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Toxicity Test of Fixed Dose Method on Ethanol Extract of Ramania Leaves (*Bouea macrophylla* Griffith.) from South Kalimantan

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ABSTRACT

Acute oral toxicity is a side effect that occurs in a short time through single oral administration or repeated doses within 24 hours and can occur in any organ of the body, one of which is the liver. Ramania (Bouea macrophylla Griffith.) is a herbal plant that has antioxidant properties and inhibits free radicals. The animals used in this study were female white rats of the Wistar strain. Acute toxicity tests were carried out orally with a fixed dose method on 4 groups of animals with dose groups of 50 mg/kgBW, 300 mg/kgBW, 2000 mg/kgBW and 5000 mg/kgBW. Each group consisted of 5 test animals. Observations were made on LD50, clinical conditions, body weight and pathological conditions of the test animals. Observations were made for 24 hours then continued until the 14 days. The results of this study were that there was no death of animals in all groups of test animals during the observation, namely the test animals did not experience diarrhea, aggressiveness, changes in breathing and significant changes in motor activity. In clinical conditions, hair loss occurred in the test animals. Based on the body weight of the test animals from 4 observation groups, a sig value of > 0.05 was obtained, meaning that there was no significant change in the body weight of the test group. From the results obtained by the BB group before treatment and the BB group on the 7th day with values of 0.921 and 0.314, there was no significant change in the body weight of the test group. However, in the BB group 14 with a value of 0.031 there was a significant difference in the body weight of the test group. Based on the toxicity test guidelines from BPOM RI, it can be concluded that it has an LD50 > 5000 mg / kg BB with a classification of non-toxic to animals and the administration of high doses of Ramania leaf ethanol extract (Bouea marcrophylla Griffith) orally to Wistar strain rats causes gastric ulcers.

Keywords : *Bouea macrophylla Griffith*, F*ixed Dose*

INTRODUCTION

The use of traditional medicine is recognized to have lower side effects than modern medicine, but it is necessary to pay attention to the safety of the active ingredients and their consistency, especially for routine use. In accordance with quality standards from the World *Health Organization (WHO),* traditional medicines must meet several requirements including quality, safety and efficacy (Aryzki & Susanto, 2019).

One of the plants commonly used as a medicinal ingredient is ramania leaves *(Bouea macrophylla Griffith)* which is a plant used as herbal medicine in Asia (Aryzki, 2019[2]). Previous research has shown that ethanol extract of ramania leaves has antidiabetic activity (Aryzki[2], 2019), analgesic (Familia, 2024) and biolarvicide (Aryzki & Budi, 2023).

Based on research (Astri *et al*., 2012) obtained the results of acute toxicity test per oral ethanol extract of dewa leaves on the condition of the gastric mucosa of male rats and female rats of the Wistar strain, can cause gastric ulcers at doses> 1.625 g / kgBW. Meanwhile, the level of safety and side effects of ramania leaf extract are not yet known so that scientific information regarding the efficacy and toxic effects that will be caused by ramania leaf extract still needs to be studied. So it is necessary to conduct this study to see the effect of toxicity of administering ethanol extract of ramania leaves on the function of the stomach of female white rats.

Considering that the use of ramania leaves is quite widespread in society, especially in the field of pharmacology. Meanwhile, the level of safety and side effects of ramania leaf extract are not yet known, so scientific information regarding the efficacy and toxic effects that will arise from ramania leaf extract still needs to be researched. Therefore, it is necessary to investigate the effect of the toxicity of acute administration of ramania leaf ethanol extract on the gastric function of female rats.

The purpose of the oral acute toxicity test is to identify the intrinsic toxicity of a substance, determine the target organ, and measure the sensitivity of the species to the substance. This test also provides information on acute hazards, safe and toxic doses, and LD50 values. In addition, the test results are used to design further toxicity tests, classify the level of hazard of the substance, and determine appropriate labeling based on the toxicity detected (BPOM, 2014; BPOM, 2022; Istiqomah, 2020; Ugwah-Oguejiofor CJ, 2019).

