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Antibacterial and Anti-Diarrheal Potential of Andaliman (*Zanthoxylum acanthopodium DC*) Methanol and Nanoemulsion Extract

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ABSTRAK

Diare, salah satu masalah kesehatan global lazim penyebab kasus kematian dan kesakitan pada berbagai kelompok usia bagi masyarakat disuluruh dunia. Andaliman merupakan tanaman herbal khas Sumatera Utara dengan berbagai manfaat yang telah diteliti sebelumnya. Penelitian ini bertujuan untuk menganalisis potensi anti-bakteri dan anti-diare dari ekstrak metanol dan nano emulsi Andaliman (Zanthoxylum acanthopodium). Penelitian ini menggunakan penelitian eksperimental laboratorium dengan desain post test only group desain. Pengujian antibakteri dilakukan dengan menggunakan metode difusi cakram dengan tiga kali pengulangan terdapat empat konsentrasi ekstrak. Kelompok standar yang digunakan ciprofloxacin 5µg dan kelompok kontrol menggunakan DMSO. Kemudian dilanjutkan dengan metode mikrodilusi dengan konsentrasi awal ekstrak 500mg/Dl untuk menentukan Kadar hambat minimum dan kadar bunuh minimum. Penelitian anti-diare menggunakan 30 ekor tikus wistar janatan dengan ekstrak nano emulsi. Kelompok standar diberikan loperamide 3 mg/KgBB dan kelompok kontrol diberikan CMC Na 0,25 ml. Tikus diinduksi dengan minyak jarak dan frekuensi buang air besar serta feses yang cair diamati selama 1 jam. Ekstrak metanol Andaliman (Zanthoxylum acanthopodium) menunjukkan efek penghambatan moderat terhadap E.Coli, dengan KHM dan KBM sebesar 55,56 mg/mL. Potensi sebagai anti-diare, ekstrak nano emulsi Andaliman menunjukkan perbedaan yang signifikan pada dosis 50mg/KgBB untuk frekuensi diare dan tinja encer. Namun, tidak ada perbedaan signifikan yang ditemukan pada hasil gastrointestinal dan entero-pooling. Berdasarkan hasil penelitian dapat disimpulkan bahwa ekstrak metanol dan nanoemulsi Andaliman (Zanthoxylum acanthopodium) menunjukkan adanya aktivitas antibakteri terhdap E.Coli dan menunjukkan potensi anti diare. Implikasi yang diharapkan adalah bahwa andaliman dapat menjadi pengobatan alternatif untuk diare, khususnya untuk diare yang disebabkan oleh Escherichia coli.

ABSTRACT

Diarrhoea is one of the prevalent global health problems, causing cases of death and morbidity in various age groups for people around the world. Andaliman is a typical herbal plant of North Sumatra with multiple benefits that have been studied previously. This study aims to analyze the antibacterial and anti-diarrheal potential of methanol extract and nanoemulsion of Andaliman (Zanthoxylum acanthopodium). This study used laboratory experimental research with a post-test-only group design. Antibacterial testing was carried out using the disc diffusion method with three repetitions of four extract concentrations. The standard group used ciprofloxacin 5µg, and the control group used DMSO. Then, we continued with the microdilution method with an initial concentration of 500mg/Dl extract to determine the minimum inhibitory and kill levels. An anti-diarrhoea study was conducted using 30 Wistar rats treated with nanoemulsion extract. The standard group was given loperamide 3 mg/KgBB, and the control group was given CMC Na 0.25 ml. Rats were induced with castor oil, and the frequency of defecation and liquid faeces were observed for 1 hour. Andaliman (Zanthoxylum acanthopodium) methanol extract showed a moderate inhibitory effect against E.Coli, with KHM and KBM of 55.56 mg/mL. Potential as anti-diarrhoea, Andaliman nano emulsion extract showed a significant difference at 50mg/KgBB dose for diarrhoea frequency and loose stools. However, no significant differences were found in gastrointestinal and entero-pooling results. Based on the results, it can be concluded that methanol extract and nanoemulsion of andaliman (Zanthoxylum acanthopodium) showed antibacterial activity against E.Coli and showed anti-diarrhea potential. The expected implication is that andaliman can be an alternative treatment for diarrhoea, especially for diarrhoea caused by Escherichia coli.

