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Acute Toxicity Test Oral Effervecent Tablet Ethanol Extract of Ramania Leaves (Bouea macrophylla Griffith.) from South Kalimantan

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Abstract

Ramania (Bouea macrophylla Griffith.) is a herbal plant that has antioxidant properties and inhibits free radicals. This research aims to evaluate the safety of effervescent tablets of the ethanol extract of ramania leaves with acute toxicity test. The experimental animals used in this research were female Wistar white rats. The acute toxicity test was carried out orally using the fixed dose method in 4 groups of animals with dose groups of 50 (I), 300 (II), 2000 (III) and 5000 mg/kgBW (IV) respectively. Observations were made on the LD50, clinical condition, body weight and pathological condition of the test animals. The results of this study were that there were no animal deaths in all groups of test animals during observation. In clinical conditions, the 4 groups experienced discoloration and hair loss, while in groups III and IV the animals were inactive (weak). 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. It can be concluded that the effervescent tablets of the ethanol extract of ramania leaves have an LD50 > 5000 mg/kg BW with a classification of non-toxic to animals.

Keywords: *Bouea macrophylla Griffith, Effervescent, Fixed 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 & Budi, 2023). 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 & Budi, 2023). Previous research has shown that ethanol extract of ramania leaves has antidiabetic activity (Aryzki et al., 2019), analgesic (Familia et al., 2023)and biolarvicide (Aryzki & Febrianti, 2023). The development of dosage forms is also carried out to make it easier to use this extract, one of which is made in the form of effervescent tablets.

In order to develop and use herbal medicines, it is not enough to just test the efficacy. It is necessary to carry out safety tests such as toxicity tests, one of which is acute toxicity tests in pharmaceutical preparations.

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 studied. Therefore, it is necessary to study the toxicity effect of acute administration of ramania leaf ethanol extract effervescent tablets on gastric function in female rats.

The oral acute toxicity test aims to detect the intrinsic toxicity of a substance, determine

target organs, species sensitivity, obtaining hazard information after exposure to a substance acute, obtain initial information that can be used to establish dose levels, design subsequent toxicity test, obtaining the LD50 value of a material/preparation, as well as determining classification ingredients/preparations and labeling (BPOM, 2014; BPOM, 2022; Istiqomah, 2020; Ugwah-Oguejiofor, 2019).

RESEARCH METHODOLOGY

Materials and methods

The tools used are glassware (Pyrex®), surgical tools (Wells Spencer®), animal balance (Presica Geinweigher GW-1500®), rotary evaporator (Heidolph VV-300®), 1 ml oral probe (Terumo®), analytical balance (Kern®).

The materials that will be used in the research are Ramania leaves, 96% ethanol, 2N HCl, Dragendroff reagent, Mayer's reagent, FeCl3, creatinine reagent, 0.9% NaCl, 10% NBF (Neutral Buffered formaline) solution, alcohol (70%,80%,90%,95% and 100%), 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. 300 g of leaf powder was extracted using the maceration method using a 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. Collection of materials and preparation of Ramania leaf extract

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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 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 (Aryzki & Susanto, 2019)

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 (Aryzki & Susanto, 2019)

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 (Aryzki & Susanto, 2019)

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 (Aryzki & Susanto, 2019)

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 (Aryzki & Susanto, 2019)

Steroids and Triterpenoids

1 mL of ethanol extract was added (CH3CHO)2O and concentrated H2SO4. 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 (Aryzki & Susanto, 2019)

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 (Aryzki & Susanto, 2019)

Making effervescent tablets from Ramania Leaf Extract begins with making effervescent granules first before pressing them. Effervescent granules are made separately between acid granules and alkaline granules to avoid premature effervescent reactions. The extract is granulated first with lactose. The resulting granules are called extract granules. Acid granules are made by mixing extract granules, citric acid, tartaric acid, and some PVP. Meanwhile, alkaline granules are made by mixing sodium bicarbonate with the remaining PVP. Making effervescent granules is carried out in a place where room temperature and air humidity are maintained. PVP was added in dry form, then moistened with 95% ethanol drop by drop. The mass to be ranulated is then sieved with a 14 mesh sieve to obtain granules of a homogeneous size. The granules are then dried in an oven at a temperature of 40-60°C. After drying, magnesium stearate was added to the granules and then their physical properties were tested (Fitria et al., 2022)

Then the granules are compressed to form effervescent tablets. The tablet pressing room was conditioned for 30 minutes by setting the room temperature below 25°C and maintaining room humidity. Tablets are made by flowing a certain amount of granule mass from the hopper into a die hole of a certain size, then the mass that has entered is compressed by pressure resulting from the meeting between the upper punch and the lower punch (Puspita Tanjung et al., 2018).

