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## The Potential Effect Of Ethanolic Extract Rambusa Leaf (*Passiflora foetida* L.) As Anti-inflammation To Male Mice (*Mus musculus*)

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**Abstract:** One of the plants known as herbal medicine in Central Java is Brambutan or Rambusa. This research conducted on experimental animal using ethanol extract of rambusa leaf (*Passiflora foetida* L.) which has an anti-inflammation effect on reducing edema. The carrageenan used-induced feet edema was 1 % and animal subjects were grouped into 5 categories for different treatments, namely the administration of 1 % colloidal Na-CMC solution, diclofenac sodium solution, ethanol extract with various doses of 250 mg/kg body weight, 500 mg/kg body weight, and 750 mg/kg body weight given orally. By the time after 1 hour, the edema volume on mice feet measured until 6 hours. The data showed that the 1 % Na-CMC colloidal solution given did not affect the percentage of edema reduction (0 %), the subject group was given 250 mg/kg body weight and 500 mg/kg body weight showed a decrease level of edema (0.01 %), the group was given 750 mg/kg body weight decreased edema (0.02 %), and diclofenac sodium solution group showed decrease level of edema (0.04 %). In this study, it can be concluded that the ethanol extract of rambusa leaf (*Passiflora foetida* L.) has the highest anti-inflammation and anti-inflammation effect at a dose variation of 750 mg/kg body weight with a 0.02 % reduction in edema

**Keywords:** anti-inflammation; rambusa; *Passiflora foetida* L

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### INTRODUCTION

Indonesia is one of the countries rich in medicinal plants so it is very useful as an alternative ingredient for treatment. Plants used as medicine are known by the name of medicinal plants. Traditional medicine is an ingredient or herb of ingredients in the form of plant ingredients, animal ingredients, mineral ingredients, galenic preparations or mixtures that have been used for health services for generations, and can be applied in accordance with the norms prevailing in society (Lambogia *et al.*, 2016)

Herbal medicine is in great demand by the public because usually the ingredients can be found easily in the environment around them. In general, the content of substances in natural ingredients is not only healing but also increases the body's resistance significantly. It is this condition that drives the development of medicine from plants and everything that is in nature (Suparni dan Wulandari, 2012).

Central Java is an island in Indonesia that is famous for its rich biodiversity. One of the plants that can be found in Central Java is Rambusa. Rambusa (*Passiflora foetida* L.) is known to the people of Central Java especially Purwodadi, Grobogan Village, Central Java as a Brambutan plant. People know this plant is a wild plant that grows in bushes and plateaus, usually the fruit is directly consumed fresh. Rambusa (*Passiflora foetida* L.) is a type of plant that is widely found creeping on other plants. Other plants are usually found in watery areas such as swamps and rivers (Lim, 2012).



In rambusa (*Passiflora foetida* L.) there is a flavonoid content which is a substance that can inhibit the inflammatory process, flavonoid compounds are said to have anti-inflammatory, antioxidant, and antimicrobial effects (Ilavarasan *et al.*, 2005). The presence of anti-inflammatory effects is thought to be due to the activity of secondary metabolites contained in the ethanol extract of rambusa leaves (*Passiflora foetida* L.) In the pharmaceutical world, increasing the efficiency of the use of traditional medicine can be done by making pharmaceutical preparations aimed at facilitating the use of these traditional medicines.

The writer wants to provide innovation by using ingredients from rambusa leaf ethanol extract from Central Java in extract preparations as an anti-inflammatory. Inflammation is usually treated using steroid-class anti-inflammatory drugs (AIS) and nonsteroidal anti-inflammatory drugs (AINS). Inflammation is a normal protective response to tissue injury caused by physical trauma, damaging chemicals, or microbiological substances. Anti-inflammatory is a designation for an agent or drug that works against or suppresses inflammatory processes (Dorlan, 2002).

Anti-inflammatory drugs from synthetic chemicals are widely used by the public because they have a rapid effect in eliminating inflammation but also have a risk of harmful side effects, including causing disturbances in the gastrointestinal tract, body circulation system, respiratory tract, metabolic processes, and hypersensitivity (Kertia, 2009). Based on the description above, researchers are interested in conducting a study on the effectiveness of rambusa leaf ethanol extract (*Passiflora foetida* L.) on male mice (*Mus musculus*) to determine the effectiveness of anti-inflammatory in terms of decreasing the volume of carrageenan-induced male mice foot edema.