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1. INTRODUCTION

Diarrhea is defined as fecal passages with a thinner consistency and more frequent frequency, occurring more than twice a day(Saragih & Arsita, 2019; Simanjuntak et al., 2023). Diarrhea can be caused by a variety of clinical factors, including viruses, bacteria, malabsorption, allergies, poisoning, and immunodeficiency. While inadequate hygiene is often blamed for the condition, it is important to consider these other potential causes as well. Andaliman (Zanthoxylum acanthopodium DC) is a spice unique to specific regions in North Sumatra (Biologi et al., 2022; Marpaung et al., 2024). Andaliman is one of the typical spices used in Toba Batak cuisine, which generally grows wild in hilly areas with low temperatures. The part of andaliman that is utilized is the fruit, which causes a distinctive effect when eaten. Andaliman has several biological activities such as larvicide, anti-inflammatory, analgesic, antimicrobial, antioxidant and antijamu (Biologi et al., 2022; Marpaung et al., 2024; Simanjuntak et al., 2023). Some of the most common diseases suffered by Indonesians include diarrhoea, acute respiratory infections and pneumonia. Escherichia coli bacteria in food and drinks that enter the human body can cause symptoms such as diarrhoea, nausea, and vomiting. Bacillus subtilis bacteria can also cause meningitis, endocarditis, eye infections, bacteremia and septicemia. Diarrhea can be caused by bacterial infections, viruses, parasites, malabsorption, allergies, poisoning, and immunodeficiency. The most common cause of diarrhea is *Escherichia coli* bacteria (Aminian et al., 2023; Gallo et al., 2020; Lim et al., 2020). Approximately two billion cases of diarrhea occur annually worldwide. Of these cases, 1.9 billion affect children under the age of five, and 580 million result in mild to severe dehydration (Biselli et al., 2022; Kambale et al., 2021). Diarrhea causes approximately 300 deaths annually per 100,000 children in countries with the poorest health indicators, such as Madagascar, Chad, and the Central African Republic. In Indonesia, the number of exceptional diarrhea cases reached its highest prevalence in 2020, at 4.00%, based on data from 2010–2020, where it was initially recorded at 1.74% (Tigerprints et al., 2020; World Health Organization, 2020). The use of medicinal plants has become a community concern, particularly for those living in rural areas. Treatment utilizing various plants is based on empirical wisdom inherited from ancestors and has been used for years. One of the medical plant is Andaliman (*Zanthoxylum acanthopodium*), a typical Indonesian spice plant. (Aritonang, 2022). The Andaliman (*Zanthoxylum acanthopodium*) plant belongs to the Rutaceae family and is typically growing wild in the Tapanuli region at altitudes of 1,500 meters above sea level. It is commonly used in various types of food, particularly in traditional Batak cuisine (Adrian et al., 2023; Nurlaeni, Iskandar, et al., 2021; Silalahi et al., 2021).

Andaliman works as an antibacterial by inhibiting cell membrane function. Antibacterials can be divided into two categories: bacteriostatic, which suppresses bacterial growth, and bactericidal, which can kill bacteria. Inhibition of the formation of complex compounds with extracellular proteins on the cell membrane and soluble components causes membrane damage, followed by the release of intracellular compounds (Agung Adha Witasa Dewana et al., 2022; Nadon et al., 2023). Bacterial infections and diarrhoea are significant global health problems. In many countries, especially developing countries, bacterial infections and diarrhoea are still important causes of morbidity and mortality (Behera & Mishra, 2022; Cohen et al., 2022; Ugboko et al., 2020). Finding effective and safe alternatives for treating infections and diarrhoea is essential to improve the quality of life and public health. The overuse and inappropriate use of antibiotics have led to increased antibiotic resistance (Ali, 2020; Dhingra et al., 2020). This makes treatment of bacterial infections increasingly difficult. Research into antibacterial compounds from natural sources such as andaliman may provide potential new alternatives and reduce dependence on synthetic antibiotics (Gunawan et al., 2024; Nugraha et al., 2023). Previous research has shown that the extract of Andaliman (*Zanthoxylum acanthopodium*) contains secondary metabolites, such as phenols, saponins, flavonoids, tannins, triterpenoids, steroids, and alkaloids. These compounds can be used as raw materials for drug production. However, further research is necessary to determine their potential. This study aims to confirm the antibacterial and anti-diarrheal potency of Andaliman (*Zanthoxylum acanthopodium*) methanol and nanoemulsion extracts. It is anticipated that the findings of this study will facilitate public comprehension and contribute to the advancement of scientific knowledge concerning the utilization of natural products as pharmaceutical agents, with a particular focus on andalimam as a potential antidiarrheal agent.

