Effectiveness Test Antidiarrhea of the Combination of Ziziphus Mauritiana Leaves and Curcuma Domestica Rhizome on Male Mice (Mus musculus)

Rita Muliyani[1], Andi Zsazsa Rafiatul Mukhlis[1], Nur Mahdi[2*]

[1]*Pharmacy Diploma Program, College of Health Darul Azhar Batulicin, Indonesia.*

[2]*Pharmacy Program, Faculty of Mathematics and Sciences, Lambung Mangkurat University, Indonesia*

Email[*]: nur.mahdi@ulm.ac.id

ABSTRACT

Diarrhea is a disease that experiences an increase in fecal mass and the number of bowel movements is more than 4 times per day. Synthetic drugs are often used to treat diarrhea, but they often cause dangerous side effects. Bidara leaves (Ziziphus mauritiana) and turmeric rhizomes (Curcuma domestica) are often used as alternative treatments to treat diarrhea because they contain potential diarrheal drugs. The purpose of this study is to determine the antidiarrheal effect of a combination of bidara leaf ethanol extract (BLEE) and turmeric rhizome ethanol extract (TREE) on male mice that were given PO with oleum ricini. The research method used was experimental with the induction of oleum ricini diarrhea with a combination dose of EEDB and EERK 4mg:2mg/20grBB, 2mg:4mg/20grBB and 4mg:4mg/20grBB. The results of the study with One Way ANOVA with a confidence level of 95% in this study were < 0.05 which means that there was a significant difference and continued LSD tests in all combination groups obtained a meaningful difference with the positive control group (attapulgite). The results of the study concluded that the combination of bidara leaves (Ziziphus mauritiana) and turmeric rhizomes (Curcuma domestica) had a stronger antidiarrheal effect than positive control (attapulgite).

Keywords: *Antidiarrheal, bidara leaves, combination, effectiveness, turmeric rhizome.*

INTRODUCTION

Diarrhea is a disease characterized by an increase in stool volume, bowel movements more than 4 times a day, or the consistency of stool becoming watery [1]. Diarrhea is a leading cause of death globally each year [2].

Currently, people around the world still rely on traditional medicine, which involves using plant extracts as remedies. Synthetic chemical drugs act quickly but have the risk of dangerous side effects [3]. Plants used to treat diarrhea include turmeric rhizome, majaan leaves, guava leaves, and bidara leaves. These plants have effects that can constrict the intestinal mucosa and reduce fluid secretion during diarrhea, as well as possess anti-inflammatory and antibacterial properties [4].

Phytochemical screening of turmeric extract shows the presence of chemical compounds such as alkaloids, triterpenoids, saponins, flavonoids, tannins, and polyphenols. The chemical compounds in turmeric rhizome include oleoresin, curcumin, resin, essential oils, desmethoxycurcumin, and bisdemethoxycurcumin [5].

Phytochemical screening of bidara leaf extract reveals the presence of flavonoids, phenols, quinones, saponins, terpenoids, and tannins [6].

Based on the background above, the researchers aim to combine bidara leaf and turmeric rhizome extracts and test them on male mice, as previous studies with single extracts showed antidiarrheal effects. The objective of this study is to analyze the effectiveness of the ethanol extract combination of bidara leaves (Ziziphus mauritiana) and turmeric rhizome (Curcuma domestica) as an antidiarrheal in male mice (Mus musculus) induced with castor oil (oleum ricini).

METHODS

This experimental method was conducted in the Laboratory of STIKes Darul Azhar Batulicin by treating experimental groups under controlled conditions.

Materials

Attapulgite tablets, Na CMC, concentrated HCL, dried bidara leaves and turmeric rhizome (simplicia), FeCl3, Mg sulfate, and 96% ethanol [7].

Extraction

Bidara leaf (Ziziphus mauritiana) and turmeric rhizome (Curcuma

domestica) samples were collected from Tanah Bumbu, South Kalimantan. The samples were wet-sorted to separate unwanted particles, cleaned, chopped, and sun-dried until reaching a moisture content of less than 10%. Extraction was performed using the maceration method: 100 grams of each dried simplicia was soaked in 96% ethanol (1:10 ratio) for 3 days with occasional stirring. After 3 days, the macerate was filtered, and the filtrate was heated in a water bath to evaporate the ethanol, yielding a thick extract. The extract yield was calculated using the formula:

% Yield = (weight of extract / weight of simplicia) x 100

Phytochemical Screening

Identification of compound groups included flavonoids, alkaloids, tannins, and saponins:

1. Flavonoid Test: Extract + 96% ethanol + magnesium powder + 3 drops of concentrated HCl. Red, orange, or yellow color indicates flavonoids [9].
2. Alkaloid Test: 2 ml of extract in three test tubes + 1 ml 2N HCl. Tube I: 2 drops Mayer reagent = white precipitate. Tube II: 2 drops Wagner reagent = orange to brown precipitate. Tube III: 2 drops Dragendorff reagent = orange precipitate [10].
3. Tannin Test: Sample boiled in 10 ml water and filtered. Filtrate + 1% FeCl = dark green color [11].
4. Saponin Test: Sample boiled in 10 ml water, filtered, shaken for ±10 seconds + 1 ml 2M HCl = foam formation [12].

