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Cream Formulation Of Methanol Extract Of Tapak Kuda Leaves (Ipomoea Pes Caprae (L.) R. Br.) As Anti-Inflammatory in Male White Mice (Mus Musculus)

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Abstract. *The study entitled "Cream Formulation of Methanol Extract of Tapak Kuda (Ipomoea pes-caprae (L.) R. Br.) Leaves) As an anti-inflammatory in male white mice (Mus musculus)" using laboratory experimental methods with posttest control group design, data analysis using SPSS 25.0 with One Way ANOVA test and Duncan test. The percentage of extract yield obtained was 3.12%, phytochemical screening test showed the presence of secondary metabolite compounds alkaloids, terpenoids, flavonoids, saponins, tannins and steroids. Cream preparation of methanol extract of Tapak Kuda leaves at concentrations of 2.5%, 5% and 10% showed anti-inflammatory activity and the maximum dose to reduce edema diameter was 10% concentration with a percentage inhibition of 87.97%. Data analysis with One Way ANOVA test with a significance value ≤ 0.05 which indicates the presence of anti-inflammatory activity in Ipomoea pes-caprae tread leaf methanol extract cream.*

Keywords: *Anti-inflammatory Test, Cream preparation, Ipomoea pes-caprae (L.) R. Br Leaf.*

1. INTRODUCTION

Indonesia is a country rich in biodiversity, especially plants that are widely used to treat various diseases and are commonly referred to as medicinal plants (Wantoro, 2023). Indonesia has approximately 7,000 of the 30,000 types of plants that are thought to have medicinal uses that encourage people to utilize them (Adiyasa & Meiyanti, 2021). One of the medicinal plants that can be used as a treatment is the Ipomoea pes-caprae (L.) R. Br..

Ipomoea pes-caprae (L.) R. Br. is a wild plant that grows creeping around rocky and sandy beaches (Safutra & Zuriat, 2018). Research by Akinniyi et al. (2022) stated that Ipomoea pes-caprae leaves are used topically to treat skin diseases, joints accompanied by inflammation, dermatitis, boils, and jellyfish stings by squeezing the leaves and applying them to the affected area. Nusaibah et al. (2022) stated that the extract of Ipomoea pes-caprae (L.) R. Br. It contains alkaloids, flavonoids, saponins, terpenoids, and tannins, which function as antimicrobials, stimulate the growth of new cells for wound healing, and can be used as anticancer and anti-inflammatory agents.

Anti-inflammatory activity of Ipomoea pes-caprae (L.) R. Br. has been carried out by Hasanah (2021) using ethanol extract of Ipomoea pes-caprae leaves in the form of a gel preparation, showing low anti-inflammatory activity. This study will be carried out using a topical

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preparation containing the leaf extract of Ipomoea pes-caprae (L.) R. Br. in cream preparation form. Previous studies have stated that there are several cream formulations for antibacterials made at concentrations of 2.5%, 5%, and 7.5%, showing that the results do not meet the parameters of pH and spreadability (Falles Raintung et al., 2013).

Therefore, it is necessary to conduct research on the formulation of Ipomoea pes-caprae leaf extract cream (Ipomoea pes-caprae (L.) R. Br.) as an anti-inflammatory agent in male white mice (Mus musculus) from Tegar Bahari Beach, so that it can be developed as an herbal medicine in the form of a cream preparation that is efficacious as an anti-inflammatory and safe for use by the community.

2. RESEARCH METHOD

This study used an experimental method of the post-test control group design type, where there were only two groups, namely the control group and the treatment group, which were selected randomly.

Time and Place of Research

This research was conducted from June 2024 to August 2024 at the Natural Material Chemistry Laboratory, Faculty of Health Sciences, Undergraduate Pharmacy Study Program, Batam University. Ipomoea pes-caprae leaf samples (Ipomoea pes-caprae (L.) R. Br.) were taken from Tegar Bahari Beach, Barelang Bridge 6, Batam City.

