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Analgesic Effect of the Ethanol Extract of Sisik Naga Leaf (*Drymoglossum* sp.) on White Male Mice (*Mus musculus*)

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ABSTRACT

Pain is a sense of discomfort and accompanied by pain due to cell damage to the body. Pain can be overcome with the use of modern medicine and folk remedies. One of the plants that are suspected to be analgesics is the sisik naga leaf (*Drymoglossum* sp.). This test aims to find out the analgesic effect of sisik naga leaf ethanol extract (*Drymoglossum* sp.) on white male mice with *the Hot Plate Test* method. The extraction method is done by maceration with ethanol solvent. A total of 25 mice is divided into 5 groups and are identified in each group. The treatment dose group is 25 mg/ Kg BW (T_2); 50 mg/ Kg BW (T_3); 100 mg/Kg BW (T_4), Na-CMC suspension of 0.5% as negative control (T_0) and antalgin suspension as a comparison (T_1) and phytochemical screening. The dose that gives analgesic effects is 100 mg / Kg BW. The content of compounds contained in sisik naga leaves in the form of flavonoids, alkaloids, glycosides and steroids. Analysis of statistical data in the form of *Parametric Test One-Sample Kolmogorov-Smirnov Test* (*p =0.05*), ANOVA, and TUKEY HSD followed by Non Parametric Test namely *Kruskhal Wallis*.

Keywords: *Analgesic, hotplate test, sisik naga leaf, pain, ANOVA*

1. INTRODUCTION

Indonesia is a country known for its abundant natural materials, both in land and in the land or also known as biodiversity. One biodiversity is a high level of plant that can be used as traditional medicine is sisik naga leaves (*Drymoglossum* sp.) [1]

Traditional medicine is an herb obtained from natural ingredients in the form of plants, animal ingredients, mineral ingredients, saurian preparations (galenik) or a mixture of ingredients used for generations by the community based on the prevailing norms in the community environment [2].

Nowadays, traditional medicine is in high demand from the wider community because it has small side effects [3]. Traditional medicine carried out by people in various regions, of course, has their own characteristics. This is influenced by the biodiversity that exists around the community to be the main solution in treating a disease [4].

In this study the plant used to test analgesic effects was the sisik naga leaf (*Drymoglossum* sp.). Sisik nagas (*Drymoglossum* sp.) are an epiphytic plant that can grow on other trees and can multiply its leaves with its roots. Sisik naga leaves contain Steroid compounds, flavonoids, alkaloids, and glycosides, essential oils, and tannins that are strongly suspected to be efficacious as analgesics [5].

Analgesics are drugs taken to relieve and cure pain. Analgesic drugs work on the system Nerves that have properties that can affect consciousness and can also not affect consciousness [6].

Pain is a sense of discomfort and is accompanied by pain due to damage to cells in the body. Pain that is felt can result in obstruction of daily activities [7]. Based on the above as a first step to research about the efficacy of sisik naga leaf plants (*Drymoglossum* sp.) conducted an analgesic effects test on white male mice (*Mus musculus*) in the form of ethanol extract and using *the Hot Plate Test method.* With this research, it is expected to be an insight for other researchers so that in the future this research can be developed into a scientific article. And can be developed into a standard herbal remedy.

2. RESEARCH METHODS

This research method is experimental. Testing of analgesic effects was conducted in the Laboratory of Pharmacology and Pharmacognocy Laboratory of the Faculty of Pharmacy, Tjut Nyak Dhien Medan University. Tests were carried out on 5 groups, each group consisting of 5 mice.The treatment dose group is 25 mg/ Kg BW (T_2); 50 mg/ Kg BW (T_3); 100 mg/Kg BW (T_4), Na-CMC suspension of 0.5% as negative control (T_0) and antalgin

suspension as a comparison (T_1). The research was conducted in February-July 2021.

2.1 Tools and Materials

The tools used in this study are analytical scales, stirrer rods, hot plates, cameras, mobile phones, animal cages, lumpangs and stamfers, a set of glasses in the form of: beaker glass and measuring glasses, injection spuit 0.5 cc and 1 cc, stopwacth, digital scales.
The ingredients used in this study are Antalgin tablets 500 mg, Akuades, Sisik naga leaf ethanol extract, 96% ethanol solution, white mice aged 8-10 weeks, weight 20-30 grams, Na- CMC 0.5%.

2.2 Preparation of Test Animal

A total of 25 white male mice is divided into 5 groups. The criteria for mice in this test are mice aged 2-4 weeks with a weight ranging from 20-30 grams. Mice is carefully processed in one week with the aim to adapt the squeak with the atmosphere it has just, before testing the analgesic effect of mice is satisfied first for 18 hours, but still given a drink.