2. METHOD

This research employs a laboratory experimental study utilizing the Posttest Only Control Group design for in vivo testing. In this experimental design, the experimental group and control group are not selected at random. Instead, a comparison is made between the control group and the experimental group. The experimental group underwent a designated treatment regimen, while the control group did not. A type of laboratory experimental research was conducted for in vitro tests using a research design based

on disc diffusion and microdilution methods. Tools and materials: an oven, an evaporator, a blender, a 40-mesh sieve, scales, a funnel, filter paper, a magnetic stirrer, a sanitizer, laboratory glassware, analytical balances, a stirrer, an incubator, a paper disc, a water bath, a mortar and pestle, a Petri dish, a 96-well macro plate, a micro pipette, an Erlenmeyer flask, an autoclave, an osse needle, a sterile swab, a measuring flask, a 96-well plate, a stirring rod, scissors/scalpel, a test tube, gloves, and documentation tools. With regard to the chemicals employed, the following drugs and reagents were utilized:The following chemicals were utilized in this study: methyl paraben, propyl paraben, aquadest, Tween 80, PEG 400, methanol, castor oil, loperamide HCl, CMC Na, Nutrient Agar, NaCl, Nutrient Broth, DMSO, ciprofloxacin, aquadest, and Na CMC. The collected data were analyzed using the software Statistical Package for Social Sciences (SPSS), version 25. Thus, the results were expressed as mean±standard error of means (SEM). The significance of differences between groups was analyzed by using kruskall wallis, one way ANOVA followed by post hoc Tukey's test. A P value of less than 0.05 was considered statistically significant. And Mann whitney if the P value > 0.05. Preparation of Andaliman Nanoemulsion. First, 2kg of Andaliman (*Zanthoxylum acanthopodium*) fruit was thoroughly cleaned and dried in an oven at 55°C for 5 hours. Next, the dried fruit was pulverized into simplisia, and 700 grams of sifted simplisia was extracted using maceration method with methanol as the solvent (1:3) for 24 hours.(Gede et al., 2022)·(Djuang et al., 2022) After filtering, the liquid extract was concentrated into a thick extract with an evaporator at 55°C. Finally, the nanoemulsion was expertly made by dissolving Methyl Paraben and Propyl Paraben in heated and cooled Aquadest, and then adding Tween 80 to Aquadest. The sample underwent magnetic stirring at 5000rpm for 30 minutes (Mass 1). Next, a mixture of PEG and Andaliman (*Zanthoxylum acanthopodium*) extract underwent magnetic stirring at 5000rpm for 20 minutes (Mass 2). Mass 1 and Mass 2 were then slowly combined using a dropper pipette and underwent magnetic stirring at 5000rpm for 8 hours, followed by sonication. (Rienoviar et al., 2019)·(Tanessa et al., 2023)· (Yuliani et al., 2022)