RESEARCH METHODS

Tools

The tools used are beakers (Pyrex®), Erlenmeyer glasses (Pyrex®), surgical tools (Wells Spencer®), filter paper, grinder, porcelain cup, mortar and stamper, animal balance (Presica Geinweigher GW-1500®), rotary evaporator (Heidolph VV-300®), 1 ml oral probe (Terumo®), analytical balance (Kern®), mikropipet, spektrofotometer UV-Vis (UV Mini SHIMADZU), funnel, hot plate, mouse cage, wire, animal feed and drink containers.

Materials

The materials that will be used in the research are Ramania leaves, 2N HCl, Dragendroff reagent, Mayer's reagent, $FeCl_3$, creatinine reagent, 0.9% NaCl, 10% NBF (Neutral Buffered formaline) solution, alcohol 96%, xylol, Haemotoxylin Eosin (HE), NA-CMC, paraffin and Aquadest, lactose (Quadrant), citric acid (Quadrant), tartaric acid (Quadrant), sodium bicarbonate (Brataco Chemica), magnesium stearate (Quadrant), aspartame (Quadrant), polyvinyl pyrrolidone (BASF), 95% alcohol (Brataco Chemica) and Wistar strain rat ethine.

Collection of materials and preparation of Ramania leaf extract

Ramania leaves were collected in Kandangan Baru Village, Pelaihari, South Kalimantan. 5 kg of leaf powder was extracted using the maceration method using a 10 L solvent, the extract was carried out for 3x24 hours with stirring every 6 hours. The liquid extract that is obtained is then collected and then evaporated over a water bath at a temperature of 50°C until a thick extract with a constant weight is obtained. The liquid extract that is obtained is then collected and then evaporated over a water bath at a temperature of 50°C until a thick extract with a constant weight is obtained.

Sample Preparation

Sample processing begins with simplicia processing from ramania leaves. The ready-made Ramania leaf simplicia was subjected to phytochemical screening to determine the content of secondary metabolites present in Ramania leaves. Then proceed with the extraction process with the maceration method. Extraction was carried out with 97% ethanolic solvent to obtain ethanolic extract of ramania leaves. Then a phytochemical screening was carried out on the ethanolic extract of ramania leaves to reconfirm the content of the compounds present in the ethanolic extract of ramania leaves (Aryzki, 2019).

Phytochemical Test Stage

The ethanol viscous extract was then taken a little and dissolved in 10 mL ethanol and then tested for its phytochemicals.

1. Flavonoids

1 mL of ethanol extract was added with 3 mL of 70% ethanol, and shaken, then heated in a water bath, and shaken again then filtered. The filtered filtrate was added with 0.1 g of Mg band and 2 drops of concentrated HCl. A positive test containing flavonoid compounds is indicated by the presence of red color (6).

2. Tannins

1 mL of ethanol extract dripped with 5 drops of 10% NaCl and filtered. The filtrate obtained was added with 1% gelatin and 10% NaCl. A positive test for the presence of tannins is indicated by the presence of a white precipitate (7).

3. Phenol

1 mL of ethanol extract plus 10 drops of 1% FeCl3. A positive test for the presence of phenolic compounds is the formation of red, blue, purple, black or green (6).

4. Flavonoids

1 mL of ethanol extract was added with 3 mL of 70% ethanol, and shaken, then heated in a water bath, and shaken again then filtered. The filtered filtrate was added with 0.1 g of Mg band and 2 drops of concentrated HCl. A positive test containing flavonoid compounds is indicated by the presence of red color (6).

5. Saponins

1 mL of ethanol extract was mixed with 2 mL of distilled water and shaken for 1 minute, then added 2 drops of 1N HCl. Positive test for the presence of saponin compounds if a stable foam is formed ± 7 minutes (6).

6. Tannins

1 mL of ethanol extract dripped with 5 drops of 10% NaCl and filtered. The filtrate obtained was added with 1% gelatin and 10% NaCl. A positive test for the presence of tannins is indicated by the presence of a white precipitate (7).

