content, obtained FI and FII results that meet the requirements set out by each test standard. The BGL from day 1 to day 7 decreased in each group, positive control (68.8±3.76), negative control (112.5±6.89), FI (81.0±4.04), and FII (72.33±5.12). Based on the statistical results of the T-test on the physical evaluation of BLE transdermal patch preparations of FI and FII there were significant differences in weight and thickness uniformity, but there were no significant differences in pH, folding power, and humidity tests. The administration of a 47% BLE in FII transdermal patch provides insignificant BGL reduction effect compared to the 30% BLE in FI transdermal patch.

Keywords: *Anredera cordifolia (ten).* Steenis, patch transdermal, physical characterization, antihyperglycemic

Corresponding author:
Iis wahyuningsih
Faculty of Pharmacy
Universitas Ahmad Dahlan
Jl. Prof. Dr. Soepomo, S.H, Warungboto, Umbulharjo, Yogyakarta, Indonesia
Email: iis.wahyuningsih@pharm.uad.ac.id

INTRODUCTION

Hyperglycemia is closely related to diabetes mellitus caused by a lack of the hormone insulin. Lack of insulin will cause glucose to accumulate in the blood which then causes blood glucose levels to increase (American Diabetes Association, 2010). The hormone insulin is produced from a group of beta cells in the pancreas gland and plays an important role in glucose metabolism in body cells. Diabetes mellitus is a disease caused by the failure of the process of breaking down glucose substances in the blood when the body is in normal condition. The sugar substance will then be broken down to be converted into glucose and glycogen by the hormone insulin produced by beta cells in the pancreas. Glycogen and glucose will go through a process that is metabolism or combustion which will be converted in the form of energy (American Diabetes Association, 2010).

Binahong leaf (*Anredera cordiflolia* (Ten.) Steenis) is a plant empirically proven to lower blood glucose levels in diabetic patients (Elisma, 2014). The main ingredients of binahong leaf extract (BLE) that can lower blood glucose levels are triterpenes, flavonoids, alkaloids, tannins, and saponins (Selawa, 2013). However, flavonoids have some problems if given orally, their stability is influenced by temperature, pH, and light so that their bioavailability is poor. In addition, the use of natural ingredients of binahong leaves is not accepted by the public, especially oral use so that the formulation is carried out using transdermal patch preparations. The optimal dose of binahong leaf extract as an antidiabetic is 400 mg/KgBW. Binahong leaf ethanol extract gel preparations at concentrations of 10% and 30% can lower blood glucose on the 8th day (Kintoko, 2015).

Transdermal patch preparations are preparations that are placed or attached to the skin to deliver a specific dose of medication and enter the bloodstream (Mane, 2021). Transdermal preparations may enhance the administration of drugs through the skin for topical and systemic administration. Patch preparations are very suitable in hyperglycemic patients because the drugs given do not undergo first-pass metabolism so that drug absorption is more optimal, can be used for long-term therapy or follow-up treatment, and is easily stopped in case of poisoning/allergy, frequency of administration can be reduced to improve patient compliance (Mane, 2021).

The development of this patch formulation uses HPMC as a polymer where the best penetration results are 99.626% in 48 hours (Mangilal et al., 2015). Glycerol as a plasticizer has the advantage that it can produce elastic and strong patches, where the patch preparation obtained does not break when folding many times in the same place (Ningsi, 2015). The combination of these two materials provides an improved kinetic effect of penetration and release when combined. HPMC and glycerol provide a discharge flux of 5.757 µg/mL hours and a penetration flux of 0.096 µg/mL hours (Gunawan, 2010). The use of oleic acid alone or in combination can increase the penetration of drugs into the skin better than other chemical enhancers such as DMSO, span 20, lauric acid, caprylic acid, capric acid and so on (Rahayu & Mita, 2016).The purpose of this study is to know if the formulation of BLE patches meets the physical characteristics of good patches and to know if the difference in BLE concentrations in transdermal patch preparations can affect BGL-lowering activity.

MATERIALS AND METHOD

Materials

Fresh binahong leaves come from Allizzu herbal garden, Bantul district, Yogyakarta, aquadest, ethanol 70% (technical grade, Merck), ethanol 96% (technical grade, Merck), glycerol (pharmaceuticals grade, Sigma), glucose (pharmaceuticals grade, Sigma), HPMC (pharmaceuticals grade, Ardrich), insulin (pharmaceuticals grade, NovoRapid), oleic acid (pharmaceuticals grade, Sigma), oral disposable, plaster (Hypafix), standard feed (hi-pro-vit), tween 80 (pharmaceuticals grade, Ardrich), and water for injection (technical grade, Merck).