2.3 Preparation of Ethanol Ekstract Sisik naga Leaf (Drymoglossum sp.)

Weighed as many as 250 g of simplicia powder of sisik naga leaves was macerated with 96% ethanol for 3 hours. Then it is percolated at a rate of 20 drops per minute. Percolation is stopped when the resulting percolate becomes clear. Percholate was distilled with a rotary evaporator at a temperature of 50^0C to obtain a thick extract, dried with a freeze dryer and obtained a dry extract from simplicia of sisik naga leaves, weighed and stored in a glass container with a good lid, then referred to as Sisik Naga Leaf Ethanolic Extract.

2.4 Preparation of Extract Solution

When the average weight of the weight of the squeak is 20 grams. So the amount of extract given at a dose of 25 mg / kg BB is 0.5 mg, the amount of extract given in the treatment group dose of 50 mg / kg BB is 1 mg. The amount of extract given in the treatment group dose of 100 mg / kg BB is 2 mg. How to make an extract solution for the mice out group given an ethanol extract of sisik nagas leaves (*Drymoglossum sp* .) the group of mice given extract 25 mg / Kg BB is 0.5 mg and dissolved suspense Na-CMC 0.5% and 70 ml a akuades. The mice, mice extract given 50 mg / kg BB is to weigh the extract as much as 50 mg dissolved in 30 ml of Na-CMC suspension 0.5% and 70 ml of a akuades, and for the administration of the extract to mice with a dose of 100 mg / Kg BB is by weighing 100 mg of extract and dissolved with 30 ml and aquedies 70 ml of akuades. Mix and dissolve until homogeneous and then taken as much as 0.5 ml and then given to mice orally in a dose of 25 mg / Kg BW. Giving ethanol extract of sisik naga leaf leaves dose 50 mg / Kg BW is given as much as 1 ml and for the dose of ethanol extract sisik nagas leaves dose 100 mg / kg BW is 2 ml.

2.5 Preparation of Comparison Solution

The dose of Antalgin used is 500 mg/70 kg of human BB. So the calculation of the dose is to use a confectionery dose in humans with a weight of 70 kg to 20 grams is 0.0026. Then the calculation of the dose of antalgin in mice is = 0.5 x 0.0026 = 1.3 grams. The antalgin in na-CMC suspension of 0.5% is:

$$\frac{1,3}{500} \text{ x } 100 \text{ ml} = 0.26 \text{ ml.}$$

2.6 Evaluation of Analgesic Activity

Mice first fasted for 18 hours to speed up the time of absorption of the drug. The administration of the test substance is done orally. Each squeak is given treatment, according to the prescribed dose. Mice are included in the *Hot Plate Test* which has been modified with a temperature of 55^0C. Dosing for each treatment was given only once in each experimental group and observed sueking response at the time of jumping and licking the legs with a duration of 60 seconds. Observations are all carried out 5 times, namely before the administration of test substances, 30^{th} minute after the administration of test substances, 60^{th} ,minute after administration of test substances, 90^{th}, minute after administration of test substances, minutes 120^{th} after the administration of the test substance .

2.7 Evaluation of Water Content

In this method of determining water content using the gravimetric method, with the principle of evaporation of water contained in the sample with a temperature of 105^0C. Heat a 30-minute flu porcelain cup in the oven, then cool it on the decikator and re-weigh the weight, then weigh the sample as much as 2 g and then put it in a porcelain cup, dry it for 5 hours to a temperature of 105^0C and then re-weighed the weight of the cup [9].
Calculation of water content

$$= \frac{W2-W3}{W2-W1} \text{x } 100\%$$

Where: W1 = empty cup weight (gram)
W2 = weight of cup + powder before heating (gram)
W3 = cup weight + powder heating results (grams).

The Simplisia quality requirement is ≤ 10%. Water content that is too high can lead to the growth of microbes that will decrease the stability of the extract [10].

2.8 Evaluation of Ashes Content

Determination of total ash levels is done by using Krush inserted into a tool in the form of a furnace at a temperature of 800°C. The determination of ash levels also aims to find out the number of foreign objects such as soil, sand left on vegetable preparations. The standard determination of The total ash content of simplisia according to Pharmacopoeia Herbal Indonesia in 2008 is for the total ash level is < 4.8% [11].

Calculation of total ash levels =

$$\frac{\textbf{weight after treat} - \textbf{sample weight}}{\textbf{sample weight}} \text{ x } 100\%$$

Based on the test the ash level obtained is 0.43%.