Disc diffusion method, Andaliman (*Zanthoxylum acanthopodium*) methanol extract was diluted with DMSO solvent in concentrations ranging from 50 mg/ml to 350 mg/ml. The control group was treated with DMSO, while the standard group received Ciprofloxacin. Agar media was prepared by mixing 20 grams of Nutrient Agar with 1 liter of distilled water and 8 grams of Nutrient Broth with 1 liter of distilled water. *Escherichia coli* was cultured on NA media by inoculating a pure dose of the bacteria and incubating it for one day. The bacterial growth was suspended in 0.9% NaCl and compared to the McFarland standard 0.5. (Chiuman et al., 2023) The disc diffusion method was performed with three repetitions. Bacteria were inoculated on the surface of NA in a Petri dish using a sterile swab, and then discs inoculated with various concentrations of extracts, controls, and standards were placed on the surface. The Petri dish was incubated for 18-24 hours, and the zone of inhibition was measured using a caliper.(Sari et al., 2017)·(Intan et al., 2021)·(Jannah et al., 2017) Microdilution method. The microdilution method using Nutrient Broth liquid media with an extract concentration of 500 mg/mL. 96-well plates were filled with 200 µL of Nutrient Broth per column. The sterile control was placed in column 1, the growth control in column 2, and the extract control in column 3. The extract was added to column 4, homogenized, and 100 µL was transferred to column 5. This process was repeated until column 12, with 100 µL being discarded in the final column. To determine the Minimum Inhibition Concentration, add 10 µL of *E. coli* suspension to columns 2, 4 through 12, incubate for 24 hours, and observe the turbidity of the media. The concentration at which no turbidity is observed is the Minimum Inhibition Concentration. To determine the Minimum Kill Concentration, re-culture columns with clear media. Next, pipette 10 µL of the mixture into the wells of the macroplate, pour over hardened NA media, and homogenize. Incubate for 24 hours. (Oltxlg et al., n.d.)·(Lolongan et al., 2016) In-vivo antidiarrheal activity test using 10% Andaliman (*Zanthoxylum acanthopodium*) nanoemulsion extract and Loperamide as positive control. Loperamide was dissolved in 0.5% Na CMC at a dose of 3 mg/kgBB. To determine the lethal dose, toxicity tests were conducted on mice. The mice were fed and then given Andaliman (*Zanthoxylum acanthopodium*) nanoemulsion extract through an oral sonde at doses of 2000 mg/kgBB, 1000 mg/kgBB, 500 mg/kgBB, 150 mg/kgBB, and 50 mg/kgBB. Signs of toxicity were observed by monitoring changes in locomotor behavior, eating, and drinking patterns for 14 days.(Abdela, 2019)· A total of 30 rats were divided into 5 groups: Group I, the Negative Control, received castor oil and Na CMC; Group II, the standard, was induced with castor oil and given loperamide HCl; Group III, Treatment I, was induced with castor oil and then given Andaliman (*Zanthoxylum acanthopodium*) nanoemulsion extract at a dose of 25 mg/kgBB; and Group IV, Treatment II, was induced with castor oil and then given a dose of 50 mg/kgBB of Andaliman (*Zanthoxylum acanthopodium*) nanoemulsion extract. Group V: Treatment III - The mice were induced with castor oil and then administered a dose of 75 mg/kgBB of Andaliman (*Zanthoxylum acanthopodium*) nanoemulsion extract. All rats in each treatment group were fasted for 18 hours. Then, they were given 0.75 ml of castor oil as the inducer of diarrhea. After 1 hour, the rats received the assigned treatment based on their respective test groups. During the observation period, the frequency of defecation,

consistency of feces, and intestinal motility of rats were recorded. (Kifle et al., 2021) After one hour, the rats were given activated charcoal as a marker, left for an additional hour, and then terminated and dissected to extract the small intestine organs (from the pylorus of the stomach to the cecum). The contents of the small intestine were squeezed and placed in a separate container to measure their volume. The weight of the small intestine was measured when full and empty.(Oltxlg et al., n.d.), (Gudeta et al., 2021) The volume and mass of the small intestine contents were used to calculate the percentage inhibition of their volume and mass using the following formula:

- *Castor oil Induced Enteropooling Activity*

$$\%\ Reduction\ in\ weight\ of\ intestinal\ content = \frac{\text{weight of intestinal content in NC} - \text{weight of intestinal content in TG x 100}}{\text{PI of Control groupweight of intestinal content in the negative group}}$$

3. RESULT AND DISCUSSION

Result

Physical Characteristics of Andaliman Nanoemulsion Extract are presented in Table 1. The methanol extract of Andaliman was then formulated into nanoemulsion with an average particle size of 0.20 µm. The smallest particle size was 0.01 µm, and the largest was 42.30 µm. Size Profile of Andaliman Extract Nanoemulsion with PSA (Particle Size Analyzer) NanoTec seen in Table 1.

