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Research Article

Antidiarrheal Potential of Maja (*Crescentia cujete*) Fruit Extract in Mice

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Abstract

Diarrhea, characterized by frequent, loose stools, can be treated with various approaches. This study investigated the antidiarrheal properties of maja (*Cresentia cujete*) fruit extract, which contains secondary metabolites like tannins and flavonoids with astringent properties. The objective was to determine the optimal dose of *C. cujete* fruit extract for treating diarrhea in mice. An *in vivo* experimental design was employed, inducing diarrhea in male mice using *oleum ricini*. The study assessed stools' onset, duration, consistency, frequency, and weight. *Cresentia cujete* fruit extract was administered orally at 125, 250, and 500 mg/kg BW doses. A comparison was made with negative control (CMC-NA) and positive controls (loperamide and attapulgite). Data analysis involved ANOVA followed by the Tukey test. The findings revealed that 500 mg/kg BW of *C. cujete* fruit extract was the most effective dose for treating diarrhea in mice. These results suggest the potential of *C. cujete* fruit extract as a promising natural antidiarrheal agent.

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INTRODUCTION

Diarrhea, a common gastrointestinal ailment characterized by increased stool frequency and altered consistency, remains a significant global health concern. While often underestimated, diarrhea is a leading cause of death, particularly among young children. In fact, it is the third most common cause of death in children under five years of age, surpassed only by pneumonia and tuberculosis[1]. Dehydration, a severe consequence of fluid and electrolyte loss, is the primary cause of mortality associated with diarrhea[2].

Diarrhea remains a significant public health concern, particularly in developing countries like Indonesia. In 2018, diarrheal diseases resulted in a substantial number of deaths, with children under five and adults over five being disproportionately affected. Diarrhea prevalence among Indonesian toddlers increased from 6.7% to 15.2% between 2013 and 2018[3]. Moreover, in 2017, 1725 diarrhea cases were reported across 12 provinces and 17 districts/cities, leading to 34 fatalities. East Java emerged as a hotspot, accounting for the second-highest number of diarrhea cases (151,878) with a prevalence of 7.6%. Surabaya, a major city in East Java, bore a significant burden, reporting approximately half of the total cases in the province[4].

Diarrhea can be treated with either specific or non-specific approaches. Non-specific treatments, such as loperamide, aim to reduce symptoms by suppressing peristalsis[5]. However, this approach is not suitable for all patients, particularly young children. Specific treatments, involving antimicrobial drugs, target the underlying bacterial infection. Yet, indiscriminate use of antibiotics can lead to antibiotic resistance and other adverse effects[6].

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Indonesia, a country renowned for its rich biodiversity, offers a wealth of potential natural remedies. Numerous plants have been traditionally used for various purposes, including medicinal applications. Unfortunately, many plant species are undervalued and face the risk of extinction due to deforestation and habitat loss[7], one of them is maja or *Crescentia cujete*. *Crescentia cujete*, commonly known as the Calabash Tree, is often overlooked due to its unpleasant fruit. The fruit's black, sticky, and malodorous nature has led to the plant being considered poisonous and undesirable. As a result, it is frequently discarded or even destroyed, limiting its potential applications[8]. While traditional uses of *C. cujete* include utilizing its fruit shells for utensils and its pulp as fertilizer or pesticide[9], there is a growing interest in exploring its medicinal properties. Recent studies have investigated the potential of *C. cujete* leaf extract as a treatment for wounds[10], diabetes[11], and cancer[12]. To further unlock the potential of this underutilized plant, additional research is necessary to investigate various aspects of its biology, chemistry, and applications.

Crescentia cujete has long been recognized for its diverse pharmacological properties. Previous studies, such as those conducted by Atmodjo[8], have identified the presence of various secondary metabolites within the plant, which contribute to its potential therapeutic applications. One such potential application is in the realm of antibacterial therapy[13]. Bacterial infections, often contracted through contaminated food or water, can lead to a range of gastrointestinal disorders, including diarrhea[14]. Previous research has demonstrated the therapeutic potential of *C. cujete*, particularly its antioxidant and antibacterial properties, attributed to its rich content of primary and secondary metabolites[15-17]. However, despite extensive studies, the specific antidiarrheal activity of *C. cujete* fruit, particularly at the molecular level, remains underexplored.

