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ANTIDIARRHEAL ACTIVITY OF ETHANOLIC EXTRACT OF *Vernonia amygdalina* Del LEAVES AGAINTS MALE MICE INDUCED BY OLEUM RICINI

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Abstract

Leaves of Vernonia amygdalina Del. is one of the alternative plants that people use as antidiarrheal in Martapura, Banjar. The purpose of research to determine the antidiarrheal activity and the effectiveness of ethanolic extract of leaves against male mice. The negative control was given NaCMC 0.5% orally, positive control was given Loperamide HCl 0.005 mg/20 g orally, and groups of ethanolic extract of leaves were given dosages in 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW orally. After 1 hour of treatment, the groups were induced by Oleum ricini orally and were observed for 4 hours. Based on three parameters were frequency, weight, and consistency of diarrhea. From the results observation of that negative control, positive control, EEDA 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW for diarrhea frequency parameter was 7.4 ± 2.30; 2 ± 1.22; 5.8 ± 3.34; 6.2 ± 4.08; 2.4 ± 1.67; the weight parameter was 1.34 ± 0.43; 0.38 ± 0.29;0.66 ± 0.56; 0.57 ± 0.38; 0.49 ± 0.33 and then consistency of diarrhea with EEDA 400 mg/kg BW improved the consistency at 180 minutes compared to control group at 210 minutes. In conclusion, from all parameters of ethanolic extract of Vernonia amygdalina Del. leaves can give an antidiarrhea effect with optimum dosage is 400 mg/kg BW.

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INTRODUCTION

Diarrhea is still a global problem and is one of the main causes of high child morbidity and mortality in the world (Nurhalimah *et al*., 2015). In general, it is estimated that more than 10 million children aged less than 5 years die every year, around 20% die of diarrhea. Diarrhea is still a public health problem in developing countries such as Indonesia (Ministry of Health of the Republic of Indonesia, 2014). The presentation of diarrhea in Indonesia was 6,897,463 cases per year (Ministry of Health of the Republic of Indonesia, 2016).

The use of traditional medicines is increasingly growing and tends to be favored by many people because it has many advantages, including relatively cheap prices, easily obtained raw materials and side effects of traditional medicines which are considered smaller than the side effects of synthetic drugs. One of the plants that can be used as traditional medicine is *Vernonia amygdalina* Del. or african leaves. *Vernonia amygdalina* Del. grow in the west, especially in tropical countries like Indonesia (Suryati *et al*., 2016). Currently, there is no research related to *V. amygdalina* as antidiarrheal. *Vernonia amygdalina* Del. are empirically used by the community of South Kalimantan, especially in the Martapura district of Banjar as an antidiarrhe with simple processing by drinking *V. amygdalina* decoction (Kharimah *et al*., 2016). From the description above, the authors would like to conduct further research with the aim of relating extraction of *V. amygdalina* as antidiarrheal and increasing knowledge and information about *V. amygdalina* as antidiarrheal.

MATERIAL AND METHODS

Tools and Materials

The materials used in this study were leaves of V. amygdalina, 96% ethanol, Loperamide HCl, aquadest, oleum ricini, Sodium-carboxy methyl cellulose (Na-CMC) 0.5%, acetic acid, magnesium powder, 1% FeCl3, HCl concentrated, Mayer reagent, Liebermen-Burchard reagent and Wagner reagent.

The tools used are rotary evaporator, stopwatch, analytical scales, and oral sonde. The study used test animals of male Balb / C strain with a weight of 20-30 grams and aged 2-3 months. Ethical clearance is

obtained from Health Research Ethics Commission, Faculty of Medicine, Universitas Lambung Mangkurat Indonesia with number 793/KEPK-FK UNLAM/EC/VII/2018.

Methods

Extraction

Vernonia amygdalina leaves dried and pollinated, then weighed as much as 200 g. The powder was macerated with ethanol 96% as much as 1 L (1 : 5) at room temperature or protected from direct sunlight for 1 x 24 hours extract was collected and the residue macerated. Furthermore, macerated 3 x 24 hours, the results of maceration were then concentrated with a rotary evaporator and waterbath at 60°C to obtain a thick extract.

Phytochemical Screening

Phytochemical Screening with reagents to determine the content of *Vernonia amygdalina* leaves. The groups of compounds tested included alkaloids, flavonoids, steroids, saponins, and tannins.

Alkaloid Test

A total of 0.5 g of extract was added with 10 drops of 2 N sulfuric acid shaken until two layers were formed. The top layer is divided into 2 parts in the test tube. The first tube is added to the Mayer reagent, white deposits will form. The second tube was added to Wegner's reagent, brown deposits will form (Lailatul *et al*., 2010).