Tools and Materials

The tools used in this study were plastic bags, scissors, analytical scales, needle scales, knitting winnowing, plastic basins, brown glass bottles, ovens, beaker glasses, test tube racks, stirring rods, spatulas, test tubes, glass funnels, filter paper, rotary evaporators, desiccators, glass droppers, object glasses, glass plates, pH meters, hot plates, cream pots, mortars, stemfers, spatulas, evaporator cups, measuring cups, porcelain crucibles, porcelain crucible pliers, Erlenmeyer flasks with glass lids, tripod supports, asbestos gauze, bunsen burners, matches, vials, pencils, TLC plates, tissues, subcutaneous syringes, razors, iron nets and animal cages. The materials used in this study were Ipomoea pes-caprae leaves (Ipomoea pes-caprae (L.) R. Br.), methanol, ethyl acetate, n-hexane, distilled water, amyl alcohol, FeCl3, Wagner reagent,

Dragendorff reagent, Meyer reagent, HCl 2M, H2SO4 2M, magnesium powder, spirit solution, clean running water, stearic acid, TEA, glycerin, nipagin, sodium tetraborate, hydrocortisone 2.5%, carrageenan. White mice were used as experimental objects for anti-inflammatory testing.

Extract Preparation

The process of making Ipomoea pes-caprae leaf extract begins by taking a sample of 2000 g and the initial treatment (wet sorting, washing, drying, and chopping). Subsequently, extraction was carried out using the maceration method with methanol solvent by shaking for 3 days, and the filtrate was filtered and concentrated using a rotary evaporator.

Phytochemical Screening

a. Alkaloid Test

The extract powder was put into a test tube, 10 ml of chloroform and 4 drops of NH4OH were added, and the mixture was filtered. Ten drops of H2SO4 were added to the filtrate, which was then stirred to produce two layers. The upper layer was separated into three different test tubes. Five drops of Meyer, Wagner, and Dragendorf reagent were added. Positive alkaloids were indicated by the presence of a white precipitate if Meyer's reagent was added, a brown precipitate if Wagner's reagent was added, and a red/orange precipitate if Dragendorf's reagent was added (Andayani & Nugrahani, 2018).

b. Terpenoid Test

Two milliliters of the extract were added to 10 drops of acetic acid (CH3COOH) and 2 drops of sulfuric acid (H2SO4). If a red or purple color is formed, it is positive for terpenoids (Sangkal et al., 2020).

c. Flavonoid Test

The extract powder (0.1 g) was placed in a beaker, and 10 ml of distilled water was added and heated to boiling temperature for 5 min. It was then filtered, and the filtrate was used as a test solution. The filtrate was put into a test tube, Mg ribbon was added, and concentrated HCl (1 ml of amyl alcohol (1 mL) were added and shaken vigorously. A positive flavonoid test result is

indicated by the formation of a red, yellow, or orange color on the amyl alcohol layer (Andayani & Nugrahani, 2018).

d. Tannin Test

The extract powder (0.1 g) was added to 10 ml of hot water, boiled for 5 min, and filtered. A 1% FeCl3 solution was then added to the filtrate. Positive results were indicated by the formation of a blackish-green color (Andayani & Nugrahani, 2018).

e. Saponin Test

The extract powder was placed in a beaker as much as 0.1 gr, and then 10 ml of hot water was added and boiled for 5 min. It was then filtered, and the filtrate was used as a test solution. The filtrate was placed in a closed test tube, shaken for ± 10 s, left for 10 min, and 1 ml of 2M HCl was added. The presence of saponins is indicated by the formation of stable foam (Andayani & Nugrahani, 2018).

Specific Parameter Test

a. Organoleptic Test

Organoleptic tests were carried out on thick extracts, which consisted of observing color, odor, taste, and texture.

