Effectiveness of Ethanol Extract of Mangosteen Leaves (*Garcinia mangostana L.*) as Anti-diarrhea in Mice

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ABSTRACT

Diarrhea is a condition where a person experiences defecation with a soft or liquid consistency, with more frequent frequency (generally three or more times) a day One of the plants used as treatment for diarrhea is mangosteen leaves (Garcinia mangostana L). The leaves of the mangosteen plant (Garcinia mangostan L.) contain tannin compounds which can be useful as an antidiarrheal. Tannin is a substance that is effective as an adstringents, so it is thought to be able to provide an anti-dare effect. To determine the effectiveness of ethanol extract of mangosteen leaves (Garciniamangostana L) as antidiarrhea in male white mice (Mus musculus) induced by oleum ricini. This research uses a type of laboratory experimental study using male mice (Muss musculus L) that have been induced with oleum ricini. There were 15 mice used and divided into 5 groups, namely: negative control group (CMC Na 0.5%), positive control group (loperamide HCl) and treatment groups with dose concentrations of 250 mg/kgBW, 450 mg/kgBW and 650 mg/ kg BW. The research results showed that mangosteen leaves contain flavonoids and tannin compounds. This study shows the antidiarrheal activity value of 3 extract dose groups. There were 4 test parameters with the greatest antidiarrheal activity results at a dose of 650 mg/kgBB. The average result of the onset of diarrhea was 62.3 minutes, then the average result of the frequencyof diarrhea was 5 times, while the duration of diarrhea was 85 minutes with an average feces weight of 0.99 grams. The ethanol extract of mangosteen leaves at a dose of 650 mg/kgBW had the most effective antidiarrheal activity in male (mus musculus) mice induced by oleum ricini.

Key words: *anti-diarrhoea, mangosteen leaves, loperamide; oleum ricini, garcinia mangostana*

INTRODUCTION

Diarrhoea is a condition in which a person experiences defecation with a mushy or liquid texture, which can even be water only, and usually occurs with a more frequent frequency (generally three or more times) a day (Ramadhina, Immawati., & Nury, 2023). The morbidity and mortality rates due to diarrhoea are still quite high (Indriyani, Rifiana, & Novitasari, 2017). Several *surveys* in Indonesia show that diarrhoea morbidity rates for all age groups range from 120 to360 per 1000 population (12%-36%). In 2016, about 8% of total deaths were caused by diarrhoea, with an estimated 450,000 people dying each year (Irma., Sabilu, Yusuf, Masluhiyah, & Erwin., 2021). In 2015, diarrhoea outbreaks occurred in 11 provinces in Indonesia, with 1,213 cases and 30 of them ended in death. Diarrhoea ranked fourth in the number of events, at 12 or 1.3%, with 131 cases and 6 deaths. According to the 2016 Indonesian health profile data, East Java reported 1,048,885 cases of diarrhoea, but only 338,806 cases were treated (Irma. et al., 2021). In Lumajang district, diarrhoea ranked fourth as the cause of death in post-neonatal infants (more than 28 days - 1 year) with a percentage of 9.3%, still below pneumonia which reached 20.9% (Irma. et al., 2021).

Acute infectious diarrhoea can be divided into two clinical and pathophysiological types, namely inflammatory diarrhoea and non-inflammatory diarrhoea. Inflammatory diarrhoea is generally caused by bacterial infections and cytotoxins, characterised by the presence of mucus and blood in the faeces (Jayanto, Ningrum, & Wahyuni., 2020). Clinical symptoms of inflammatory diarrhoea involve changes in heartburn, colic-like pain, nausea, vomiting, fever, tenesmus, as well as signs of dehydration (Iqbal, Setyawati, & Towidjojo, 2022). In non-inflammatory diarrhoea, diarrhoea is caused by enterotoxins resulting in large volumes of liquid diarrhoea without mucus and blood. Abdominal complaints are usually minimal or nonexistent, but symptoms and Signs of dehydration develop quickly, especially in cases that do not receive replacement fluids (Iqbal et al., 2022). In adults, acute diarrhoea is often caused by spicy food, where diet is highly influential. If left untreated, acute diarrhoea can lead to death, either due to biochemical disorders such as severe metabolic acidosis (Darvid, Khuluq, & Rahayu, 2023). Therefore, vigorous and timely treatment is essential to prevent the serious consequences of acute infectious diarrhoea.