7. Steroids and Triterpenoids

1 mL of ethanol extract was added $(CH_3CHO)_2O$ and concentrated H_2SO_4. The presence of steroid compounds is indicated by the formation of green or blue color. The presence of triterpenoid compounds is indicated by the formation of a golden yellow, yellow or purple color (6).

8. Alkaloids

1 mL of ethanol extract was added with 2 mL of 2N HCl and shaken. The mixture was then divided into 3 different tubes. In each tube, 1 drop of Mayer's reagent is

added to the first tube, 1 drop of Dragendorff's reagent to the second tube, and 1 drop of Wagner's reagent to the third tube.

The presence of alkaloid compounds if the addition of Mayer's reagent forms a yellow precipitate, the addition of Dragendorff's reagent forms a red precipitate and the addition of Wagner's reagent forms brown or red precipitate (7).

Adaptation of Test Animals

Before the treatment was carried out on the mice, acclimatization was carried out for 7 days. The acclimatization process is carried out so that the mice can adapt to the new environment and to determine the suitability of the mice to be used. The criteria for mice selected were mice that behaved normally, were healthy and did not increase their body weight by more than 10% (8). The test animals are given BR-1 feed and water. The test animal cages are cleaned every 2 days.

Division of Test Animal Groups

The animals used in this study were female white rats of the Wistar strain. Acute toxicity tests were conducted orally with a fixed dose method on 4 groups of animals with doses of 50 mg/kgBW, 300 mg/kgBW, 2000 mg/kgBW and 5000 mg/kgBW. Each group consisted of 5 test animals. Observations were made for 24 hours then continued until the 14th day. There was no control group in this study.

Acute Toxicity Test

Dissolved 100 mL of 0.5% Na CMC suspension. Then proceed with making a suspension of ramania leaf extract weighed at a dose of 50, 300, 2.000, and 5.000mg/kg BW each, put into a mortar and add 10mL of 0.5% Na-CMC to each -each dose of extract and grind until homogeneous. LD50 measurements and delayed toxicity testing (decreased movement activity, diarrhea, sleep, respiratory movements and death) refer to BPOM (10), namely that mice were divided into 4 groups with each group consisting of 5 mice then fasted before being given treatment but drinking water. may be given. After fasting, the mice were weighed and given the test preparation in a single dose using an oral probe to the test animals.

Symptoms of toxicity were observed in mice periodically during the first 4 hours at 5, 30, 60, 120, 240 minutes and once a day for 14 days. Then visual observations were carried out, namely that the mice experienced a decrease in motor activity, aggressiveness, respiratory movements, sleep, diarrhea and death. During the experiment, the animals were weighed every 48 hours, food and water intake was monitored. After 14 days, the number of dead and living mice was counted to calculate the LD50 value, then surgery was carried out on the test animals to remove the gastric ulcer organs and carry out a histology examination of the organs (10,11).

RESULTS AND DISCUSSION

Collection of Ingredients and Processing of Simplicia Powder

Samples and ramania (*Bouea Marophylla Grifth*) were collected in Kandanagn Baru Village, Pelaihari, South Kalimantan. 5 kg of ramania leaves are picked directly from the tree, then wet sorted and washed. The ramania leaves are then chopped to get a smaller size, then dried in a drying cabinet at 50°C.

Dried Bouea marcrophylla Griffith leaves were obtained as much as 2,336 g and then powdered. The powder obtained was 504.81 g, so it can be concluded that 21.60% of the weight of fresh Bouea marcrophylla Griffith leaves was lost in the drying and pollination process.

Table 1. Results Of Extract Processing

Simplicia Weight	Extract Weight	Rendeman
2.336 gram	504.81gram	21.60%

The yield value functions to determine how much secondary metabolite levels are carried by the solvent (Sari et al., 2021). The higher the yield value, it indicates that the extract contains high levels of compounds (Wijaya et al., 2018). The requirement for a good yield is not less than 10% (Badriyah & Farihah, 2022).