Water and Ethanol Soluble Essence Level Test

The ethanol and water-soluble essence levels were tested by soaking 5 g of extract in the solvent, followed by shaking for 6 h, and then leaving it for 18 h. After that, the filtrate was filtered, and 20 ml of filtrate was removed and evaporated over a Bunsen burner until the remaining essence was attached to the evaporator cup. The percentage of essence level was calculated based on the following equation:

$$\% \text{ Ethanol/Water Essence Level} = \frac{solvent\ used}{extract\ used}\ x\ weight\ of\ pure\ extract.$$

After calculating the percentage of ethanol or water-soluble extract, the percentage yield was calculated using the following equation:

$$\% \text{ Yield} = \frac{total\ juice\ weight}{sample\ used} \text{x } 100\%.$$

Chromatography (TLC)

Thin-layer chromatography testing was carried out by spotting the sample on the TLC plate, then the TLC plate was placed in a chamber containing a solvent and observed against the solvent that carries the sample to the upper limit mark, and observing the spots formed under ultraviolet light. The spots that appeared were then calculated for the Rf value using the following equation:

$$\text{Rf: } \frac{Stain\ height\ from\ the\ bottom\ limit}{Path\ length\ passed\ by\ the\ solvent}$$

Non-Specific Parameter Test

a. Drying Shrinkage Test

The drying shrinkage test was performed by heating the extract in an oven at a temperature of 105 °C. A drying shrinkage test was performed until a constant weight was obtained. The percentage calculation was then performed based on the following equation:

$$\%\ \text{Drying shrinkage} = \frac{m1-m2}{m1} \times 100\%.$$

Description:

m1= weight of sample used in the test.

m2= average cycle value, where the weight of each cycle was subtracted from the weight of the empty porcelain crucible and then divided by the number of cycles.

b. Water Content Test

The water content test was performed by heating the extract in an oven at 105 °C. A water content test was performed until a constant weight was obtained. Then, the percentage calculation was performed based on the following equation:

$$\%\ \text{Water content} = \frac{m1-m2}{m1} \times 100\%.$$

Description:

m1= weight of sample used in the test.

m2= average cycle value, where the weight of each cycle was subtracted from the weight of the empty porcelain crucible and then divided by the number of cycles.

c. Ash Content Test

The ash content test was carried out by burning the extract at a high temperature (250–500 °C). until the simplicia changes into a grayish-white ash form. Then, the percentage calculation is carried out based on the following equation:

$$\% \text{ Ash content} = \frac{Stain\ height\ from\ lower\ border}{Length\ of\ solvent\ travelled\ path} \times 100\%$$

Cream Formulation

The methanol extract of Ipomoea pes-caprae leaves was formulated into a cream preparation made at concentrations of 2.5, 5, and 10% with the following formulation:

Table 1. Preparation formulation

Ingredients	Concentration		
	F1	F2	F3
Ipomea leaf extract	2,5%	5%	10%
Stearic Acid	142 g	142 g	142 g
TEA	10 g	10 g	10 g
Sodium tetraborate	2,5 g	2,5 g	2,5 g
Glycerin	100 g	100 g	100 g
Nipagin	0.1 g	0.1 g	0.1 g
Aquadest	100 g	100 g	100 g

After the cream was made, organoleptic observations (color, odor, texture), pH, homogeneity, spreadability, and preference tests were performed.

d. Anti-inflammatory Activity Test

The test animals used in this study were healthy male white mice (Mus musculus) aged–2-3 months and weighing–20-30 grams. Before testing, the experimental animals were acclimatized for 7 days. The mice were divided into five groups, namely group I positive control (+) administered 2.5% hydrocortisone cream, group II negative control (-) given cream base, treatment group III given a 2.5% concentration test cream preparation, treatment group IV given a 5% concentration test cream preparation, and treatment group V given a 10% concentration test cream preparation. The diameter of edema formed on the soles of the mice's feet was measured using a caliper at 30, 60, 90, 120, 150, 180, 240, 300, and 360 min after being induced by 1% carrageenan (BPOM, 2021).