Transdermal patch preparation

The transdermal patch formula of binahong leaf extract tested for antihyperglycemic activity can be seen in Table 1. BLE transdermal patches are created by mixing HPMC into glycerol as a mixture of 1. Oleic acid was mixed with tween 80 and then dissolved in 5 mL as a mixture of 2. BLE has dissolved

in alcohol as much as 5 mL then added mixture 1 then mixture 2 and stirred until homogeneous, the mixture is poured into a mold then dried at room temperature for 30 minutes to evaporate the alcohol and ventilated at 50°C for 24 hours so that a layer of film is obtained then wrapped in aluminium foil and then stored in a desiccator.

Table 1. Transdermal patch formula of binahong leaf extract

Material	Concentration	
Binahong leaf extract	30%	47%
HPMC(*Stabilizer*)	0.2 g	0.2 g
Glycerol (*Plasticizer*)	0.2 g	0.2 g
Oleic Acid (*Enhancher*)	0.1 g	0.1 g
Tween 80 (*surfaktan*)	0.06 g	0.06 g

Test characteristics of the preparation

Organoleptic test

Testing was carried out visually in the form of color, texture, and aroma (Kumar et al., 2013).

Weight uniformity test

The five patch matrices of each formulation are weighed and averaged, and then calculated the standard deviation and relative standard deviation (Kumar et al., 2013).

Patch thickness test

The five patch matrices of each formulation are measured in thickness using calipers. Calculated average patch matrix thickness, standard deviation and relative standard deviation (Kumar et al., 2013).

pH test

The five patch matrices of each formulation are tested using a special pH meter (pH meter surface) tool on the surface. Calculated the average value of the pH obtained (Kumar et al., 2013).

Fold durability test (Folding Endurance)

It is done by means of one patch of each formulation folded in the same place several times until it looks visibly torn. The number of times a patch can be folded in the same place without tearing occurring is calculated as the value of the durability of the fold (Kumar et al., 2013).

Moisture absorption test

The five patch matrices of each formulation are weighed and placed in a desiccator containing silica gel in them at room temperature for 24 hours. The patch matrix is then weighed and calculated the percentage of moisture absorption (Kumar et al., 2013).

BGL reduction activity test

The number of experimental animals is based on the calculation formula of Freed (n-1) (t-1) ≥ 15. The test animals used in this study were 24 male white rats (*Rattus nervogicus*) weighing 200-300 grams which were divided into 4 groups, namely:

a. Negative control group (glucose + placebo)
b. Positive control group (glucose + insulin)
c. FI group (glucose + patch BLE 30%)
d. FII group (glucose + patch BLE 47%)

The test animal was acclimatized for 7 days. Each group of test animals were fasted for 10-18 hours and then measured the initial blood glucose level using a glucometer. Glucose administration is done

orally for 8 days at a dose of 4.5 mg/kgBW. The rats were characterized to be diabetic if the blood glucose level is >124 mg/dL using a glucometer autocheck device (Rohma et al., 2015). Then the rat's hair is shaved in the back with a size of 3x5 cm as a place for applying transdermal patch preparations. Each rat measured its blood glucose levels after 6 hours of treatment on days 1, 3, 5 and 7 (Rohma et al., 2015).

Data Analysis

The data obtained in the form of patch physical characteristics using the t-test and the time of decrease in BGL of male white rats (*Rattus norvegicus*) was analyzed statistically using the one-way ANOVA test with a 95% confidence level. Sig. <0.05 Signification is established if p<0.05.

RESULTS AND DISCUSSION

Characterization of transdermal patches

The formulated BLE transdermal patch is followed by physical tests including organoleptic tests, weight uniformity tests, patch thickness tests, pH tests, folding endurance tests, and moisture content tests.

Organoleptic

In organoleptic tests of transdermal patch preparations of binahong leaf extract include aroma, color, and texture. The organoleptic test results of each extract patch formula appear in Figure 1. Based on the organoleptic test above FI (binahong extract 30%) and FII (binahong extract 500 g) have the same characteristics of odor (typical of extract) and texture (smooth, homogeneous, elastic surface), the difference only occurs in the color of the preparation where FI the amount of binahong extract is 30% brighter than FII with binahong extract 47%. This happens because binahong extract has a natural green-black color, the higher the amount of extract used to eat the darker the color of the preparation follows the natural color of the extract.