Plant Determination

Determination aims to find out the true identity of the plants used. The determination was carried out at the Ministry of Research, Technology and Higher Education, Lambung Mangkurat University, FMIPA Basic Laboratory. Based on letter number 086/LB.LABDASAR/III/2019 issued in Banjarbaru on March 25 2019, it states that the plant used is the Bouea marcrophylla Griffith species.

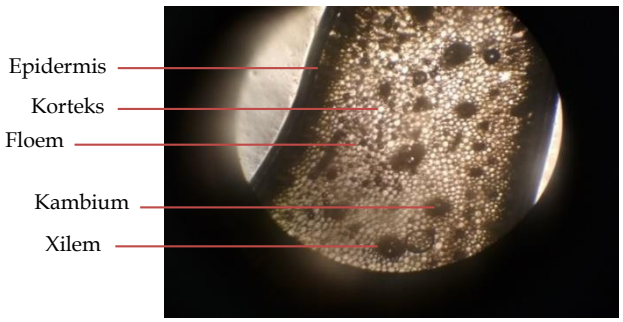
Simplicia Standardization

Organoleptic examination of wet simplicia ramania leaves is that they have a dark green color, with a characteristic smell of ramania leaves, the bitter and astringent taste of simplicia ramania leaves is thought to contain alkaloid compounds (Kuspradini *et al*, 2016) and saponins (Lien *et al*., 2013).

Meanwhile, the organoleptic examination of dried simplicia ramania leaves is that they have a dark green color, with a characteristic smell of ramania leaves, a bland and astringent taste. Microscopic examination of ramania leaves is 20 cm long, 6.8 cm wide, the tip of the leaf is cirrhose, green with a smooth texture, the base of the leaf is obtuse, the edge of the leaf is clenulate, the leaf veins are undulate.

Picture 1. Characteristics of Ramania Leaves

Based on the results of the microscopic examination, there are upper epidermal cells and lower epidermal cells, transport bundles in the form of xylem and phloem and cortex, while in the longitudinal section, the cell walls, cytoplasm and stomata are visible on the lower surface of the leaf with an anomocytic type.



Picture 2. Longitudinal cross-section of a Ramania (*Bouea marcrophylla Griffith)* leaf

The test results for levels of dissolved compounds in certain solvents aim to provide an overview of the number of compounds contained in the sample. The value of the watersoluble essence of Bouea marcrophylla Griffith leaves is greater than the value of the ethanol soluble essence so that the number of compounds contained is more soluble in nature.

Compounds that are thought to dissolve in water solvents are carbohydrates, saponins, tannins, quaternary alkaloids, sugars, amino acids and some vitamins. Compounds that are thought to be dissolved in ethanol solvents include terpenoids, alkaloids, phenols, wax glycosides, lipids and volatile oils.

Extract Standardization

The ethanol extract of Ramania (*Bouea marcrophylla Griffith)* leaf on organoleptic examination showed the same results. The ethanol extract of Ramania (*Bouea marcrophylla Griffith)* leaf has a dark green color, the characteristic smell of ramania leaves is quite strong and has a bitter, astringent taste. The bitter taste is caused by the presence of secondary metabolites such as alkaloids and saponins found in the ethanol extract of Ramania (*Bouea marcrophylla Griffith)* leaf.

This was proven by the phytochemical screening carried out which showed it was positive for containing alkaloids, salkowaski, tannins, triterpenoids, glycosides, interquinones, saponins, phenolics, flaphonoids. The results of phytochemical screening from the ethanol extract of Ramania (*Bouea marcrophylla Griffith)* leaf can be seen in Table 2.