3. RESULTS AND DISCUSSION

Tapak kuda leaves (2100 g) were obtained from Tegar Bahari Beach, Jembatan 6 Barelang, Batam. The samples were first determined at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, West Sumatra. The results of the Tapak kuda plant determination showed that the sample used was indeed a plant named Ipomoea pes-caprae (L.) R. Br.

In this study, the initial treatment of the sample consisted of several steps, namely wet sorting, chopping, washing, drying, and dividing the mass of the sample to be made into simple and thick extracts.

Extract Preparation

A sample of 2000 g was extracted using the maceration method and concentrated using a rotary evaporator. The extract weight was 31.27 g with a yield of 3.12%.

Table 2 Results of Extract Making

Extract Preparation Data	Results
Weight of sample taken	2100 g
Weight of sample after initial treatment	2000 g
Weight of macerated sample	2000 g
Weight of extract	31,27 g
Yield	3,12 %

Phytochemical Screening

The results in Table 2 show that the Ipomoea pes-caprae leaf extract contains alkaloids, terpenoids, steroids, flavonoids, tannins, and saponins.

Table 3 Phytochemical Screening Results

Test Type	Reagent	Result
Alkaloids	Meyer	+
Terpenoids	Dragendorf	+
	Wagner	+
Steroids	Aquadest+ H_2SO_4+CH_3COOH	+
Flavonoids	Aquadest +H_2SO_4+CH_3COOH	+
Tannins	Aquadest +Serbuk mg+HCl+amil alkohol	+
Saponins	Aquadest +$FeCl_3$	+
Test Type	Aquadest	+

Specific Parameters

Specific parameter testing in the form of organoleptic tests, water and ethanol soluble extract levels, and thin-layer chromatography (TLC) tests

Table 4 Specific Parameter Test Results

Type	Data	Results
Organoleptic	Color	Brown
	Smell	aroma of tea
	Taste	Bitter
	Texture	Thick
Water soluble extract content	% sample results	19%
Ethanol soluble extract content	% sample results	9%
Thin layer chromatography	Rf1 Value	0,78
	Rf2 Value	0,85

The results in Table 4 show that the extract obtained had a brown color, and the smell of the extract was almost similar to the aroma of tea and had a bitter taste and a thick texture. The percentage of water-soluble extract content was 19%, whereas the percentage of ethanol-soluble extract content was 9%. The results of the TLC test showed an Rf value of 0.78, indicating the presence of tannins, and an Rf value of 0.85, indicating the presence of flavonoids.

Non-Specific Parameters

Non-specific parameter testing in the form of drying shrinkage, water content, and ash content tests.

Table 5 Results of Non-Specific Parameter Tests

Test Type	%
Drying shrinkage	9,54 %
Water content	0,011%
Ash content	0,44%

The results in Table 5 show a drying shrinkage percentage of 9.54%, water content percentage of 0.011%, and ash content percentage of 0.44%.

Physical Evaluation of Cream

The methanol extract cream of Ipomoea pes-caprae leaves was prepared at concentrations of 2.5, 5, and 10%. Cream testing was carried out to determine the quality of the cream, including organoleptic, homogeneity, pH, spreadability, and preference tests.

Table 6 Results of Physical Evaluation of Cream

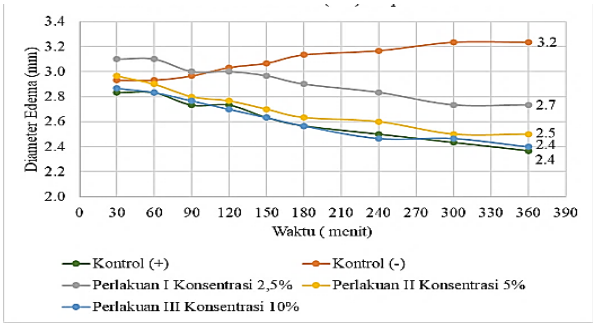
Test	Type	2.5%	5%	10%
Organoleptic	Color	Cream	Light yellow	Brownish yelow
	Odor	Odorles s	Odorles	Odorles
	Texture	Semi solid	Semi solid	Semi solid
	Preferenc e Test (Value)	53	54	59
pH		7	6	6
Homogenitas		Homo gen	Homogen	Homogen
Spread power test		6,89 cm	6,86 cm	6,64 cm