## METHODS

This research is experimental with a laboratory approach that will be carried out with a series of experiments at the Pharmacology and Pharmaceutical Biology Laboratory of STIKES Telogorejo Semarang. Rambusa leaves (*Passiflora foetida* L.) used in the study were obtained from Grobogan Purwodadi Village, Central Java. The selected leaves are taken at 08.00-10.00 WIB. The test animals used were male mice (*Mus musculus*), with bodyweight 20-30 g, and aged 2-3 months. The condition of the animal is healthy. The number of male mice (*Mus musculus*) used was 15 heads which were divided into 5 groups and each group consisted of 3 mice.

## RESULT AND DISCUSSION

The result of the yield value is calculated based on the initial powder weight value and then compared with the total extract weight that has been obtained, the calculation of the yield value is empty bottle = 14.55 g, empty bottle + extract = 26.95 g, Extract weight = 12.4 g. Phytochemical tests used ethanol extract of rambusa leaves that have been dissolved with 96% ethanol solvent. The dissolved extract is then reacted with various reagents of the tested phytochemical compounds. The data for the description of the phytochemical test results can be seen in the following table.

**Table 1. Phytochemical Compound of Ethanolic Extract Rambusa Leaf (*Passiflora foetida* L.)**

Phytochemical Compound	Reagen	Result
Alkaloid	Mayer: brown Wagner : darkolive green	+ Alkaloid
Flavonoid	Ethanol + magnesium + concentrated HCl: The change of the surface layer to red.	+ Flavonoid
Tanin	The filtrate is filtered, then + 2 FeCl3 drops: Formed brown color.	+ Tanin
Terpenoid/Steroid	Extract + 3mL CHCL3 + 2 drops H2S04 and + CH3CO2 concentrated : Formed green color with blue top layer.	+ Terpenoid/Steroid
Saponin	Filtrate + warm water later strongly shaken for 1 minute, + concentrated HCL 2 drops: The foam persists for 1 minute.	+ Saponin



**Table 2. Rambusa Leaf Extract Results (*Passiflora foetida* L.)**

Sample	Wight Sample (g)	Weight Ekstrak (g)	Volume of Etanol 96 % (mL)	Duration of maceration (hours)
Rambusa leaf ( <i>Passiflora foetida</i> L.)	500	26,95	1500	72

**Table 3. Average Percentage decrease in Edema Volume of Soles of Feet Mice Treated With Peroral Administration of Test Preparations, Compared To Control Preparation**

Groups	Average (MI)
Na-CMC 1 % Solution	0
Rambusa leaf extract (250 mg/kg BB)	0,01
Rambusa extract (Dose 500 mg/kg BB)	0,01
Rambusa extract (Dose 750 mg/kg BB)	0,02
Diclofenac Sodium	0,04

**Table 4. Decreased Volume of Edema On Feet Mice After Given Peroral Administration Of Extract And Control Groups (mL)**