Flavonoid Test

A total of 0.5 g of extract was put into a test tube. The filtrate is added 2-3 drops of concentrated sulfuric acid and 0.2 g of Magnesium powder, then shake vigorously. Changing the color of the solution to orange to brick red indicates the presence of flavonoids (Noer *et al*., 2018).

Steroid Test

A total of 0.5 g of extract was put into a test tube, then added 10 drops of anhydrous acetic acid, 2 drops of concentrated sulfuric acid, and several Lieberman-Burchard reagents. The solution is shaken slowly and left for a few minutes. The presence of steroids is shown in blue or green (Lailatul *et al*., 2010).

Saponin Test

A total of 0.5 g of extract is put into a test tube, then 2 mL of water is added and shaken vigorously for 10 seconds. The presence of saponins is indicated by the formation of stable foam for 10 minutes with height of 1-3 cm (Noer *et al*., 2018).

Tannin Test

A total of 0.5 g of extract is put into a test tube, then were added by 2 or 3 drops of 1% $FeCl_3$ solution. The blackish green or dark blue solution formed indicated the precence of tannins (Noer *et al*., 2018).

Antidiarrheal Activity Test

Test animals were divided into five groups consisting of five mice adapted and fasted for one hour before treatment. Furthermore, each group was treated orally consisting of a control group (0.5% Na-CMC), a test group with extracts (100, 200 and 400 mg/kg BW), and a comparison of Loperamid HCl (0.005 mg/kg BW). After that all test animals were given induction of Oleum ricini 0.75 ml orally. After the test animals were treated, four hours of observation were carried out with the observed parameters including diarrhea frequency, faecal weight and stool consistency.

Data Analysis

Data from stool consistency observation, diarrhea frequency and faecal weight were tested statistically with variance analysis method at 95% confidence level, followed by One Way ANOVA SPSS version 19.0.

RESULTS AND DISCUSSION

Extraction

Of the 200 g of *V. amygdalina* leaf powder, 31.43 g of thick ethanol extract was obtained with the extract yield of 15.67%.

Phytochemical Screening

Phytochemical screening is a qualitative test which aims to determine the active compound content of secondary metabolites in plant extracts. Phytochemical screening tests showed positive results for all tested components, namely alkaloids, flavonoids, steroids, saponins, and tannins. The test results can be seen in Table 1.

Table 1. Phytochemical screening results of ethanol extract from *V. amygdalina* leaves

Compound groups	Reagent	Reaction results	Description
Alkaloid	Mayer	White precipitated	+
	Wegner	Brown precipitated	+
Flavonoid	Mg metal	Brick red solution	+
Steroid	Lieberman-Burchard	Greenish solution	+
Saponin	Water	Foam	+
Tanin	1% $FeCl_3$	Blackish green	+

Antidiarrheal Activity Test

Testing this antidiarrheal activity uses a protection method. In the protection method oleum ricini is used as the inducer. Oleum ricini or castor oil is a triglyceride that is efficacious as laxansia. In the small intestine, this oil undergoes hydrolysis and produces risinoleic acid which stimulates the intestinal mucosa, thereby accelerating its peristalsis and resulting in rapid bowel evisceration, in the form of dilute discharge of bowel movements (Guo *et al.*, 2014). Determination of antidiarrheal activity of ethanol extract of *V. amygdalina* leaves was carried out by observing the frequency of diarrhea, consistency, and weight of faeces

Frequency of Diarrhea

The first parameter observed in the protection method induced by oleum ricini was the frequency of diarrhea. Where in determining the frequency of diarrhea, obtained the average value of each group of negative control showed a significant difference ($p < 0.005$) to Loperamid HCl and extract of 400 mg/kg BW, while extracts of 100 mg/kg BW and extracts of 200 mg/kg BW shows differences that are not significantly different ($p > 0.005$). This shows that Loperamid HCl and extract of 400 mg/kg BW can prevent diarrhea significantly better than extract 100 mg/kg BW and extract 200 mg/kg BW. The average frequency of diarrhea can be seen in Table 2.