Table 1. Size Profile of Andaliman Extract Nanoemulsion with PSA (Particle Size Analyzer) NanoTec

Parameter	Value
Mean (µm)	0.20
Median (µm)	0.03
Standard Error (µm)	0.89
Min (µm)	0.01
Max (µm)	42.30

The disc diffusion method was used to test the antibacterial properties against *Escherichia coli.* The results showed significant differences between each test group with a value of P < 0.05 as shown in Figure 1. The group treated with 250 mg/ml had the largest inhibition zone diameter compared to the standard group, while the group treated with 50 mg/ml had the smallest inhibition zone, the calculating result presented in Table 2.

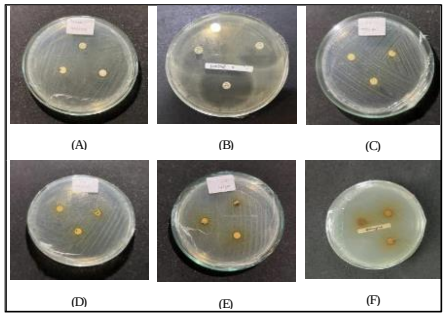


Figure 1. The results of Testing with the Disc Diffusion Method with Three Repetitions are Seen Visually the Figure Above Shows (A) Negative Control, (B) Positive Control (Ciprofloxacin); (C) 50 mg/ml Concentration; (D) 150 mg/ml Concentration; (E) 250 mg/ml Concentration; (F) 350 mg/ml Concentration

Table.2 Quantitative Test Results of Inhibition Zone with Shapiro-Wilk test, where P Value is Obtained from One-Way

Group	Inhibition Zone Diameter (mm)		P Value
	Mean	Std. Deviation	
Standard	36.97	2.20	
Control	6.00	0.00	< 0.01
350 mg/ ml	8.10	1.07	

Group	Inhibition Zone Diameter (mm)		P Value
	Mean	Std. Deviation	
250 mg/ml	8.48	1.11	
150 mg/ml	6.98	0.59	
50 mg/ml	6.62	0.25	

The Tukey HSD Post Hoc Test results indicate that significant differences only exist in the Standard treatment group (Ciprofloxacin) compared to the treatment group at all concentrations and the control group. These results are presented in Table 3.

Table 3. Post Hoc Test and Tukey HSD Results

Treatment Group	Comparison	P Value	Interpretation
Standard	Control	0.01	Significant
	350 mg/ ml	0.01	Significant
	250 mg/ml	0.01	Significant
	150 mg/ml	0.01	Significant
	50 mg/ml	0.01	Significant
Control	350 mg/ ml	0.27	Insignificant
	250 mg/ml	0.14	Insignificant
	150 mg/ml	0.88	Insignificant
	50 mg/ml	0.98	Insignificant
350 mg/ ml	250 mg/ml	0.99	Insignificant
	150 mg/ml	0.82	Insignificant
	50 mg/ml	0.60	Insignificant
250 mg/ml	150 mg/ml	0.60	Insignificant
	50 mg/ml	0.38	Insignificant
150 mg/ml	50 mg/ml	0.99	Insignificant

Microdilution method. The results of the observations of bacterial growth are presented in Figure 2 and Table 4. Bacterial growth was observed on a 96-well macroplate at concentrations of 18.52 mg/ml, 6.17 mg/ml, 2.06 mg/ml, 0.69 mg/ml, 0.23 mg/ml, and 0.08 mg/ml. The Minimum Inhibitory Concentration was determined to be 55.56 mg/ml.