Groups	Animal Subject	Feet Volume (Every 1 Hour)								Level of edema
		Pre treatment	Induction	1	2	3	4	5	6	
Na-CMC	1	0,08	0,20	0,09	0,23	0,24	0,23	0,21	0,20	0,00
	2	0,09	0,18	0,21	0,20	0,25	0,23	0,21	0,21	-16,6
	3	0,09	0,16	0,20	0,23	0,21	0,23	0,24	0,24	-50
<b>Average</b>		<b>0,08</b>	<b>0,18</b>	<b>0,16</b>	<b>0,22</b>	<b>0,24</b>	<b>0,23</b>	<b>0,22</b>	<b>0,21</b>	<b>-16,6</b>
Dose 250 mg/kg	1	0,06	0,12	0,15	0,13	0,11	0,12	0,11	0,11	8,3
	2	0,10	0,13	0,18	0,20	0,17	0,15	0,14	0,13	7,6
	3	0,08	0,15	0,23	0,21	0,17	0,15	0,14	0,13	13,3
<b>Average</b>		<b>0,05</b>	<b>0,13</b>	<b>0,19</b>	<b>0,18</b>	<b>0,15</b>	<b>0,14</b>	<b>0,12</b>	<b>0,12</b>	<b>7,6</b>
Dose 500 mg/kg	1	0,06	0,10	0,16	0,17	0,15	0,13	0,11	0,09	10
	2	0,06	0,12	0,14	0,14	0,13	0,12	0,12	0,11	8,3
	3	0,10	0,14	0,18	0,12	0,17	0,15	0,14	0,12	14,2
<b>Average</b>		<b>0,07</b>	<b>0,12</b>	<b>0,16</b>	<b>0,14</b>	<b>0,15</b>	<b>0,13</b>	<b>0,12</b>	<b>0,10</b>	<b>16,6</b>
Dose 750 mg/kg	1	0,10	0,15	0,18	0,20	0,18	0,16	0,14	0,13	13,3
	2	0,07	0,12	0,19	0,22	0,17	0,12	0,11	0,10	16,6
	3	0,07	0,14	0,15	0,18	0,16	0,15	0,13	0,12	14,2
<b>Average</b>		<b>0,08</b>	<b>0,13</b>	<b>0,18</b>	<b>0,2</b>	<b>0,17</b>	<b>0,14</b>	<b>0,13</b>	<b>0,11</b>	<b>15,3</b>
Diclofenac sodium	1	0,06	0,15	0,18	0,17	0,15	0,13	0,12	0,10	33,3
	2	0,08	0,15	0,20	0,21	0,19	0,17	0,15	0,11	26,6
	3	0,06	0,17	0,18	0,13	0,11	0,12	0,11	0,12	29,4
<b>Average</b>		<b>0,07</b>	<b>0,16</b>	<b>0,19</b>	<b>0,17</b>	<b>0,15</b>	<b>0,14</b>	<b>0,13</b>	<b>0,11</b>	<b>31,2</b>



**Table 5. Percent Decrease Volume of Edema On Feet Mice After Induction**

Groups	Animal Subject	Volume of Edema (mL)			Level of Edema After Treatment*	Decrease of Edema (%)
		Pre treatment	Induction	Treatment		
Na-CMC	1	0,08	0,20	0,20	0	0
	2	0,09	0,18	0,21	0	0
	3	0,09	0,16	0,24	0	0
Dose 250 mg/kg	1	0,06	0,12	0,11	0,01	8,3
	2	0,10	0,13	0,12	0,01	7,7
	3	0,08	0,15	0,13	0,02	13
Dose 500 mg/kg	1	0,06	0,10	0,09	0,01	10
	2	0,06	0,12	0,11	0,01	8,3
	3	0,10	0,14	0,12	0,02	14
Dose 750 mg/kg	1	0,10	0,15	0,13	0,02	13%
	2	0,07	0,12	0,10	0,02	16%
	3	0,07	0,14	0,12	0,02	14%
Diclofenac sodium	1	0,06	0,15	0,10	0,05	33%
	2	0,08	0,15	0,11	0,04	26%
	3	0,06	0,17	0,12	0,05	29%

\*Level of Edema After Treatment =  $V_{induction} - V_{treatment}$

Inflammation is a normal protective response to tissue injury caused by physical trauma, damaging chemicals, or microbiological substances. Inflammation is usually divided into three phases, namely acute inflammation, immune response, and chronic inflammation. The symptoms that arise in inflammation are redness (rubor), heat (color), pain (dolor), swelling (tumor), and function (functio laesa). Rambusa leaves (*Passiflora foetida* L.) it is efficacious as an anti-inflammatory, sedative, urinary decay (diuretic), as well as cleansing heat and toxins. The fruit part is also efficacious in relieving pain (analgesics) and strengthening the lungs (Dalimartha, 2007). The extraction method used in this study is maceration extraction. The maceration method is a cold method (extraction process without heating) and is suitable for soft-textured samples. In addition, heating can lead to the destruction of the chemical content in simplicia. This method has the advantage that all parts of the sample can be in contact with the solution