Table 2. Results Of Standardization Parameters Of Bouea Marcrophylla Griffith Leaf Simplicia

No.	Standardization Parameters	Result	Requirement (MMI & BPOM RI)
1	Extract yield	21.50%	-
2	Water content	2.1%	≤10%
3	Acid insoluble ash content	26.5%	≥0,7%
4	Results of water soluble essence content	23.7%	≥16,00%
5	Results of ethanol soluble essence content	15.6%	≤8,00%
6	Cu levels	0.02 ppm	<10mg/kg
7	Mn levels	1.80 ppm	<11mg/kg

The drying shrinkage parameter aims to determine the amount of compounds lost or evaporated during the drying process. If the value of the drying shrinkage is smaller, the better the drying process carried out on the sample. This means that the water content in the sample will be smaller, thereby reducing the possibility of fungus growing on the simplicia. The compounds lost during the drying process include water, essential oils and volatile compounds (Rizqa, 2010).

Determination of the total ash content shows the inorganic compounds contained in the leaf simplicia of Ramania (*Bouea marcrophylla Griffith)* leaf. The higher the total ash content in a sample, the worse the sample quality (Apriyantono *et al*., 1989; Nugraheni *et al*., 2015).

Determination of acid insoluble ash content shows the presence of acid insoluble inorganic compounds such as soil or sand that are still attached to the leaf simplicia of Ramania (*Bouea marcrophylla Griffith)* leaf. This can be caused by contamination that occurs through the air or the sample treatment area during the process of taking the leaves until they become powder. Determining the ash content is one of the most important parameters in evaluating raw materials for traditional medicine, because it is related to the level of safety of using simplicia as a raw material for traditional medicine.

The Cu content of Ramania (*Bouea marcrophylla Griffith)* leaf simplicia has the highest Cu content, namely 0.022 ppm. This is because the location where Ramania (*Bouea marcrophylla Griffith)* leaf grows is close to lake construction activities. These construction activities use intensively moving machines, where one source of Pb pollution is the combustion of vehicle fuel (Reffiane *et al*., 2011). Cu metal can contaminate plants through stomata that are open during the day on the surface of the leaves (Antari & Sundra, 2002).

The Mn content of Ramania (*Bouea marcrophylla Griffith)* leaf simplicia has a relatively low Mn content of 1.80 ppm. This can be seen from the fact that the Mn content in the plantation area is relatively low and below the quality standard. The results of phytochemical screening from Ramania (*Bouea marcrophylla Griffith)* leaf can be seen in Table 3.

Table 3. Results Of Phytochemical Screening Of The Ethanol Extract Of Ramania (*Bouea Marcrophylla Griffith)* Lef

No.	Phytochemical Compounds	Results
1	Alkaloids	+
2	Salkowasaki	+
3	Tannin	+
4	Triterpenoids	+
5	Glycosides	+
6	Interquinone	+
7	Saponins	+
8	Phenolic	+
9	Flaphonoid	+

The results of observing the TLC pattern showed that samples taken from Kandangan Village, Baru Pelaihari, South Kalimantan showed almost the same chromatogram pattern. Based on the elution results, in the nonpolar mobile phase there were 5 spots on the sample, while in the polar mobile phase there were 3 spots on the sample. The results of observing the Rf value show that the chromotogram profile of the sample contains compounds. The Rf value of the ethanol extract Ramania (*Bouea marcrophylla Griffith)* leaf can be seen in Table 4.

Table 4. Rf Value Of Ethanol Extract Of Ramania (*Bouea marcrophylla Griffith)* Leaf With H2SO4 Spray Reagent

No.	Rf value of Ramania (*Bouea marcrophylla Griffith)* leaf extract n-hexane acetate mobile phase	Rf value of Ramania (*Bouea marcrophylla Griffith)* leaf extract, mobile phase chloroform: methanol
1	0.24	0.07
2	0.41	0.21
3	0.77	0.97
4	0.9	
5	0.94	

Determination of water content in this study used the distillation method using water-saturated toluene solvent. The water content is determined to maintain the quality of the extract and avoid rapid growth of fungi in the extract (Arifin et al, 2006). The water content of the ethanol extract of Ramania (*Bouea marcrophylla Griffith)* leaf is 2.1% - 21.50%, which means it meets the specified standard requirements. The higher the water content, the easier it is for fungus or mold to grow, thereby reducing the biological activity of the extract during storage.