2.9 Macroscopic Test and Microscopic Test

Macroscopic examination of fresh sisik nagas leaves aims to find out the characteristics of the leaves which include: leaf shape, leafflexibility, thickness, stability, texture, color, taste, and aroma of the leaves [12].

2.10 Phytochemical Screening

Phytochemical screening is the identification of secondary metabolite compounds with the aim of knowing the content of chemical compounds contained in the plant. Identification of compounds performed includes: flavonoid tests, alkaloid tests, steroid tests, saponins, and triterpenoids according to [13].

Flavonoids are effective as analgesics by inhibiting the work of cyclooxygenase enzymes by reducing prostaglandin production by arachidonic acid thereby reducing pain. Flavonoid compounds also play a role in stopping neutrophil degranulation causing inhibition of cytokines, free radicals, and enzymes that act as inflammation [14].

Alkaloid compounds are rationed as a trigger for the nervous system, increase blood pressure and reduce pain [15].

Steroids can prevent the formation of phospholipidenzymes thereby blocking the formation of prostaglandins and leukotrietes that result in analgesics being better than NSAIDs.

2.10.1 Flavonoid Test

As many as 1 g of simplisia powder is soaked in methanol, added 2N hydrochloric acid, heated. Filtered when hot, if the filtrate is red, orange, then it needs to be splattered with water until it is colorless. However, if the filtrate is green or brown, it does not need to be diluted. Puted into filtrate distilled water and added a solution of hexane, then shaken and removed the gas until the gas is lost. Taken the lower layer (methanol). Evaporated at a temperature of 40 ^{0}C then dissolved with ethyl acetate and filtered. The Filtrate is divided into 2 and used as a test solution.

a. Filtrate 1 ml is evaporated until dry and then dissolved in 2 ml of ethanol and then added 0.5 g of zinc powder and 2 ml of concentrated hydrochloric acid. It will give you a red solution.
b. Filtrate 1 ml is evaporated until dry and then dissolved in ethanol ml and then added 0.5 g of magnesium powder and 2 ml of concentrated hydrochloric acid. It will give it a red, purple, yellow or orange color.

2.10.2 Glycoside, Steroid and Triterpenoid Testing

As much as 2 grams of sisik naga leaf ethanol extract is dissolved in 2 ml of chloroform. Add a few drops of anhydrous acetic acid followed by the addition of concentrated sulfuric acid through the walls of the reaction tube on the chloroform extract. Positive glycosides are indicated by the onset of a bluish green ring between two layers [16]. Several grams of each simplisia powder are accelerated by 20 ml *n*-hexane for 2 hours, then filtered. The resulting filtrate is evaporated and the rest is added to the The Liberman-Bouchard reagent. If the purple or red color is formed indicates the presence of a free triterpenoid group and if there is a green or turquoise color indicates the presence of a group of free steroid compounds.

2.10.3 Saponin Testing

A sum of 0.5 g of simplisia powder, inserted in a test tube is 10 ml of hot water and shaken for 10 minutes, until foamy or more is formed and then dripped with HCl 2N then the extraction is positive to contain saponins.

2.10.4 Tannin Testing

A sum of 0.5 g of simplisia powder is filtered with 10 ml of distilled water and then filtered, the filtrate is diluted with distilled water until it is colorless. The solution is taken as much as 2 ml and added 1 to 2 drops of iron (III) chloride reagent 1%. If there is a blue or black color indicates the presence of tannins.

2.10.5 Alkaloid Examination

A total of 1 g of simplisia powder is put into the Erlenmeyer and dissolved with ethanol, added with 2N hydrochloric acid, then measured the acidic pH using litmus paper, heated over a water handler for 30 minutes, cooled and filtered. The obtained filtrate is used to examine alkaloids. Taken 3 test tubes as much as 6 drops. Alkaloids are positive if there are white deposits in Meyer reagents and there are brown or yellow deposits in dragendorf and bouchardat reagents. If it has not obtained results, then on the remaining filtrate contained in the Erlenmeyer added concentrated ammonia to base (check litmus paper). Put in a split funnel added 20 ml diethyl ether-chloroform with a ratio of 3: 1 then shake while expelling the gas occasionally until the gas is gone. Let stand for a while and then form 2 layers. Taken the bottom layer (*dietary-chloroform*) look in the cup vaporized, evaporated over the water handler until

the remaining 1/3 of the initial reservoir. Then added 2N hydrochloric acid to the acid. Take 1 ml inserted into the test tube which has each was marked with Erlenmeyer, dragendorf and bouchardat. Then tested each reagent as much as 6 drops. Alkaloids are positive if white deposits occur in reagent as much as 6 drops. Alkaloids are positive if white deposits occur in Mayer reagent and brown or brown yellow deposits at dragendorf and bouchardat reagents.