### Sample collection

The obtained leaves of rambusa (*Passiflora foetida* L.) are collected and wet dissertation. The purpose of wet sorting is to separate the leaves from dirt or pebbles that are still attached to the rambusa leaves (*Passiflora foetida* L.). Then the leaves are separated from the stalk. Rambusa leaves (*Passiflora foetida* L.) then dried in a container then dried in the sun and covered with a black cloth. Then, rambusa leaves (*Passiflora foetida* L.) are reduced in size to a powder using a blender to facilitate contact with larger solvents to obtain good yields. Furthermore, in the test of the moisture content of rambusa leaves, a result of 7.31% was obtained, the results obtained were by following the standards of the Indonesian Herbal Pharmacopoeia Edition II (2017), namely the moisture content in simplicia  $\leq 10\%$ .

### Making Rambusa Leaf Ethanol Extract

Rambusa leaf powder (*Passiflora foetida* L.) of 500 g is put into a maceration vessel, then 96% ethanol solvent is introduced as much as 1500 mL. the sample is allowed to stand for 3 x 24 hours while occasionally stirring. The marinade is filtered to obtain its filtrate. The powder from the previous marinade was macerated again by adding 1500 mL of 96% ethanol solvent and allowed to stand for 3 x 24 hours while occasionally stirring. The result of the marinade is then filtered to get a second filtrate. The extract obtained is collected and then filtered and evaporated using a saucer on a water bath until it produces a concentrated extract, then evaporated again using a water bath until a viscous extract is obtained. Maceration is carried out by soaking simplicia as much as 500 g in a 96% ethanol coating liquid.

### Phytochemical screening



Phytochemical screening is carried out to give an idea of the compounds contained in the ethanol extract of rambusa leaves. The phytochemical screening results of rambusa leaf extract contain alkaloid compounds, flavonoids, tannins, terpenoids, and steroids, and saponins. In the wagner test, the K<sup>+</sup> metal ions will form a coordinate covalent bond with nitrogen in the alkaloid forming a precipitating potassium-alkaloid complex. In alkaloid tests with Mayer reagents, there is a white precipitate, it is estimated that nitrogen in alkaloids will react with K<sup>+</sup> metal ions from potassium tetraiodomercurate (II) to form a potassium-alkaloid complex that precipitates (Marliana et al., 2005).

The flavonoid identification test on the ethanol extract of rambusa leaves (*Passiflora foetida* L.) showed positive results in the presence of a change from dark-green to red-orange. This group of compounds is easily extracted in ethanol solvents which have polar properties due to the presence of hydroxyl groups so that hydrogen bonds can be formed (Sriwahyuni, 2010).

Phytochemical assay of tannin compounds by adding ethanol extract of rambusa leaves (*Passiflora foetida* L.) with FeCl<sub>3</sub> solution showed positive results. Phytochemical tests using FeCl<sub>3</sub> can show the presence of a phenol group, if there are phenol compounds, it is also possible that there are tannins, because tannins are polyphenol compounds. Greenish-brown discoloration occurs due to the formation of complex compounds between tannins and FeCl<sub>3</sub> (Harborne, 1987).

In terpenoid and steroid filtrate tests the extract was reacted with a lieberman-burchad reagent. According to Robinson (1995), when a triterpenoid compound is dripped lieberman-burchad reagent through its walls it will give a reaction to the formation of a brownish-green color, while the steroid will produce a bluish-green color.

In the saponin test, a positive result was obtained, namely the formation of foam. The formed foam remains viable for 1 minute after strong shaking. After the foam is formed, a 2-drop concentrated HCL is added to see if the foam drops back into a filtrate other wise the foam sample remains stable with the addition of a concentrate HCL.

### Testing of Anti-Inflammatory Effects

Anti-inflammatory testing is carried out using male mice (*Mus musculus*) as a test animal because male mice (*Mus musculus*) biological conditions are stable when compared to female mice whose biological conditions are affected by their cycle period (estrus). Anti-inflammatory testing on mice is based on the rat hind paw edema method, which is an artificial inflammatory swelling of the sole of the left foot of the carrageenan-induced test animal. Before treatment, each mice has fasted for 8 hours. Then weighed the body weight, to find out the volume of administration of the appropriate drug, then measured the initial volume of the left leg of the mice using a plethysmoneter. After that, each treatment group was carrageenan-induced by being injected intraplanelically in the left leg of the mice.