Table 2. Average Frequency of Diarrhea

Test Group	Dosage (mg/kg BW)	Frequency ± SD
Negative Control	-	7.4 ± 2.30
Loperamide HCL	0,005	2 ± 1.22*
Ethanol extract of	100	5.8 ± 3.34
V. amygdalina	200	6.2 ± 4.08
leaves	400	2.4 ± 1.67*

Description: 0.5% Na-CMC Negative Control; * Significantly different p (<0.05) with controls

Stool Consistency

The next parameter observed was stool consistency as can be seen in Figure 1. The consistency of faeces was determined from the start of being given induction of Oleum ricini until the occurrence of diarrhea, which was observed every 30 minutes for four hours. In determining the consistency of faeces done by looking at the shape that occurs it can be categorized into four groups, namely not chapter (0), normal (1), soft (2), and slimy/watery (3). Consistency seen visually was assessed using Scoring, so that groups that were declared to have antidiarrheal effects were the group that showed the smallest consistency rate (Suherman *et al*, 2013).

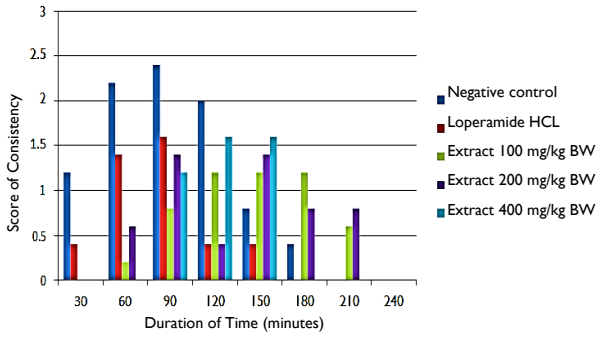

Figure 1. Stool consistency per time

In Figure 1 shows that Loperamide HCl improves stool consistency at minute 150; extract 100 mg/kg BW improves stool consistency at 210 minutes; extract 200 mg/kg BW improves stool consistency at 180 minutes; extract of 400 mg/kg BW improved stool consistency in the 150th minute compared to the negative control

group. The smaller the number of stool consistency, the greater the antidiare effect produced (Suherman *et al*., 2013). The results of the study of ethanol extract of the leaves of *V. amygdalina* 400 mg/kg BW which have the best effect as antidiarrheal are associated with a corresponding increase in dosage, the effect also increases (Nurhalimah *et al*., 2015).

Stool Weight

The last parameter observed in the diarrhea protection method induced by oleum ricini was faecal weight to determine the presence or absence of antidiarrheal effects of the ethanol extract of the leaves of *V. amygdalina*. The average stool weight can be seen in Table 3.

Table 3. Average Stool Weight

Test Group	Dosage (mg/kg BW)	Weight (g) ± SD
Negative Control	-	1.34 ± 0.43
Loperamide HCL	0,005	0.38 ± 0.29*
Ethanol extract of	100	0.66 ± 0.56
V. amygdalina	200	0.57 ± 0.38
leaves	400	0.49 ± 0.33*

Description: 0.5% Na-CMC Negative Control; * Significantly different p (<0.05) with controls

The results showed that faecal weight showed a significant difference (p <0.005) against Loperamide HCl, extract 200 mg/kg BW and 400 mg/kg BW while extract 100 mg/kg BW showed no significant difference with negative controls. This shows that extract 200 mg/kg BW and extract 400 mg/kg BW can prevent diarrhea.

The results showed that the ethanol extract of leaves of V. amygdalina Del. at a dose of 400 mg/kg BW is very good to use as an antidiarrheal because it contains secondary metabolites such as steroids, tannins, and flavonoids. The higher the dose, the better the activity as antidiarrheal because the extract used contains more secondary metabolites which have properties as antidiare. Steroid compounds as antidiarrhea can increase the absorption of water and electrolytes in the intestine, resulting in normal absorption of water and electrolytes in the intestine (Anas *et al*., 2012). Tannin in the sample can provide antidiarrheal activity (Tadesse *et al*., 2017). The mechanism of the tannin as an antidiarrheal is by shrinking the intestinal mucous membrane and shrinking the pore so that it will inhibit the secretion of excess electrolyte fluid (Tjay & Rahardja, 2007). The mechanism of saponin as antidiarrheal by inhibiting histamine release which triggers a decrease in the absorption of fluids so that inhibition can normalize the absorption of fluids (Anas *et al*., 2012). The mechanism of flavonoids as antidiarrhea can inhibit intestinal motility, reduce the secretion of water and electrolytes and prolong intestinal transit time so that body fluids can be absorbed properly (Derebe *et al*., 2018).

CONCLUSION

The chemical compounds contained in the ethanol extract of leaves of V. amygdalina positively contain flavonoids, steroids, tannins, saponins and alkaloids. There is an influence of giving the ethanol extract of leaves of V. amygdalina to the frequency of stool (sig 0.040), stool consistency (sig 0.089) and stool weight (sig 0.071). The results of the study of ethanol extract of leaves of V. amygdalina at a dose of 400 mg/kg BW have an antidiarrheal effect.

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