Acute Toxicity Test

Dissolve 100 mL of 0.5% Na CMC suspension. Then proceed with making a suspension of ramania leaf extract effervescent tablets weighed at a dose of 50, 300, 2,000, and 5,000mg/KgBW 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 (2022), 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 kidney organs and carry out a histology examination of the organs (BPOM, 2022).

RESULT AND DISCUSSION

Collection of Ingredients and Processing of Simplicia Powder

Samples and ramania (*Bouea Marophylla Grifth*) were collected in Kandangan 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 offresh *Bouea marcrophylla Griffith* leaves was lost in the drying and pollination process.

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). Characteristics of Ramania Leaves can be seen ini **Figure 1**.

Figure 1. Characteristics of Bouea marcrophylla Griffith Leaves

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

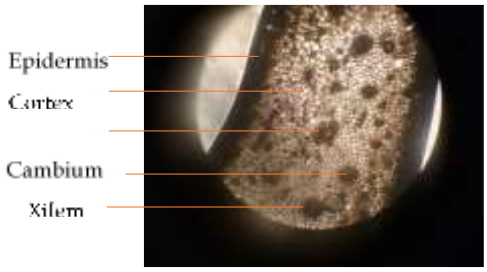

Figure 2. Longitudinal cross-section of a *Bouea marcrophylla* Griffith Leaves

are undulate. Longitudinal cross-section of a *Bouea marcrophylla Griffith* leaf can be seen in **Figure 2.**

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.

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 water soluble 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 *Bouea marcrophylla Griffith* leaves on organoleptic examination showed the same results. The ethanol extract of *Bouea marcrophylla Griffith* leaves 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 *Bouea marcrophylla Griffith*. This was proven by the phytochemical screening carried out which showed it was positive for containing alkaloids, salkowaski, tannins, triterpenoids, glycosides, interquinones, saponins, phenolics, flavonoids. The results of phytochemical screening from the ethanol extract of *Bouea marcrophylla Griffith* leaves can be seen in **Table 1.**

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 *Bouea marcrophylla Griffith*. The higher the total ash content in a sample, the worse the sample quality (Apriyantono et al., 1989; Nugraheni et al., 2015).

Table 1. Phytochemical Screening of ethanol extract of *Bouea marcrophylla* Griffith leaves

Phytochemical Compounds	Results
Alkaloids	+
Salkowasaki	+
Tannin	+
Triterpenoids	+
Glycosides	+
Interquinone	+
Saponins	+
Phenolic	+
Flavonoid	+

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 *Bouea marcrophylla Griffith*. 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 *Bouea marcrophylla Griffith* leaf simplicia has the highest Cu content, namely 0.022 ppm. This is because the location where *Bouea marcrophylla Griffith* 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 *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 *Bouea marcrophylla Griffith* can be seen in **Table 2**.

Table 2. Results of standardization parameters of *Bouea marcrophylla* Griffith leaf simplicia

Standardization Parameters	Result	Requirement (MMI & BPOM RI)
Extract yield	21.50%	-
Water content	2.1%	≤10%
Acid insoluble ash content	26.5%	≥0.7%
Results of water soluble essence content	23.7%	≥16.00%
Results of ethanol soluble	15.6%	≤8.00%
essence content		
Cu levels	0.02 ppm	<10mg/kg
Mn levels	1.80 ppm	<11mg/kg

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 results of stains that appear to be reddish in color and the stains on the graded macerated ethanol extract are 5, while the results of the stains on the total macerated methanol extract are 3. Rf value of *Bouea marcrophylla Griffith* leaf extract n-hexane acetate mobile phase is 0.24; 0.41; 0.77; 0.9 and 0.94. Rf value of *Bouea marcrophylla Griffith* leaf extract, mobile phase chloroform: methanol is 0.07; 0.21 and 0.97.