Testing the total ash content and acid insoluble ash content in the extract aims to determine the content of

inorganic compounds or total minerals and acid insoluble inorganics mixed in the sample during the extraction process. The total ash content and insoluble ash content of the extract acid obtained were smaller than the total ash content and insoluble ash content of simplicia acid. This shows that during the process of making the extract, inorganic compounds such as minerals or sand and soil that are not soluble in acid are not absorbed during the extraction process. The value of total ash content and acid insoluble ash content should have a small value because these parameters are related to the safety level of the extract as a raw material for traditional medicine (Sapna *et al*., 2008).

The results of determining the yield, water content, total ash content and acid insoluble ash content from the ethanol extract of Ramania (*Bouea marcrophylla Griffith)* leaf are presented in Table IV. Based on this data, it can be said that during the extraction process, several minerals, both internal and external, such as sand and soil, were not absorbed along with the extract. The total ash content and acid insoluble ash content of the ethanol extract of Ramania (*Bouea marcrophylla Griffith)* leaf meet the maximum ash content limits regulated in the Indonesian Materia Medika.

Acute Toxicity Test

This research was conducted to detect the intrinsic toxicity of a substance and the effect of administering Ramania (*Bouea marcrophylla Griffith)* leaf on gastric function in mice. This research has passed the ethical test of the Research Ethics Commission of Sari Mulia University Banjarmasin with No. 032/KEP-UNISM/XI/2023. The test animals used were 20 animals which were divided into 4 groups randomly.

The acute toxicity testing method used is based on the BPOM non-clinical in vivo toxicity test guidelines (8). In this acute toxicity test research, the fixed dose method was used. This method is used for test materials with a moderate degree of toxicity and the dose chosen is one that does not cause death, severe pain or is irritating/corrosive.

Acute toxicity testing of ramania leaf extract effervescent tablets was carried out with four dose variations, namely doses of 50mg/KgBW, 300mg/KgBW, 2.000mg/KgBW, and 5.000mg/KgBW. From the four doses tested, it was seen that the test animals did not experience diarrhea, aggressiveness, or respiratory changes. and significant changes in movement activity. Mice seem to sleep more, this is natural because mice are animals that are active at night or nocturnal animals. The treatment also did not cause death in the test animals, however the administration of ethanol extract Ramania (*Bouea marcrophylla Griffith)* leaf had an effect on kidney function as seen from the creatinine values and kidney histology. The observations made on the organ systems are as in the Table 5 (BPOM, 2014; 2020).

Table 5. Observations On Test Animals

No.	Organ Systems	Examination Organ Systems
1	CNS & Samatomotor	Behavior
		Movement
		Activeness to stimuli
2	Breathing	Eel and breathing rate
3	Cardiovascular	Regional palpitations cardiac
4	Gastrointestinal tract	Incident
		Stool consistency
5	Skin and Fur	Color and wholeness
6	Mucous membrane	Conjunctiva, mouth
7	Eye	Eyelid
		Eyelashes
		Transparency

LD50 Value Between Low Dose and High Dose Groups

Table 6. LD50 Values Between Groups

No.	Group	LD50	
		Treatment	Number of Dead Rats
1	I	Ethanol Extract of Ramania Leaves (*Bouea macrophylla Griffith)* 50 mg/kgBB	0
2	II	Ethanol Extract of Ramania Leaves (*Bouea macrophylla Griffith)* 300 mg/kgBB	0
3	III	Ethanol Extract of Ramania Leaves (*Bouea macrophylla Griffith)* 2.000 mg/kgBB	0
4	IV	Ethanol Extract of Ramania Leaves (*Bouea macrophylla Griffith)* 5.000 mg/kgBB	0

Behavior

Qualitative observations based on observations after oral administration of ethanol extract Ramania (*Bouea marcrophylla Griffith)* leaf were carried out on the LD_{50}, clinical condition, body weight and pathological condition of the test animals. Observations were made at 5, 30, 60, 120,

240 and once a day for 14 days for 24 hours then continued for up to 14 days. The results of this study were that there were no animal deaths in all groups of test animals during observation. In the clinical condition of the test animals, Group I on the 14th day there was a change in color of the feathers, group II on the 10-14th day there was a color change in the feathers and on the 14th day the feathers fell out; group III animals are inactive (weak) and there is a change in color of the fur and hair loss; group 4 on days 7-14 there was a change in color of the feathers and feather loss. Observations of sensory, motor activity, changes in the neuromuscular system, eyes, respiratory, skin, gastrointestinal and gastrourinary changes as well as body posture show that they are normal. Results showed no deaths.