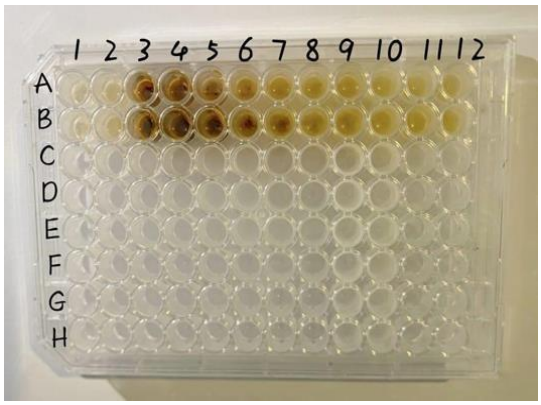


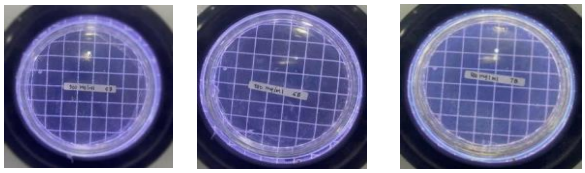
Figure 2. Micro Dilution Test Results on 96-Well Macroplate

Table **4.** result of Turbidity Observation Using the Micro-Dilution Method

Concentration	Turbidity
Media control	-
Bacterial growth control	+
Extract control	-
500 mg/ml	-
166,67 mg/ml	-
55,56 mg/ml	-

Concentration	Turbidity
18,52 mg/ml	+
6,17 mg/ml	+
2,06 mg/ml	+
0,69 mg/ml	+
0,23 mg/ml	+
0,08 mg/ml	+

Bacterial re-cultivation was performed in column wells 5B, 6B, and 7B, as indicated in the Table 4. The results indicate bacterial growth at a concentration of 18.56 mg/ml. The minimum concentration that does not support bacterial growth is 55.56 mg/ml, which is referred to as the Minimum Kill Concentration.



166.67 mg/ml (A) 55.56 mg/ml (B) 18.52 mg/ml (C)

Figure 3. Reculturing Results on NA Media

Antidiarrhea Activity.

Toxicity testing was conducted on mice using doses of 2000mg/KgBB, 1000mg/KgBB, 500mg/KgBB, 150mg/KgBB, and 50mg/KgBB. Mice exposed to concentrations of 2000mg/KgBB, 1000mg/KgBB, 500mg/KgBB, and 150mg/KgBB experienced convulsions and death within 1 to 5 minutes. Observations were made for 14 days on mice with a concentration of 50mg/KgBB. The results of these observations are presented in Table 5, which demonstrates that no deaths occurred during this period, this concentration was deemed to be a therapeutic dose. The LD50 was established at 150mg/KgBB.

Table 5. Observation Result for the Occurence/Absence of Toxicity Characteristic in Mice

No	Dose	1-5 minutes	➢ 5 minutes- 14 days
1	2000	+	Mice Died
2	1000	+	Mice Died
3	500	+	Mice Died
4	150	+	Mice Died
5	50	-	No toxicity features

In vivo Antidiarrheal Results on Frequency and Liquid Stool Frequency

The frequency of defecation assessed is the number of times defecation occurs within 1 hour of observation. The liquid stool frequency assessed was the number of stools in a more liquid or thinner consistency. The study found a significant difference ($P < 0.005$) in the frequency of defecation and the number of liquid feces between treatment groups. The group treated with 75mg/ml had the highest total defecation frequency, while the group treated with 25mg/ml had the highest frequency of defecation with liquid feces. The result presented in Table 6 and Table 7 below.

Table 6. Observations of Stool Consistency of Rats, Indicating Diarrhea or Not

No	Group	Diarrhea				
		1	2	3	4	5
1	Standard	-	-	-	-	-
2	Control -	+	-	+	+	+
3	75 mg/kg BB	+	+	+	+	+
4	50 mg/kg BB	-	+	-	-	-
5	25 mg/kg BB	+	+	-	+	+

Table 7. Shapiro Wilk Test and One Way ANOVA.