The results in Table 6 show that the methanol extract cream of Ipomoea pes-capraeleaf has a different color for each concentration, where the higher the concentration of extract added to the cream base, the darker the color produced. A methanol extract cream of Ipomoea pes-capraeleaf (Ipomoea pes-caprae (L.) R. Br.) produces a thick cream, and its viscosity does not change when stored. The results of the pH test of the methanol extract cream of Ipomoea pes-capraeleaf at various concentrations are safe for use. The results of the homogeneity test of the methanol extract cream of Ipomoea pes-capraeleaf are good at various concentrations, where the cream that has been made does not experience any physical changes even though it has been stored for several days. The results of the cream spreadability test with various concentrations show that the cream that has been made does not exceed the spreadability requirements. The results of the preference test from the 10 respondents in this study showed that in terms of color, aroma, and texture, the most preferred was at a concentration of 10%. This is because, at a concentration of 10%, it has a high extract concentration so that it can produce quite attractive colors, with a distinctive aroma of the Ipomoea pes-capraeleaf, which has a soft texture and is easily absorbed when applied to the skin. Antiinflammatory Cream Activity tests

The results obtained from Figure 1 show that the positive control, Treatment I (2.5% concentration), Treatment II (5% concentration), and Treatment III (10% concentration) groups experienced a decrease in the average diameter of edema to the lowest value at 360 min. In

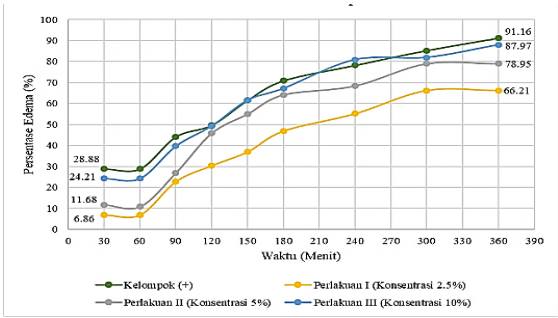
contrast, the negative control group experienced an increase in the average edema diameter until the 360th minute.

Figure 1 Graph of Average Edema Diameter



The results obtained from Figure 4.2 show that the positive control group showed the highest percentage of edema inhibition at the 360th minute (91.16%), followed by Treatment III (10% concentration) group (87.97%), Treatment II (5% concentration) of 78.95% and Treatment I (2.5%) (66.21%).

Figure 2 Graph of Edema Inhibition Percentage



Data Analysis

Data analysis was performed using SPSS with a one-way analysis of variance and Duncan's test. The results obtained significance value in the one-way ANOVA test showed a value of ≤0.05; in other words, the H0 data were rejected; namely, the anti-inflammatory activity of the methanol extract cream of Ipomoea pes-capraeleaves (Ipomoea pes-caprae (L.) R. Br.) in white male mice (Mus musculus). Duncan's test was carried out with the results showing that the positive group, Treatment I (concentration 2.5%), Treatment II (concentration 5%), and Treatment III (concentration 10%) were in the same subset column, indicating that the test group did not show a significant difference.

4. CONCLUSION

The methanol extract cream showed anti-inflammatory activity, with edema inhibition percentages of 2.5% (66.21%), 5% (78.95%), and 10% (87.97%). The ANOVA test results obtained a significance value of ≤0.05, indicating that H0 was rejected. Duncan's test showed positive group results; Treatment I (2.5% concentration), Treatment II (5% concentration), and Treatment III (10% concentration) were in the same subset column, indicating that the test group did not show a significant difference. SUGGESTIONS

It is better to use a large number of test samples to produce a sufficient amount of extract and increase the yield value of the extract. It is necessary to carry out an extraction method using different solvents. It is necessary to prepare an anti-inflammatory cream of Ipomoea pes-caprae leaf extract with different formulations and concentrations.

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