Oleum ricini is a triglyceride of ricinoleic acid that can be hydrolysed in the gut by lipase into glycerin and ricinoleic acid. As an anionic surfactant, it reduces the net absorption of fluids and electrolytes and stimulates intestinal peristalsis, so oleum ricini can cause diarrhoea (Darvid et al., 2023). Oleum ricini is used as a diarrhoea inducer. This is because oleum ricini is more effective in accelerating intestinal peristalsis so that the expulsion of intestinal contents can be faster (Darvid et al., 2023).

The biodiversity that exists in tropical countries such as Indonesia is a major factor in the discovery of various compounds that have benefits and can be used inmedicine (Setiawan, 2022). These discoveries generally already have a basis of knowledge gained from the experiences of our ancestors in the past. Along with the times, thefield of health and medicine has progressed rapidly, and the emergence of various synthetic drugs is one of the impacts. However, synthetic drugs have side effects for their users. One example of a synthetic diarrhoeal drug is loperamide, which is indicated to control and manage the symptoms of acute non-specific diarrhoea, with side effects of dizziness and symptoms of weakness. (Setiawan, 2022).

One of the medicinal plants derived from nature is mangosteen leaves *(Garcinia mangostana L)* which are commonly grown in the yard. The part of the mangosteen plant commonly used is the fruit (Ansori et al., 2020). In addition to m a n g o s t e e n fruit, mangosteen leaves can also be utilised. The leaves of the *mangosteen* plant *(Garcinia mangostan L.)* contain tannin compounds that can be useful as antidiarrhoeal. Tannins are one of the substances that are efficacious as *adstringents* so that they are thought to be able to provide antidiarrhoeal effects (Saril, Hesturini, & Azhar, 2019). The advantages of using mangosteen leaves compared to mangosteen peel as an antidiarrhoeal drug could be related to the more abundant content of certain compounds in that part. Mangosteen leaves, as mentioned earlier, contain flavonoids and tannins, which have antidiarrhoeal properties by reducing inflammation and inhibiting the growth of bacteria that cause diarrhoea (Saril et al., 2019). Although mangosteen peel also contains beneficial compounds, the concentration or type of compounds may be different from those found in the leaves. In particular, if traditional use or local scientific research supports the effectiveness of mangosteen leaves for antidiarrhoea, it could be a further consideration (Ansori et al., 2020).

Based on the description above, it is necessary to conduct research on "The Effectiveness of *Mangosteen* Leaf Ethanol Extract *(Garcinia mangostana L.)* as Antidiarrhoea in Mice Induced by Oleum Ricini" to examine the potential antidiarrhoeal activity with natural ingredients of mangosteen leaf extract extract extracted using 70% ethanol by maceration method and it is hoped that thisresearch can be useful to provide information related to the use of natural ingredients that can be used as antidiarrhoea..

MATERIAL AND METHODS

Plant Determination

Plant determination will be carried out at the Biology Laboratory of the Faculty of Applied Science and Technology, Ahmad Dahlan University No 138/Lab.Bio/B/III/2024. The purpose of determination is to ensure that the plant is really a species of (*Garcinia mangostana L*).

Mangosteen Leaf Extraction

A total of 500 grams of mangosteen leaves were macerated using 70% ethanol as much as 3000 mL, with a ratio of (1:6) then soaked for 3 days whileoccasionally stirring. The resulting macerate was collected, and the residuewas soaked or remacerated twice. The resulting macerates from both processeswere then collected and evaporated using a *rotary evaporator* at 50°C to obtain a thick extract of mangosteen leaves.

Phytochemical Screening

- *Flavonoids*

  A total of 0.5 grams of ethanol extract from thickened mangosteen leaves was put into a test tube, then dissolved using hot water and filtered. The filtrate was added with 0.1 gram of Magnesium powder and 5 drops ofconcentrated HCL. If there is a colour change to red or yellow, it indicates that the extract is positive for *flavonoids* (Setyani, Setyowati, & Ayuningtyas, 2016).

- *Tannins*

  A total of 1 mL of mangosteen leaf ethanol extract was mixed with 1% iron (III) chloride solution. If there is a colour change to greenish black, it indicates the presence of tannin content in the solution (Pangow, Widdhi Bodhi, & Queljoe, 2018).