Based on the results of the studies obtained it was seen that the administration of a colloidal solution of Na-CMC did not affect the decrease in the percentage of inflammation of the legs of mice. This is because Na-CMC is only a solvent for medicinal media so that there is no stimulation in the form of drugs to reduce edema so that the edema will continue to increase and the process of eliminating inflammatory mediators in the body of mice only occurs naturally, so the percentage of edema reduction is 0%.

At the administration of ethanol extract of rambusa leaves (*Passiflora foetida* L.) at doses of 250, 500, and 750 the average inflammation increases slowly and continues until the 2nd hour and begins to decrease at the 3rd hour and continues until the 6th hour. In rambusa leaf ethanol extract (*Passiflora foetida* L.) at a dose of 250 there was a decrease in the average edema volume by 8.3 %, 7.6 %, and 13 % Rambusa leaf ethanol extract (*Passiflora foetida* L.) at a dose of 500 there was a decrease of 10 %, 8.3 %, and 14.2 % while in the ethanol extract of rambusa leaves (*Passiflora foetide* L.) at a dose of 750 there was a decrease of 13.3 %, 16.6 %, 14.2 % of the percentage decrease in edema volume was seen the presence of anti-inflammatory activity produced.



This is due to the possibility of flavonoid compounds contained in the ethanol extract of rambusa leaves (*Passiflora foetida* L.) which is known to play an important role in the inhibition of prostaglandins (PGE) and lipoxygenase (LOX). Flavonoids mainly act on the microvascular endothelium to reduce the occurrence of hyperpermeability and inflammation. Some flavonoid compounds can inhibit the release of arachidonic acid and the secretion of lysosome enzymes from the membrane by blocking the cyclooxygenase pathway. Cyclooxygenase reactions are the first step on the pathway leading to eicosanoid hormones such as prostaglandins and thromboxanes.

In the comparison group (diclofenac sodium) inflammation increases slowly and continues until the 2nd hour and begins to decrease at the 3rd hour and continues until the 6th hour. the decrease in the volume of edema of the comparison group was greater than that of the test solution with a percentage decrease in edema volume by 0.01 %, 0.01 %, 0.02 % meaning that the potential for inhibition of diclofenac sodium was greater than that of the test solution. This is because diclofenac sodium works by stabilizing the lysosomal membrane, inhibiting the release and activity of inflammatory mediators (histamine, serotonin, prostaglandins), inhibiting cell migration to the site of inflammation, and suppressing pain.

### **Average decrease in edema**

The decrease in edema was seen from the results of the decrease in swelling on the soles of the male mice measured in the negative control (Na-CMC 1 %) was 0 while the rambusa leaf ethanol extract (dose 250 mg/kg BB) was 0.01 and the rambusa leaf ethanol extract (dose 500 mg/kg BB) was 0.01 in rambusa leaf ethanol extract (dose 750 mg/kg BB) was 0.02 While in the positive control of diclofenac sodium was 0.04

## **CONCLUSION**

Ethanol extract of rambusa leaves (*Passiflora foetida* L.) provides anti-inflammatory effect. In rambusa leaf ethanol extract (*Passiflora foetida* L.) at a dose of 250 mg / kg BB, a dose of 500 mg / kg BB, and a dose of 750 mg / kg BB has an anti-inflammatory effect and the greatest anti-inflammatory effect is at a dose of 750 compared to other doses. The effectiveness of Decreased edema on the soles of male mice feet in negative control (Na-CMC 1 %) was 0 %while rambusa leaf ethanol extract (dose 250 mg /kg BB) was 0.01 % and rambusa leaf ethanol extract (dose 500 mg / kg BB) was 0.01 % in rambusa leaf ethanol extract (dose 750 mg / kg BB) was 0.02 % While in positive control diclofenac sodium was 0.04 %.

## **AUTHOR CONTRIBUTION**

O and TS carried out study concept, design and drafting of the manuscript. O and DA provide treatment to animal subject and prepared the extract. DA participated in statistical analysis with TS. All authors read and approved the final manuscript

## **CONFLIC OF INTEREST**

The declare that the research was conducted without any commercial or financial relationship that could be construed as a potential conflict of interest

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