Antidiarrheal Effect

The dosage used was based on previous best-performing studies, increased and combined for oral administration. Test animals were 25 healthy male mice (20–30 g, 2–3 months old), divided into 5 groups (5 mice/group):

Group I: Negative control (0.5% Na CMC)

Group II: Positive control (attapulgite 2.47 mg/20 g BW)

Group III: Combination 2:1 (bidara leaf extract 4 mg : turmeric extract 2 mg)

Group IV: Combination 1:2 (bidara leaf extract 2 mg : turmeric extract 4 mg)

Group V: Combination 2:2 (bidara leaf extract 4 mg : turmeric extract 4 mg)

Mice were given test solutions orally,

followed 30 minutes later by oral castor oil. Antidiarrheal testing was based on time to onset of diarrhea, defecation frequency, stool consistency, and duration of diarrhea, monitored at 15-minute intervals up to 120 minutes.

Stock Solution Preparation

Positive Control (Attapulgite)

- Dose conversion: usual dose × conversion factor = 600 mg × 0.0026 = 1.56 mg/20 g BW
- Weighed amount Attapulgite (950 mg) = (1.56 mg ÷ 600 mg) × 950mg = 2.47 mg
- Stock Solution in 10 ml= (2.47/0.5ml) × 10 ml = 49,4 mg

Extracts

From previous studies: turmeric extract (80 mg/kg BW) [1], bidara leaf extract (180 mg/kg BW) [7].

- Dose 2 mg → 10 ml stock solution (2mg/0.5ml) × 10 ml = 40 mg
- Dose 4 mg → 10 ml stock solution (4mg/0.5ml) × 10 ml = 80 mg

Data Analysis

Cumulative data were analyzed using the Shapiro-Wilk Test for normality, followed by One-Way ANOVA (95% confidence level) to determine treatment differences, and LSD Post-Hoc test to identify significant differences [13].

RESULTS AND DISCUSSION

Chemical Component Test

The components found in bidara leaves and turmeric rhizomes in this study were alkaloids, flavonoids, saponins, and tannins, consistent with previous research [1][7].

Table 1. Result of Phytochemical Screening

Groups	Phytochemical Screening		Description
	Bidara	Turmeric	
Alkaloid	+	+	White sediment
Flavonoid	+	+	Orange
Saponin	+	+	Foamy
Tannin	+	+	Blackish green

Data Analysis Results

Onset of Diarrhea

Measured by the time to first defecation after castor oil administration. Combination extract groups showed a longer onset compared to the positive control, indicating stronger antidiarrheal effect [7].

Table 2. Result of Diarrhea Onset

Onset of Diarrhea	Average/min
Group I (Control -)	13
Group II (Control +)	17
Group III (Comb 2:1)	35
Group IV (Comb 1:2)	18
Group V (Comb 2:2)	22

Stool Consistency

Observed from mucous to normal consistency. Combination groups showed lower mean values than the positive control, indicating stronger antidiarrheal effects [7].

Table 3. Result of Stool Consistency

Stool Consistency	Average
Group I (Control -)	7
Group II (Control +)	6.6
Group III (Comb 2:1)	4
Group IV (Comb 1:2)	4.4
Group V (Comb 2:2)	5.2

Defecation Frequency

Measured by the number of defecations per minute. Combination groups had lower mean frequencies than the positive control, supporting their stronger antidiarrheal effect [7].

Table 4. Result of Defecation Frequency

Defecation Frequency	Average/min
Group I (Control -)	6.8
Group II (Control +)	6.4
Group III (Comb 2:1)	4.2
Group IV (Comb 1:2)	4.4
Group V (Comb 2:2)	5.2

Duration of Diarrhea

Positive control had the longest diarrhea duration, while combination groups had shorter durations, indicating stronger antidiarrheal effect [7].

Table 5. Average of Diarrhea Duration

Duration of Diarrhea	T2	T1	T1-T2/min
Group I (Control -)	119	33.12	85.88
Group II (Control +)	119	39.28	79.72
Group III (Comb 2:1)	100	29.16	70.84
Group IV (Comb 1:2)	95	22.05	72.95
Group V (Comb 2:2)	106	31.25	74.75

Statistical analysis with SPSS at 95% confidence level showed normal and homogeneous data (significance >0.05). One-Way ANOVA and LSD Post-Hoc tests confirmed the effectiveness of the extract combination as an antidiarrheal [15].

CONCLUSION

Based on phytochemical screening, the ethanol extracts of bidara leaves and turmeric rhizomes contain flavonoids, alkaloids, saponins, and tannins.

The combination of ethanol extracts of bidara leaves and turmeric rhizomes has a synergistic antidiarrheal effect, which is stronger than the positive control (attapulgite).

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