Treatment group	Liquid stool frequency		Total defecation frequency	
	Mean	Std. Deviation	Mean	Std. Deviation
Control	6.2	1.92	8	0.7
Standard	0.6	0.548	2.4	1.94
Dosis 25 mg/KgBB	4.8	3.19	6.6	3.84
Dosis 50 mg/KgBB	2.4	2.7	3.6	2.19
Dosis 75 mg/KgBB	4.6	1.14	8.8	3.7
Nilai P*	**0.004**		**0.005**	

Invivo Antidiarrheal Test Results on Gastrointestinal Motility. The data in Table 8 demonstrate that the experimental groups of rats showed a significant difference in total intestinal length. However, the charcoal displacement distance did not show a significant difference between the test treatment and castor oil induction (P>0.005). The charcoal displacement distance is consistent with the peristaltic index, where higher or faster peristalsis results in longer charcoal displacement distance.

Table 8. Quantitative Results of Charcoal Displacement Distance and Length of Whole Intestine of the Treatment Groups After Induction

Treatment group	Charcoal moving distance		The length of the entrice small intestine		Peristaltic index	
	Median	Min-Max	Mean	Std.	Median	Min-Max
Control	67	31-78	118.4	4.23	58.01	26.50-68.12
Standard	69	42-81	119.2	11.71	64.46	32.56-67.68
25mg/KgBB	20	16-53	103.8	10.18	19.37	16.84-44.12
50mg/KgBB	110	47-120	121.3	2.51	90.53	39.17-99.17
75mg/KgBB	57	41-61	111.7	4.63	49.57	37.96-53.04
Nilai P	**0.46**		**0.01**		**0.009**	

Test Results for Antidiarrheal Medications and Entropoling Activity presented in Table 9. According to the Shapiro-Wilk normality test, the data on the relationship between the treatment group and the weight of the intestinal contents is not normally distributed. Therefore, the Mann-Whitney non-parametric test yielded a P-value of 0.34, indicating that there is no significant difference in the weight of the intestinal contents between the treatment group and the control group.

Table 9. Analysis of Treatment Group Effect on Gut Content Weight

Treatment group	Weight of intestinal contents		Percent inhibition of small intestinal mass Contents	
	Median	Min-Max	Median	Min-Max
Control	3.03	3.03-5.02	36.14	30.70-36.64
Standard	2.51	2.51-5.18	33.29	30.50-38.29
25mg/KgBB	1.97	1.97-5.82	38.01	22.26-42.27
50mg/KgBB	3.86	3.86-7.60	35.73	31.12-47.68
75mg/KgBB	0.99	0.99-4.06	17.33	11.01-40.40
Nilai P	**0.34**		**0.564**	

Discussion

This research utilizes nanoemulsion extract aiming to reduce the particle size of the extract to facilitates quicker delivery to targeted areas. (Djuang et al., 2022)(Kifle et al., 2021) Thereby, to achieve therapeutic effects more quickly and using smaller dose. Previous studies have shown that Andaliman (*Zanthoxylum acanthopodium*) contains secondary metabolites, including flavonoids, quinones, steroids, terpenoids, and tannins.(Yuliani et al., 2022)(Windy et al., 2022) Andaliman (*Zanthoxylum acanthopodium*) is used in the community as a food preservative and for the treatment of toothache and diarrhea.(Nurlaeni, Amilia Pratiwi Kebun Raya Cibodas -Pusat Riset Konservasi Tumbuhan dan Kebun Raya Badan Riset dan Inovasi Nasional Jl Kebun Raya Cibodas, et al., 2021) It has also been researched for its antibacterial, antidiabetic, and anticancer properties. The antibacterial activity of Andaliman (Zanthoxylum acanthopodium) methanol extract varied among the test groups. The diameter of the inhibition zone against bacteria increased with the concentration of the extract, which was attributed to the higher amount of secondary metabolite content. The study used Pseudomonas Aeruginosa bacteria with Andaliman *(Zanthoxylum acanthopodium*) extract