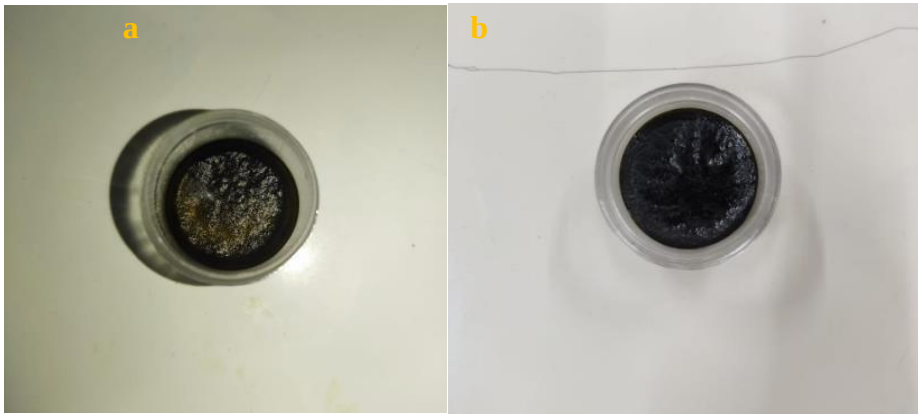

Figure 1. (a) FI of transdermal patch of binahong leaf extract 30%; (b) FI of transdermal patch of binahong leaf extract 47%

Uniformity of weights

Measurements were made using 10 replications in each formula. The weight of each patch should not deviate from the standard deviation of <0.05 (Yadv & Jain, 2011). Significant, FI (SD ±0.018, CV ±2.20%) and FII (SD ±0.020, CV ±1.83%) are still in the stipulated provisions (Table 2), namely the value of SD <0.05 and CV <5% (Baharudin & Maesaroh, 2020). This difference in patch weight affects the amount of active substance used so it affects the uniformity of the content.

A good patch thickness size is in the range of 0.5–1.0 mm, if it is smaller it will make it difficult to use (Nuryanti et al., 2016), if the patch is too thick, it will be difficult to release the active substance from the patch (Shirsand et al., 2012). Testing the thickness of the patches in each formula is to measure the thickness one by one 3 replication patches (Nurahmanto, 2016). Patch thickness is measured at 5 different points using Vernier calipers (Baharudin & Maesaroh, 2020). The requirement for a good range of durable patches is CV <2% (Nurahmanto, 2016) and an elementary < score of 0.05 (Baharudin & Maesaroh, 2020). The value obtained from the patch thickness test (Table 3) shows that the patch results made met the patch thickness requirements.

Table 2. Uniformity of weights of transdermal patch preparations binahong leaf extract

Replication	FI (30%) (g)	FII (47%) (g)
Average ± SD	0.8443 ± 0.018	1.1058 ± 0.020

Table 3. Thickness of transdermal patch preparation binahong leaf extract

Replication (mm)	FI (mm)	FII (mm)
Average ± SD	0,526 ± 0.005	0.946 ± 014

pH

PH testing determines whether the transdermal patch preparation has a pH of the preparation used for the skin, which is 4.5-6.5. If the pH of the patch is too acidic and too alkaline it can cause the skin to become dry (Mappa et al., 2013). The test results of the two formulas (Table 4) are known to meet the pH requirements of transdermal preparations, namely 4.5-6.5 (Mappa et al., 2013).

Table 4. pH transdermal patch preparation binahong leaf extract

Formula	Average	CV (%)
FI	6.23	2.45
FII	6.53	2.66

Folding endurance test

This approval aims to see if the patches made have resistance to folds. Patches are said to meet the criteria when they withstand folds more than 300 times (Lakhani et al., 2015). It can be seen Table 5 that the two patch formulations made are qualified for good patch fold resistance, which is > 300 times (Ningsi, 2015).

Table 5. Folding power of transdermal patch preparations binahong leaf extract

Formula	Average	CV (%)
FI	355.66	6.47
FII	343.33	5.86

Moisture absroption test

The prepared patches are each weighed (initial weight) and stored in a desiccator containing silica gel at room temperature for 24 hours. The patch is then re-weighed (final weight) (Shabbir et al., 2017). The required moisture content range is 1 - 10% (Kumar et al., 2013). The results of testing percent moisture content show that Formula I and Formula II have values that already meet the required range of 1 - 10% (Table 6) (Kumar et al., 2013).