Preparation of 0.5% CMC-Na Solution

CMC-Na 0.5% solution was prepared by weighing 500 mg of CMC-Nainto 50 mL of hot distilled water and then left for approximately 15 minutes until clear in colour and gel-like. Then stirred until it became a homogeneous mass and diluted with distilled water in a 100 mL volumetric flask.

Preparation of Loperamide HCl Suspension

Weighed 20 tablets of loperamide HCl. The tablets were crushed andweighed 110 mg of powder. The powder was put into a mortar and added0.5% CMC suspension little by little while being crushed homogeneously to 10 mL. The type of test animal used is mice, based on the dose calculation conversion table for various types of test animals of various species and humans, the dose conversion of humans weighing 70 kg to mice with a body weight of 20 grams is 0.0026.

Antidiarrhoeal Effectiveness Test

In this study, animals received ethical approval from KEPK Dr. Soebandi University with No. 218/KEPK/UDS/III/2024, the results of the ethical feasibility test are listed in appendix 1. Test animals were induced using oleumricni at a dose of 0.5 ml orally, then allowed to stand for 1 hour with the estimation that within 1 hour oleum ricini had worked in the body of mice. The mechanism of action of oleum ricini is to stimulate fluid and electrolyte secretion and

stimulate intestinal peristalsis. Mice are said to have diarrhoea if there is an increase in the frequency of defecation, a more liquid faecal consistency, and an increase in faecal weight (Ambari, 2018).

The test animals used totalled 15 mice, with each group containing 3 mice,After treatment, observations were made on the test parameters, namely the time of onset of diarrhoea, frequency of diarrhoea, faecal weight, and duration of diarrhoea.

The onset of diarrhoea was observed with the help of a stopwatch after treatment until the mice excreted faeces in a liquid consistency for the first time (mice suffering from diarrhoea) about 60 minutes after being induced with oleum ricini. Then the frequency of diarrhoea was observed by counting the number of times diarrhoea occurred in mice after treatment. The frequencyof diarrhoea was observed at 30-minute intervals for 5 hours. Furthermore, the frequency of diarrhoea in each dose ranking group was compared with the control group. After 5 hours the faeces were collected using tissue, the weight of the faeces can be calculated from the weight of the tissue after being used tocollect faeces minus the weight of the initial tissue before being used to collectfaeces. Then the duration of diarrhoea was calculated from the beginning of diarrhoea to the last time of diarrhoea. Furthermore, the duration of diarrhoea in each dose-ranked group was compared with the control group (Ambari, 2018).

Data Analysis Technique

The data analysis technique in this study is using the IBM SPSS version 25.0 programme using the ANOVA test. The data obtained were then tested for normality first using the Saphiro Wilk method. Then the homogeneity test is carried out, the data is said to be normally distributed and homogeneity if the p value is> 0.05. The purpose of normality and homogeneity tests is as a requirement to be able to use the *one-way* ANOVA parametric test with a 95% confidence level to see the significance of each group. If obtained If the p value is <0.05, a further *post hoc* test is carried out, namely LSD (*Least Significant Differences*) to see the difference between each sample.

RESULT AND DISCUSSION

Research with the title of the effectiveness of ethanol extract ofmangosteen leaves (*Garcinia mangostana L*.) as antidiarrhoeal in mice induced by oleum ricini was conducted at the Clinical and Community Pharmacy Laboratory of Dr. Soebandi University in May-June. This study aims to determine the effectiveness of mangosteen leaf ethanol extract *(Garcinia mangostana L)* as antidiarrhoea in male white mice (*Mus musculus*) induced by oleum ricini. Plant determination was carried out at Ahmad Dahlan University with the aim of proving the correctness of the samples used in the study with No. 138/Lab.Bio/B/III/2024. The identification results showed that the plants used were true mangosteen leaves. The results of mangosteen leaf determination can be seen in appendix 2.

Ethanol Extraction of *Mangosteen* Leaves (*Garcinia mangostana L*.).

The results obtained from the maceration process of mangosteen leaf simplisia (*Garcinia mangostana L*.). After the determination of mangosteen leaves w a s processed into powder and macerated using 70% ethanol solvent, the percentage yield value obtained was 31.6%. Therefore, the yield of the crude extract obtained was declared goodbecause the yield was >10% (Syamsul, Anugerah, & Supriningrum, 2020). The calculation of yield is the ratio of the dry weight of the product produced to the weight of the simplisia. The yield value shows the amount of active compound content in a plant. The higher the yield value produced, the more active compounds contained therein.