(Agung Adha Witasa Dewana et al., 2022; Hutapea et al., 2024). Andaliman (*Zanthoxylum acanthopodium*) contains alkaloids that prevent the formation of the cell wall layer in bacteria, leading to their death (Amalia et al., 2023; Hutapea et al., 2024; Muzafri & Karno, 2022). Additionally, flavonoids found in Andaliman (*Zanthoxylum acanthopodium)* increase the permeability of the cell wall, microsomes, and lysosomes of bacteria. Tannins also contribute to the antibacterial activity by inhibiting reverse transcriptase and DNA topoisomerase enzymes (Rizqoh et al., 2024; Susanti et al., 2020).

Previous studies have found that phenolic compounds at high concentrations can damage bacterial cell walls and the proteins within them. At low concentrations, phenolic compounds form protein complexes that result in denaturation, activate bacterial enzymes, and act as antibacterials (Lobiuc et al., 2023; Yilmaz et al., 2022). The secondary metabolite content of Andaliman *(Zanthoxylum acanthopodium)* contains these compounds. Endophytic bacteria from Andaliman are capable of inhibiting the growth of E. coli bacteria. The inhibition is due to the presence of alkaloids and flavonoids in Andaliman (Zanthoxylum acanthopodium) (Hutapea et al., 2024; Sibero et al., 2020). In addition to its pharmacological effects as an antibacterial, Andaliman (*Zanthoxylum acanthopodium)* has been found to have anti-inflammatory, anticancer, cardioprotective, hepatoprotective, nephroprotective, and wound healing properties(Mutmainah & Warditiani, 2022) Castor oil, known for its diarrhea-inducing properties, is hydrolyzed by lipase in the small intestine into glycerol and ricinoleic acid (Adrian et al., 2023; Barus, 2020; Frederick et al., 2021). This stimulates fluid and electrolyte secretion and intestinal peristalsis. Loperamide is used as a standard antidiarrheal agent. It reduces the motility of the intestine and blocks the secretion of the intestine, which slows down the peristalsis of the intestine(Frederick et al., 2021; Hao et al., 2023). The study found that a concentration of 50 mg/kg of body weight resulted in the least frequent occurrence of liquid feces defecation. The tannins present in Andaliman (*Zanthoxylum acanthopodium)* nanoemulsion extract can cause intestinal shrinkage and reduce peristalsis during diarrhea, resulting in feces with lower water content. But, the observation of gastrointestinal motility and enteroopoling activity showed that the extract did not have an antidiarrheal potential by inhibiting intestinal motility (Fan et al., 2023; YE, 2021). The Andaliman (*Zanthoxylum acanthopodium)* nanoemulsion extract does not affect small intestinal muscle movement. Tannins increase calcium outflow, which reduces peristalsis and intestinal secretions. Flavonoids inhibit the synthesis of the inflammatory mediator prostaglandin E2. The reaction between protein tannate and tannins results in reduced secretion (Harefa et al., 2021; Setiadi et al., 2022). Methanol is often used as a solvent to extract various active compounds from plants, including flavonoids, alkaloids, and saponins that have antibacterial and anti-diarrheal activities. Using methanol extract, the study can identify and evaluate the main active components of andaliman. Nanoemulsions can increase the solubility of active compounds that may be difficult to dissolve in traditional forms. This increases the effectiveness of the treatment and allows for lower doses with the same or even better results. The implication is that this study may increase public awareness and acceptance of using local medicinal plants, strengthening the integration of herbal medicine with conventional medical practices. The limitation in this research is that no specific tests were conducted to quantitatively determine the amount of secondary metabolites in Andaliman. It is hoped that future researchers will conduct quantitative tests on secondary metabolites in Andaliman so that a more accurate dosage can be obtained for more precise formulation.

4. CONCLUSION

Andaliman methanol extract and andaliman nanoemulsion extract have antibacterial effects against E. coli bacteria and have potential antidiarrheal effects. That methanol extract and nanoemulsion of andaliman (Zanthoxylum acanthopodium) showed antibacterial activity against E.Coli and showed anti-diarrhea potential.

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