2.10.6 Data Analysis Setup

Data from the observation of the mice response will be tested using statistical tests with parametric methods in the form of *Shapiro Wilk*Test, *Kolmogorov-Smirnov* Test ANOVA, and TUKEY HSD test and then continued with testing of non-parametric methods in the form of *Kruskhal Wallis* to find out the variability of treatment treatment data. Parametric test is a statistical analysis that aims to find out which data variables have a normal value *(p => 0.05*) or not*(p = <0.05). Saphiro-Wilk* test is a method used to test basic data in small amounts so that the results obtained are distributed normally or not [17].The *Kolmogorov-Smirnov* test is a method used to test data that has a degree of similarity as well as to compare data that is empirical and based on the results of the treatment obtained by establishing a picture of the differences. The value p*(p=0.05)* states that the data is distributed normally [18]. ANOVA (*Analysis of Variance)* is data testing that aims to find out the exist or absence of data that has been distributed normally and homogeneously. The ANOVA test is divided into two tests. namely *One-Way* and *Two-Way* [19]. The Tukey HSD test is a data test that aims to find out the level of uniformity or high level of trust [20].

Non-Parametric test is a test that aims to find out the difference in data or in treatment related to the normality of the data. *Kruskhal-Wallis* is one of the non-parametric methods carried out with the aim of finding out the comparison of treatment and variations of data related to the normality of the data. Generally the Kruskhal Wallis method is tested on abnormal data *(p=<0.05)* [21].

3. RESULTS AND DISCUSSIONS

Condensed extracts obtained as much as 63 grams with a yield of 25.2%. The yield is the comparison of the amount (quantitative) extracts to be produced. The higher the yield value produced, the more the value of the extract will be. If the requirement of a good yield is 17% - 33%.

The results of phytochemical screening, it is proven that Sisik nagas Leaf contained alkaloids, flavonoids, glycosides and steroids

Water content of simplicia leaves of sisik nagas (Drymoglossum sp.) the results were 2.63%. Water content is a parameter to determine the residual water after the drying process. The water content obtained in the simplicia of sisik nagas leaves has met the requirements. The simplicia quality requirement is 10%

The result of ash level obtained is 0.43%.

The result of the macroscopic test, the leaves of sisik nagas are round and partially oval, green in color, odorless and taste bitter.

The result of microscopic test, the stomata shape of the sisik nagas leaves is anomocytic. Anomocytic stomata are guard cells surrounded by several neighboring cells of the same size. Stomata are plant organs that are used to obtain food and adapt to their environment. The function of stomata is as a place for gas exchange, such as CO2 which is needed by plants in the process of photosynthesis [22].

The results of this study are in the form of a squeaking response by licking the legs and jumping using the *Hot Plate Test* method at a temperature of 55^0C against white male mice*(Mus musculus).* For observations can be seen in **Table 1** and **Figure 1.**

Table 1. The average results of the hotplate method of testing the response of mice

Group	Before treatment	The average number of responses in mice After treatment			
		30 minutes	60 minutes	90 minutes	120 minutes
Treatment 0	42.4	47.2	31.4	36.6	43.2
Treatment 1	14	85.8	21.4	28	190
Treatment 2	45.6	29	30.6	30.2	26
Treatment 3	38.8	9.4	20	38.8	32.8
Treatment 4	15.6	14.2	0.4	1.4	0.2