Table 6. Moisture preparations transdermal patch BLE

Formula	Average (%) (x ±SD)
FI	2.54±0.34
FII	2.85±0.35

The activity test of lowering blood glucose levels (BGL)

Hyperglycemia Rats

After 8 days of the administration, blood glucose measurements are carried out using an autocheck glucometer tool. Rats were said to be hyperglycemia if blood glucose levels exceeded BGL values >135 mg/dL or >124 mg/dL based on the maximum value of the glucometer autocheck tool (Wolfensohn, 2013). The results of 0-8 days of administration obtained seen in Table 7.

Table 7. Blood glucose level of glucose induced rat treated by transdermal patch of binahong leaf extract

Formula	Blood glucose levels (mg/dL)	
	Day 0 (x ± SD)	Day 8 (x ± SD)
Positive control (insulin)	100.5 ± 8.09	171.83± 43.3
Negative control (placebo)	94.7 ± 14.13	163.33± 11.21
Trandermal patch extract (30%)	103.7 ± 4.41	166.33± 44.2
Trandermal patch extract (47%)	88.2 ± 3.65	162.50± 38.6

From Table 7 obtained after oral administration of 4.5 g / kg BW glucose, the blood glucose levels of rats in each group increased in value, namely ≥124 mg/dL on the 8th day. Oral administration of glucose for 8 days makes blood glucose levels rise, insulin hormone in charge of introducing glucose into cells cannot work optimally because the amount of glucose is too much. So that there is an increase in glucose levels in the blood (Wolfensohn, 2013).

Binahong leaf transdermal patch preparation

Transdermal patching of binahong leaves is carried out once every 2 days. It is based on the function of the patch preparation which is to reduce the frequency of use and the ability of the HPMC polymer, namely the formula with a single polymer HPMC able to release excellent drugs, namely 99.626% in 48 hours (Mangilal et al., 2015).

Treatment for 7 days can be concluded for positive control to provide a significant reduction of FI (72.33 mg/dL) and FII (68.8 mg/dL). From Figure 2, it can be seen that the difference in the concentration of BLE extract used can provide a different decrease in blood glucose levels. The greater the concentration, the higher the percentage decrease in BLS. Although the FI gives a slightly lower decrease than the FII the decrease that occurs is within the normal limits of blood glucose levels. This signals FI is effective for lowering BGL in mice. Meanwhile, the negative control decrease was 112.5 mg/dL. The low decrease in BGL in negative control compared to other groups is caused by the absence of substances that can help reduce BGL. However, there is still a decrease in BLS in the negative control, this is likely to occur due to the role of the body's metabolism from mice that can reduce BGL by converting it into energy (Supriyanto et al., 2022).

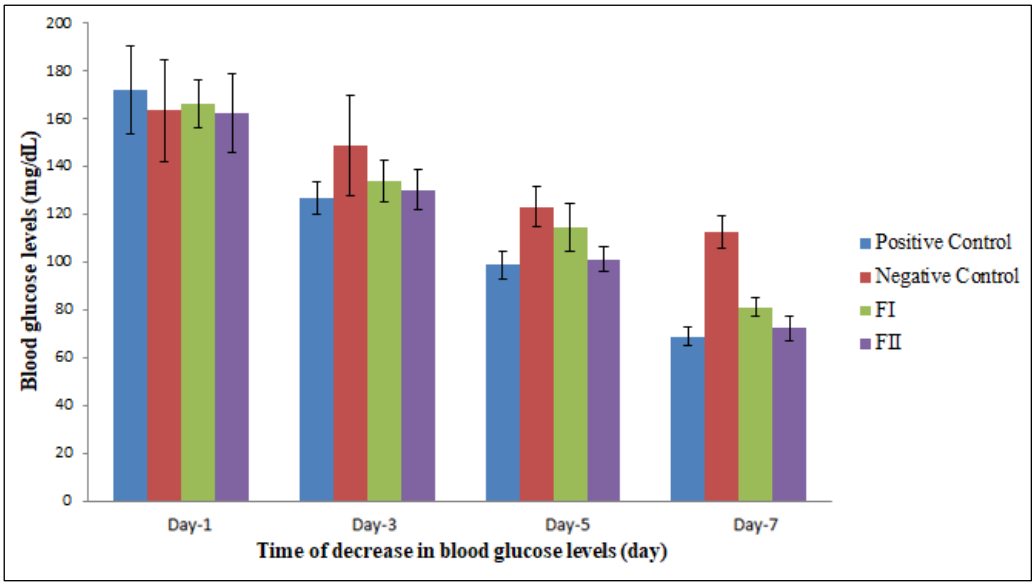

Figure 2. Graph of decrease in blood glucose levels against time (day)

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