This study aimed to investigate the anti-diarrheal potential of *C. cujete* fruit pulp extract prepared using 70% ethanol. To assess its efficacy, an *in vivo* model of castor oil-induced diarrhea in male mice was employed. The parameters evaluated included the onset of diarrhea, fecal consistency, fecal weight, frequency of defecation, and duration of diarrhea. This research provides valuable insights into the potential therapeutic applications of *C. cujete* fruit pulp and encourages further investigation into its underlying mechanisms of action at the molecular level.

MATERIALS AND METHODS

Materials

Analytical balances (Wiggen Hauser), a rotary evaporator (Buchi b-740), and standard laboratory glassware (Pyrex) were employed in this study. The chemicals used were of analytical grade and included: 70% ethanol, 1 N sodium hydroxide, 1% aluminum chloride, 2 N hydrochloric acid, Mayer's reagent, Dragendorff's reagent, 1% ferrous chloride, 1% sodium carboxymethylcellulose, acetate anhydride, sulfuric acid, loperamide hydrochloride (Lodia®), attapulgite (Diatab®), and castor oil. The primary test substance was the fruit flesh of *C. cujete*. Fruits were collected from the campus of the National Institute of Science and Technology, South Jakarta, Indonesia. The correct species identification of *C. cujete* was confirmed by the Indonesian Institute of Science, Bogor, and the Biology Research Center, West Java, Indonesia. This identification was officially recognized with Certificate Number 1083/IPH.1.01/if.07/XI/2020.

Methods

Preparation and extraction of plant materials

Five kilograms of fresh *C. cujete* fruit were thoroughly washed with running water and then peeled, sliced, and dried in an oven at 60°C for 48 hours. The dried fruit was ground into a fine powder using a 40-mesh sieve, yielding approximately 2 kg of dried fruit powder. The powdered fruit was macerated in 70% ethanol at room temperature for three days, protected from light. The resulting extract was filtered and concentrated using a rotary evaporator under reduced pressure to remove the ethanol.

Preliminary phytochemical screening

Preliminary phytochemical screening was conducted to identify the presence of alkaloids, flavonoids, saponins, and tannins in the extract. This screening aimed to establish a pharmacognostic profile for the plant material[18].

Alkaloids: Alkaloids were detected using a standard procedure. One gram of *C. cujete* fruit extract was moistened with 5 mL of 25% ammonia solution and extracted with 20 mL of chloroform. The mixture was heated, filtered, and the filtrate was concentrated to half its original volume. The concentrated extract was acidified with 1 mL of 2 N hydrochloric acid and

partitioned into two layers. The aqueous layer was divided into two portions and tested with Mayer's and Dragendorff's reagent. The formation of white, brown, and red precipitates, respectively, in these tests is indicative of the presence of alkaloids. A positive alkaloid test was confirmed if at least two of these reagents yielded a positive result.

Tannins: One gram of *C. cujete* fruit extract was extracted with 100 mL of hot water. A 5 mL aliquot of the filtrate was mixed with a few drops of 1% ferric chloride solution. A green-purple color indicates the presence of tannins.

Flavonoids: One gram of *C. cujete* fruit extract was filtered into 100 mL of hot water. A 5 mL aliquot of the filtrate was mixed with 1 mL of 5% sodium nitrite solution and 1 mL of 10% aluminum chloride solution. Subsequently, 2 mL of 1 N sodium hydroxide solution was added. A positive test for flavonoids was indicated by the development of a red or orange color.