Changes in body weight

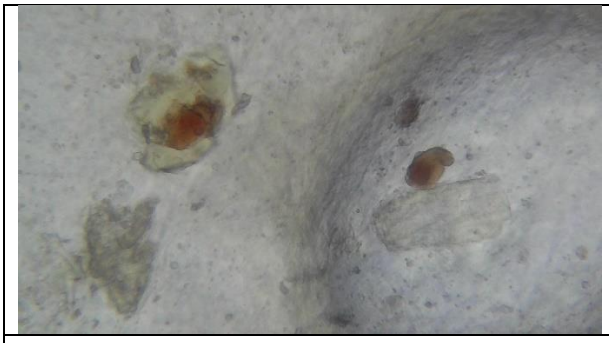
Based on the body weight of the test animals from the 4 observation groups, the sig value > 0.05 means there is no significant change in the weight of the test group, on day 7 the values are 0.921 and 0.314, meaning there is no significant change in the weight of the test group. However, in the weight group 14 day with a value of 0.031, it means there is a significant difference to the weight of the test group.

Tabel 7. Test Animals Weight

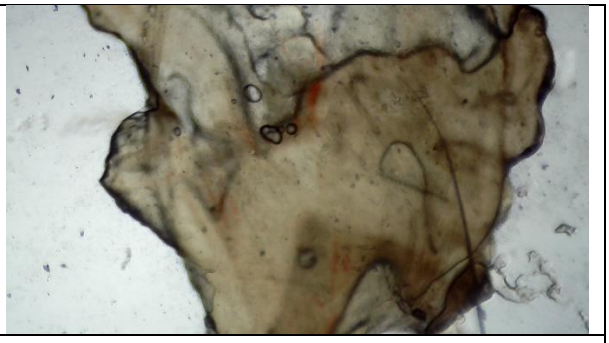
No.	Animals Group	Avarage BW		
		Before	hari ke-7	Before
1	Group I (50mg/KgBW)	223.8±41	234.4±42.16	227.2±57.69
2	Group II (300mg/KgBW)	177.2±27.64	196.4±33.55	214.2±35.42
3	Group III (2.000mg/KgBW)	192.8±29.93	193±36.54	221.8±24.69
4	Group IV (5.000mg/KgBW)	180.8±37.96	208.8±31.08	224±33.18

Macroscopic Observation Of The Gastric Ulcer

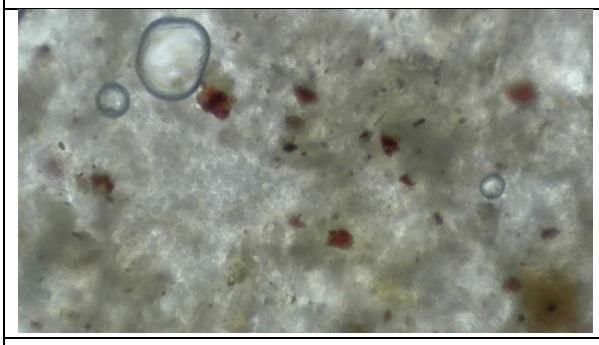
Organ observations were carried out on the stomach. The observation results can be seen in Picture 3.