Phytochemical Screening of Ethanol Extract of *Mangosteen* Leaf (*Garcinia mangostana* L.).

Phytochemical screening carried out on the group for two classes of compounds, namely flavonoids and tannins. The results of phytochemical screening show that the ethanol extract of mangosteen leaves contains secondary metabolites inthe form of flavonoids and tannins shown in table 3 below.

Secondary metabolites that are thought to have antidiarrhoeal activity are flavonoids and tannins, which have antidiarrhoeal properties by relieving inflammation and inhibiting the growth of bacteria that cause diarrhoea. The results of this phytochemical screening are the same as the results of research conducted by Pangow (Pangow et al., 2018) which states that the phytochemical identification of mangosteen leaf ethanol extract is positive for tannins and flavonoids.

Parameters of Antidiarrhoeal Effectiveness Test of Ethanol Extract of *Mangosteen* Leaf (*Garcinia mangostana L.*)

The antidiarrhoeal effectiveness test uses oleum ricini as a diarrhoea inducer. In the small intestine, oleum ricini will be broken down by lipase enzymesinto ricinoleic acid. This ricinoleic acid will accelerate intestinal peristalsis, leading to increased intestinal motility and thus diarrhoea. Diarrhoea is characterised by liquid stools and increased stool frequency (Musdar, 2020).The antidiarrhoeal activity was observed with several parameters, namely the onset of diarrhoea, frequency of diarrhoea, duration of diarrhoea, and stool weight.

This is because Na CMC does not contain active substances that can reduce antidiarrhoeal activity so that diarrhoea will continue toincrease. All treatment groups have significantly different values with Na CMC ($p<0.05$). The onset of diarrhoea in mice is characterised by several clinical symptoms, such as the consistency of faeces becoming more liquid, increased frequency of defecation, and mice appearing less active or lethargic. At the beginning of diarrhoea, the consistency of normal faeces is like faeces type 1 with the characteristics of hard, nut-like (round), and black. After the induction of oleum ricini, the consistency of the faeces produced is slimy, such as faeces type 7with the characteristics of a smooth surface, liquid, slimy, and brown. This can occur because oleum ricini has a strong laxative effect. After being treated with mangosteen leaf extract, the consistency of the faeces gradually improved back to faecal type 1. This can occur because mangosteen leaf extract contains flavonoid and tannin compounds that are efficacious as *adstringents* that can provide antidiarrhoeal effects (Inderiyani & Sulastri, 2021).

In the treatment group of mangosteen leaf ethanol extract dose, the dose of 650 mg/kgBB showed the longest diarrhoea onset time (62.3 minutes). The faster the onset of diarrhoea, the stronger the antidiarrhoeal activity. Conversely, the longer the initial time of diarrhoea, the weaker it will be(Inderiyani & Sulastri, 2021). In the frequency of diarrhoea, the dose of 650 mg/kgBB showed the lowest decrease in frequency (5 times). Determination of the frequency of diarrhoea carried out shows that the smaller the frequency of diarrhoea, the stronger the antidiarrhoeal activity.Vice versa, the greater the frequency of diarrhoea, the weaker the antidiarrhoeal activity (Manek, 2019). In the duration of diarrhoea, the dose of 650 mg/kgBB is the fastest treatment group to hold diarrhoea (85 minutes). Determination of the duration of diarrhoea shows that the faster the duration of diarrhoea, the stronger the antidiarrhoeal activity. Vice versa, the longer the duration of diarrhoea, the weaker the antidiarrhoeal activity (Darwis, 2023). In the weight of faeces, the dose of 650 mg/kgBB showed the lowest average decrease in faeces weight (0.18 grams). Determination of faecal weight

shows that the smaller the value of faecal weight, the stronger the antidiarrhoeal activity. Vice versa, the higher the value of faecal weight, theweaker the antidiarrhoeal activity (Ambari, 2018).

Of all treatment groups, the 650 mg/kgBB dose has a value equivalent to loperamide. This is due to the use of loperamide as a comparator which has a mechanism of action to suppress peristalsis and reduce secretion in intestinal motility (Fadilah, Agustien, & Rizkuloh, 2022). These results are comparable to research conducted by Irfayanti,that the best dose for antidiarrhoeal activity is at a dose of 650 mg/kgBB (Irfayanti, Zam, & Andriani, 2022).