Saponins: One gram of *C. cujete* fruit extract was extracted and purified using 100 mL of hot water. A 10 mL aliquot of the filtrate was subjected to a foam test to identify saponins. A persistent foam formation of 1-10 cm height indicates the presence of saponins. To confirm this, a drop of 2 N HCl was added to the test tube; the formation of a stable foam (±1 cm) further supports the presence of saponins.

Evaluation of antidiarrheal potential

Ethical approval for this study was obtained from the Medical and Health Research Ethics Committee of Jakarta Veterans University (Letter No. 144/I/2021/KEPK). This approval ensured adherence to ethical guidelines for human and animal research[19]. Male mice (*Mus musculus*) aged 2-3 months and weighing 20-30 g were used for *in vivo* testing. Mice were chosen as the animal model due to their physiological similarity to humans, ease of procurement and care, and rapid regeneration rate. Additionally, male mice were selected to minimize hormonal fluctuations, which can potentially influence experimental outcomes.

Mice were acclimatized to the laboratory environment for one week prior to the experiment. On the day of the experiment, mice were fasted for 30 minutes and then randomly divided into six groups of four mice each, using Federer's formula[20]. In this study, *oleum ricini* (castor oil) was administered orally to induce diarrhea at a dose of 0.8 mL/mouse. Upon ingestion, lipase enzymes in the small intestine hydrolyze castor oil into glycerol and ricinoleic acid. Ricinoleic acid, a potent laxative, irritates the intestinal mucosa and stimulates peristalsis, leading to the characteristic laxative effect. Loperamide and attapulgite were employed as standard antidiarrheal agents for comparison. Thirty minutes post-*oleum ricini* administration, each group received a different treatment: Group I (negative control; 1% CMC-Na), II (loperamide HCl; 7 mg/kg BW), III (attapulgite; 1200 mg/kg BW), and IV to VI (*C. cujete* fruit extract; 125, 250, and 500 mg/kgBW, respectively), as shown in **Table I**.

Table I. Treatment group.

Groups	Treatment - dose administrated (per oral)	Total mice
I	1% CMC-Na 1000 mg	4
II	Loperamide 7 mg/kg BW	4
III	Attapulgite 1200 mg/kg BW	4
IV	*Crescentia cujete* fruit extract 125 mg/kg BW	4
V	*Crescentia cujete* fruit extract 250 mg/kg BW	4
VI	*Crescentia cujete* fruit extract 500 mg/kg BW	4

Mice were monitored for seven hours at 30-minute intervals. Diarrhea was defined as an increase in stool frequency and a change in consistency to a softer or liquid state[21]. Observations included the onset of diarrhea, stool consistency (normal, soft, or watery/mucous), stool weight, stool frequency, and diarrhea duration. The onset of diarrhea was recorded as the time (in minutes) after *oleum ricini* administration when the first instance of diarrhea occurred. Stool weight was measured every 30 minutes. Diarrhea duration was calculated as the difference between the onset of diarrhea and the time when stool consistency returned to normal.

Data analysis

To analyze the diarrhea effect, one-way ANOVA followed by Tukey's HSD post-hoc test was employed using SPSS version 25.0. This statistical analysis allowed for the comparison of mean differences between the treatment groups.

RESULTS AND DISCUSSION

The maceration method was employed for extraction, as it avoids the use of heat, thereby preserving the integrity of heat-sensitive bioactive compounds present in the *C. cujete* fruit. This approach is in line with previous studies[22-24] that have highlighted the benefits of non-thermal extraction methods. Ethanol, a polar solvent, was chosen as the extraction solvent due to its ability to dissolve both polar and non-polar compounds present in *C. cujete* fruit. Additionally, ethanol's inert nature minimizes the risk of unwanted chemical reactions. The use of fungal fermentation, a non-toxic and sustainable method, was optimized to enhance the extraction process and maximize the yield of bioactive compounds[25]. The resulting extraction process yielded a viscous extract weighing 320 g from 545 g of *C. cujete* fruit powder, as detailed in **Table II**.

Table II. Organoleptic analysis and yield of *C. cujete* fruit extract.