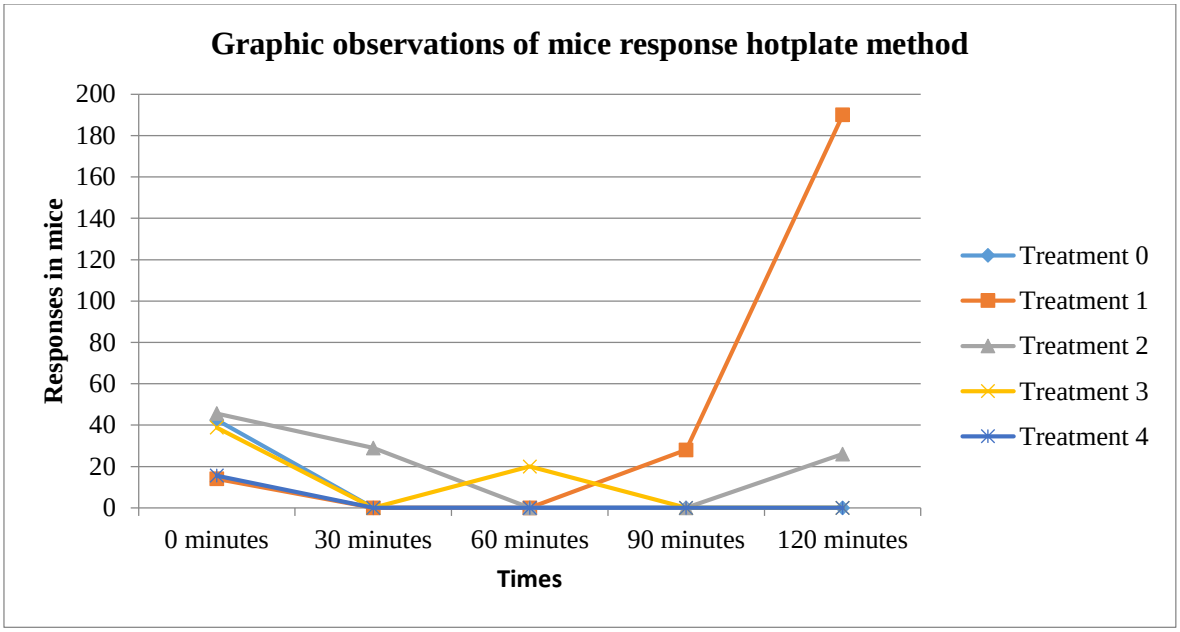

Figure 1 The results of observing the response of mice

In this study, the administration of test substances was done orally using oral sonde. After all the test substances are given to the mice, the mice are given time to rest for 15 minutes for the process of absorbing the drug into the body. The selection of male mice is done because the nature of the male sex is calmer and less aggressive so it is easy to do the treatment. While in mice the female can experience an estrus cycle that can make the female aggressive and stressed from time to time. Siklu's this hormone that can affect the results of research [23].

Pain caused by heat related to the ability to damage tissue. The reaction time required between the placement of the mice above *the Hot plate Test* and the appearance of the first response to the mice in the form of jumping or licking his legs as a reaction to reduce pain.

The results of the study obtained in three groups of mice experimental treatment given ethanol extract sisik naga leaf saw a decrease and an increase in average pain from mice before treatment and after treatment.
The results of analgesic testing with *the Hot Plate Test* method can be seen on the graph as in Figure 1, The average number of mice before administering Na CMC 0.5% is 42.4 times. At 30 minutes after cmc administration, the squeaking response increased to 47.2 times. In the 60th minute the squeak response decreased to 31.4 times, in the 90th minute it increased to 36.6 times and in the 120th minute experienced a significant increase of 43.2 times this proves that the tent is unstable This is caused by factors that affect the metabolism of drugs or extracts that are given in mice, among others, namely genetics or heredity, differences age, food, and disease [24].

The results of the study obtained in three groups of mice experimental treatment given ethanol extract sisik naga leaf saw a decrease and an increase in average pain from mice before treatment and after treatment. This average decrease in pain response of mice indicates the analgetic effect of sisik naga leaf ethanol extract. Based on the experimental group of extracts tested, an ethanol extract of sisik nagas leaves with a dose of 100 mg/kg BW produced a better effect compared to the dose of sisik naga leaf extak 25 mg / kg BW and a dose of sisik naga leaf extract 50 mg/ Kg BW. Data obtained from the results of the analgesic effect of sisik naga leaf ethanol extract on male mice, then tested with the statistical method *On- Sample Kolmogorov-Smirnov Test*, and *On Way* ANOVA *(Analysis of Variance)* with a confidence level of 95% and continued with a non-parametric test with *the Kruscal Wallis* method to see a meaningful comparison. The result is an abnormal distribution ($p < 0.05$). The Kruskal-Wallis test is therefore conducted to compare three or more sample data groups. *Kruskal-Wallis is* used when the assumption of normality is not met or the variable values are not the same [25].

4. CONCLUSION

The results of phytochemical screening, it is proven that Sisik nagas Leaf contained alkaloids, flavonoids, glycosides and steroids

Based on the results of data testing showed that in ethanol extract Sisik nagas with a dose of 100 mg / Kg BW were shown to show analgesic effects in the 30th minute and 60th minute with an average value of 14.2 times and 0.4 times and in the spss data results showed values of 0.143 in the

30th minute and 0.084 in the 60th minute. This is in line with research conducted by reseach of Antalgin reaching peak levels in blood plasma within 32 minutes and has a short half-life of 1-3 hours [26].

AUTHOR'S CONTRIBUTIONS

The author realizes that this journal still has many shortcomings, so it is expected of the reader to provide input for the sake of the perfectness of this journal.

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