The results of the Rf value will be compared with the Rf value from the standard Rf value comparison for chemical compounds. From the visible stains, 3 of the 5 stains identified in the ethanol extract of ramania leaves have the same Rf value as flavonoids, namely 0.24; 0.41 in n-hexa and acetic acid solvents and 0.21 in chloroform and methanol solvents.

The Rf value of flavonoids according to Rahayu et al., (2015) is a stain with an Rf value between 0.2 – 0.75 indicating a stain containing flavonoids. The stain results visible at UV 366 nm show reddish fluorescence indicating that it contains flavonoid compounds. According to Yuda et al., (2017) suggest that there is an interpretation of the color of the spots in terms of flavonoid structure, where in 366 nm UV light there is a light blue fluorescence stain. Some compounds (flavonols, chalcones) will fluoresce under 366 nm UV light while other

compounds (flavonol glycosides, anthocyanins, flavones) absorb light and appear as dark spots on a fluorescent background. Flavone glycosides and flavonols fluoresce yellow, flavonols appear pale yellow, catechins pale blue, anthocyanins grey-blue, chalcone and aurone red.

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 *Bouea marcrophylla Griffith* leaves 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 *Bouea marcrophylla Griffith* leaves are presented in Table 4. 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 *Bouea marcrophylla Griffith* leaves meet the maximum ash content limits regulated in the Indonesia Materia Medika. Results of standardization parameters of *Bouea marcrophylla Griffith* leaf simplicial can be seen in **Table 3**.

Table 3. Bouea marcrophylla Griffith Leaf Extract Effervescent Tablet Formulation

Formulasion	%
Ramania Leaf Extract	15
Lactose	39.7
Citric Acid	7.2
Tartric Acid	11.2
Na Bicarbonate	25.6
Aspartame	0.1
PVP	1
Total	0.2

Effervescent Tablet Formulation

Ramania leaf ekstrak effervescent tablet formulasion can be seen in Table 3. This formulation is taken from research conducted by (Puspita Tanjung et al., 2018). This tablet has undergone a physical evaluation, namely tablet thickness, but it has no effect on water content, angle of repose, granule compressibility, tablet diameter, pH, dissolution time and tablet hardness. The test results that have been carried out show water content test results of 3.41 ± 0.03 %, angle of repose 26.55 ± 1.14 %, granule compressibility 13.58 ± 1.09 %, tablet size uniformity 1.21 ± 0.03 cm, tablet hardness 4.95 ± 0.89 kg, tablet firmness 1.57±0.26%, pH 5.67 ± 0.58, tablet dissolution time 4 minutes 32 seconds ± 27 seconds, and weight uniformity 516.47±34.31. From the results obtained, it can be said that the ramania leaf extract effervescent tablets have met the physical quality of the tablets.

Acute Toxicity Test

This research was conducted to detect the intrinsic toxicity of a substance and the effect of administering ramania leaf ethanol extract effervescent tablets 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. 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% (Istiqomah, 2022).

The acute toxicity testing method used is based on the BPOM non-clinical in vivo toxicity test guidelines (BPOM, 2022). 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 sungkai leaf ethanol extract 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 below (BPOM, 2014; 2020).

Behavior

Qualitative observations based on observations after oral administration of effervescent tablets of ethanol extract of ramania leaves (*Bouea macrophylla Griffith.*) were carried out on the LD50, 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. Observatios on test animals can be seen in Tabel 6. 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

Table 4. Profile body of weight before and after treatment

Animals Group	Avarage BW		
	Before	Day 7	Day 14
Group I (50mg/KgBW)	201.8±24.23	197.4±25.46	227.4±24.31
Group II (300mg/KgBW)	203.2±34.68	163.4±33.87	161.6±25.41
Group III (2.000mg/KgBW)	205.4±14.38	200.6±23.24	226.8±27.31
Group IV (5.000mg/KgBW)	196.4±21.47	184.4±21.89	205.4±46.82

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. Result for test animal weight can be seen in **Table 4**. A sig value > 0.05 means there is no significant change in the weight of the test group. From the results obtained by the BB group before treatment and the BW group on day 7 with values of 0.921 and 0.314, meaning there was no significant change in the BW of the test group. However, in the BW group 14 with a value of 0.031, it means there is a significant difference to the BW of the test group.

CONCLUSIONS

It can be concluded that the effervescent tablets of the ethanol extract of ramania leaves (*Bouea macrophylla Griffith*.) have an LD50 > 5000 mg/kg BW with a classification of non-toxic to animals.

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