Plant identification	Results	Plant	Extract
Color	Blackish green		
Form	Thick extract		
Flavor	Bitter		
Powder weight (g)	545		
Extract weight (g)	320		
Yield (%)	58.7		

To ensure the safety and reliability of the anti-diarrheal testing, the ethanol extract was subjected to a drying process to remove residual solvent. Ethanol traces in the extract could potentially interfere with the study by affecting the central nervous system, leading to ataxia or sedation, and thus skewing the results. Prior to testing, phytochemical screening was conducted to identify the bioactive compounds present in the *C. cujete* fruit extract. Phytochemical screening of *C. cujete* fruit extract revealed the presence of a diverse range of bioactive compounds, including alkaloids, saponins, tannins, and flavonoids. These findings align with previous studies[17,26] that have reported the presence of similar phytoconstituents in other plant species. The positive results for these compounds suggest the potential of *C. cujete* fruit extract for various pharmacological activities, particularly due to the known antioxidant and antimicrobial properties of these phytochemicals.The results of this analysis are summarized in **Table III**.

Table III. Phytochemical screening of *C. cujete* fruit extract.

Phytochemicals		Results	Description
Alkaloids	Mayer's	White precipitate	+
	Dragendorff's	Redbrick precipitate	+
Saponins		Stable foam ± 1 cm	+
Tannins		Greenish black color	+
Flavonoids		Reddish orange color	+

+: presence of phytochemical

The selection of loperamide and attapulgite as comparators was informed by previous studies demonstrating the difference between loperamide and attapulgite in terms of their antidiarrheal mechanism[27]. To comprehensively assess the antidiarrheal effects of *C. cujete* fruit extract, various parameters were monitored, including the onset of diarrhea, stool weight, frequency, duration, and consistency. Administration of *oleum ricini* induced diarrhea in the mice, as evidenced by the onset of liquid fecal excretion. As depicted in **Figure 1**, the successful induction of diarrhea was confirmed by the observation of liquid stool within a specific timeframe following *oleum ricini* administration.

Treatment with 500 mg/kg BW of *C. cujete* fruit extract significantly suppressed the onset of diarrhea, comparable to the effects of 1200 mg/kg BW attapulgite and 4 mg/kg BW loperamide. However, lower doses of the extract (125 and 250 mg/kg BW) exhibited reduced antidiarrheal efficacy. The weight of fecal output was monitored every 30 minutes for 180 minutes post-*oleum ricini* administration (**Figure 2**). The results indicate that the antidiarrheal activity of the extract was dose-dependent. While lower doses (125 and 250 mg/kg BW) exhibited a less pronounced effect on stool weight reduction compared to the standard drugs, the highest dose (500 mg/kg BW) demonstrated comparable antidiarrheal activity to loperamide and attapulgite.

Additionally, the frequency of diarrhea episodes was determined by counting the number of occurrences during the observation period. The results showed that extracts administered at doses of 125 and 250 mg/kg BW exhibited lower

efficacy in suppressing diarrhea compared to the standard drugs, loperamide and attapulgite. However, the 500 mg/kg BW extract demonstrated comparable antidiarrheal activity to both standard drugs in reducing diarrhea frequency. The reduction in diarrhea frequency, as depicted in **Figure 3**, supports the efficacy of the extract at the 500 mg/kg BW dose.