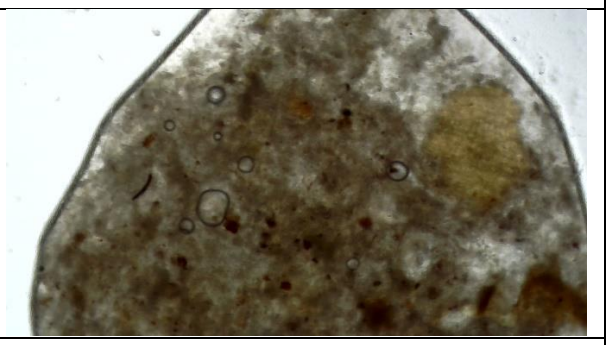
Group I Rat gastric ulcer
Ethanol extract Ramania (*Bouea marcrophylla Griffith)* doses 50 mg/kbBB



Group II Rat gastric ulcer
Ethanol extract Ramania (*Bouea marcrophylla Griffith)* doses 300 mg/kgBB



Group III Rat gastric ulcer
Ethanol extract Ramania (*Bouea marcrophylla Griffith)* doses (Bouea macrophylla griffith) 2,000 mg/kgBB



Group IV Rat gastric ulcer
Ethanol extract Ramania (*Bouea marcrophylla Griffith)* doses 5,000 mg/kgBB

Picture 3. Macroscopic Examination Of The Gastric Ulcer Of Test Animals

In research (Mustapa, 2018) active compounds contained in medicinal plants are almost always toxic when given in high doses. All poisoning occurs due to the reaction between toxic substances and receptors in the body. Oral administration of clove flower ethanol extract causes the active substances contained in clove flower extract to be absorbed in the digestive tract and then undergo a distribution and metabolism process. Toxic metabolic products work as enzyme inhibitors for the next stage of metabolism. The reaction between the active substance and the receptor in the effector organ causes symptoms of poisoning.

Gastric ulcer is a tear in the gastric mucosa that penetrates the muscularis mucosa and extends to more than 5 mm in diameter. If there is a change in the gastric defense mechanism, it can cause changes in the gastric mucosa which will eventually result in erosion and then ulceration. In this study, The flavonoid content in ethanol extract Ramania (*Bouea marcrophylla Griffith)* leaf has an inhibitory effect on lipoxygenase and cyclooxygenase (17). As a product of the cyclooxygenase pathway, prostaglandins have a protective effect on the stomach.

Decreased prostaglandins will cause a decrease in mucus production, phospholipids, HCO_3^- secretion, mucosal cell proliferation, and gastric microvascular flow. This causes discontinuity in the gastric mucosal epithelium or even deeper, which is known as a gastric ulcer (Schaimman, 2009). This mechanism may be the cause of gastric ulcers after administration of ethanol extract Ramania (*Bouea marcrophylla Griffith)* leaf. Based on the microscopic results of gastric ulcers in all groups of test animals there were ulcers as in Picture 3. This is because the compounds contained in medicinal plants do not work alone to produce a cumulative (not parallel) effect (18).

Gastric toxicity which appears as erosion and ulcers is caused by compounds that damage the surface of the gastric membrane (Picture 3B), in the form of hyperemic mucosa. Damage to the walls of the gastrointestinal tract can be assessed by dissecting experimental animals and observing lesions on the mucosa. This method is based on observing bleeding areas caused by cell damage and blood vessel damage. 13 To confirm the damage that has occurred, a histopathological examination is carried out and it appears that the damage is limited to the mucosa (Picture 4A), so it is referred to as erosion. Damage that occurs has penetrated the deeper mucosal layer of the stomach, which is called an ulcer (Picture 4B).

Based on the results of the acute oral toxicity test of the ethanol extract Ramania (*Bouea marcrophylla Griffith)* leaf on the condition of the gastric mucosa of male rats and female rats of the Wistar strain, it can be concluded that high doses of ethanol extract Ramania (*Bouea marcrophylla Griffith)* leaf given orally to rats of the Wistar strain cause gastric ulcers.

CONCLUSIONS

Based on the toxicity test guidelines from BPOM RI, it can be concluded that it has an LD50 > 5000 mg/kg BW with a classification of non-toxic to animals and high doses of ethanol extract of Ramania leaves (Bouea marcrophylla Griffith) given orally to Wistar strain rats cause gastric ulcers.

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