Based on the above discussion, it is found that mangosteen leaf ethanolextract has effectiveness as an antidiarrheal agent by reducing the onset of diarrhoea, frequency, duration of diarrhoea, and faecal weight more quickly. Thiseffect is due to mangosteen leaves containing chemical compounds that are proven as antidiarrhoeal, namely flavonoids and tannins. Flavonoids work byinhibiting intestinal motility so that fluid and electrolyte secretion can be reduced. (Lina & Astutik, 2020). Tannins are ***adstringents***, which are substances that cause density and narrowing of the cell layer so that they function to inhibit tissue secretion (Lina & Astutik, 2020). Inaddition, the higher the dose of mangosteen leaf ethanol extract, the more flavonoid and tannin compounds contained in the extract and the greater theactivity as antidiarrhoeal (Pangow et al., 2018).

The results of the *one way* ANOVA test showed that there were significantdifferences in the onset of diarrhoea with a *p-value* of 0.000 (*p-value* <0.05), frequency of diarrhoea with a *p-value* of 0.000 (*p-value* <0.05), stool weight witha *p-value of* 0.000 (*p-value* <0.05). <0.05), and duration of diarrhoea with *p-value* 0.000 (*p-value* <0.05), then continued with LSD test. The LSD test results showed a significant difference between the positive control and dose 650 at the onset of diarrhoea with a *p-value* of 0.035 (*p-value* <0.05), positive control and dose 650 at the frequency of diarrhoea with a *p-value* of 0.017 (*p-value* <0.05), positive control and dose 650 on stool weight with *p-value* 0.034 (*p-value* < 0.05), positive control and dose 650on diarrhoea duration with *p-value* 0.032 (*p-value* < 0.05). This indicates that mangosteen leaf ethanol extract at a dose of 650mg/kgBB has antidiarrhoealeffectiveness.

Based on the results of the study, mangosteen leaf ethanol extract can provide antidiarrheal effectiveness based on the parameters of the onset of diarrhoea, frequency of diarrhoea, duration of diarrhoea, and stool weight with an average value equivalent to loperamide is mangosteen leaf ethanol extract at adose of 650 mg/kgBB.

CONCLUSION

Mangosteen leaf ethanol extract (*Garcinia Mangostana L*) has antidiarrhoeal activity against male mice *Musculus.* Based on statistical analysisof antidiarrhoeal effectiveness on mangosteen leaf ethanol extract (*Garcinia Mangostana L*) dose of 650mg / kgBB provides the most effective antidiarrhoeal effectiveness in male mice induced by oleum ricini.

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TABLE AND FIGURE

Table 1 Test Group

Group	Treatment
Negative control	CMC Na 0.5%
Positive control	Loperamide HCL 10 mg/kgBB
Treatment 1	Mangosteen leaf extract at a dose of 250 mg/kgBB
Treatment 2	Mangosteen leaf extract at a dose of 450 mg/kgBB
Treatment 3	Mangosteen leaf extract at a dose of 650 mg/kgBB

Table 2 Mangosteen Leaf Extraction Results

Weight of simplisia (gram)	Weight of condensed extract (grams)	Yield (yield)
500	158	31,6%

Table 3 Phytochemical screening of mangosteen leaf extracts

Compound Reagents	Reagents	Changes that happens	Results
Flavonoids	Magnesium+ concentrated HCl	Yellow	+
Tannins	FeCl3 1%	Blackish green	+

Table 4 Diarrhoea parameters of oleum ricini-induced mice (*mus musculus*)

Group	Initial Occurrence Diarrhoea ± SD (minutes)	Frequency Diarrhoea ± SD (times)	Duration of diarrhoea ± SD (minutes)	Faecal Weight ± SD (grams)
Na CMC 0.5%	26,6±7,63	27,6±2,08	220±11,53	0,65±0,10
Loperamide HCL	62,3±2,51	8,6±1,52	107±11,01	0,31±0,05
250 mg/kgBB dose	37,7±2,3	20,3±1,52	192±5,7	0,48±0,01
450 mg/kgBB dose	40,6±4,04	13,6±1,52	150±15,30	0,43±0,07
650 mg/kgBB dose	62,3±2,51	5±1	85±9,6	0,18±0,40