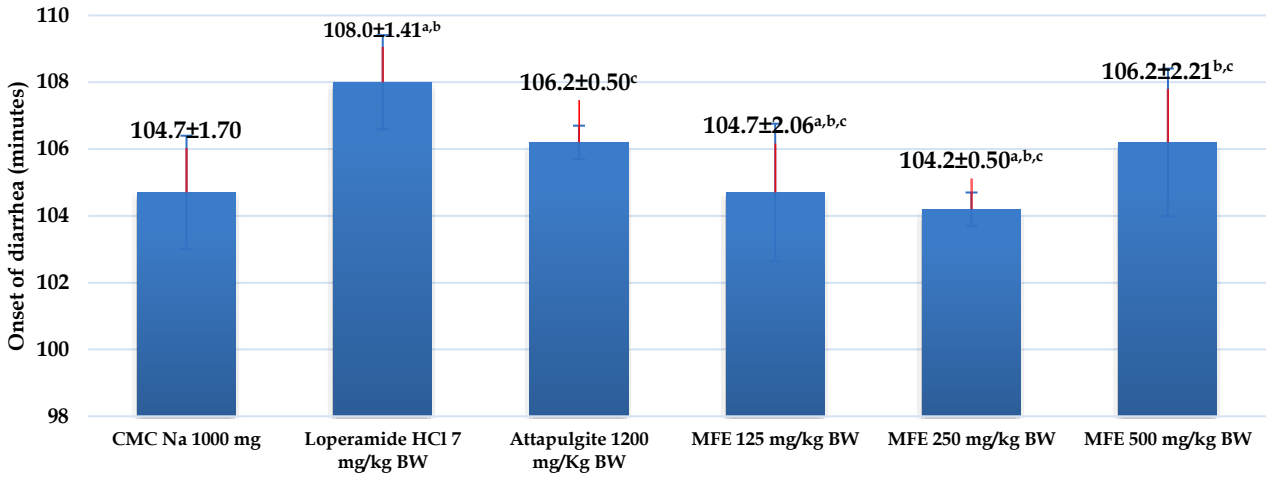

Figure 1. Diarrhea onset of each test group. **a**: There is a significant difference p <0.05; **b,c:** There is no significant difference p >0.05. MFE: *C. cujete* fruit extract.

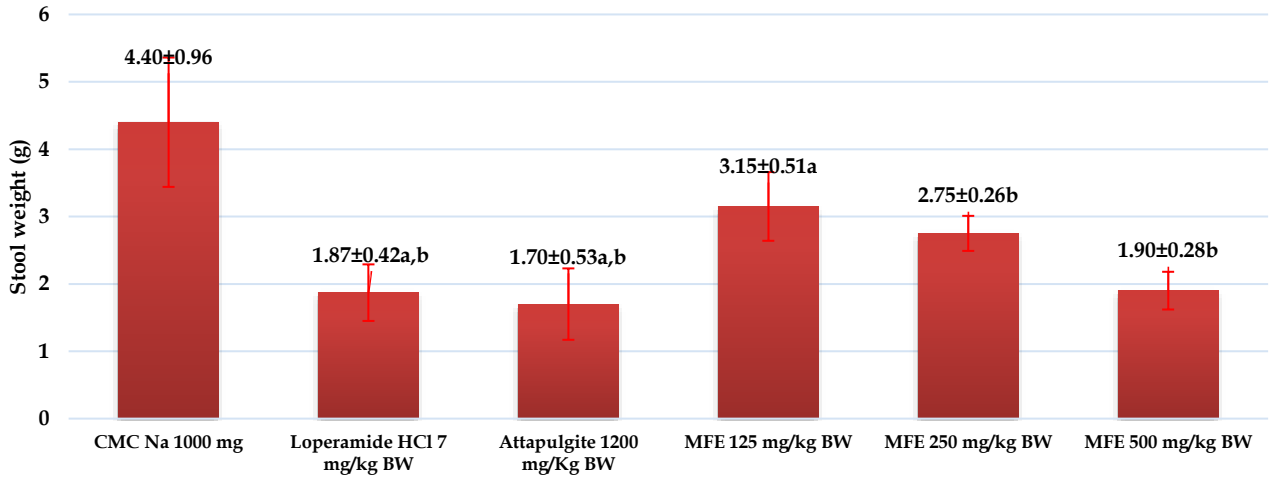

Figure 2. Stool weight of each test group. **a**: There is a significant difference p <0.05; **b,c:** There is no significant difference p >0.05. MFE: *C. cujete* fruit extract.

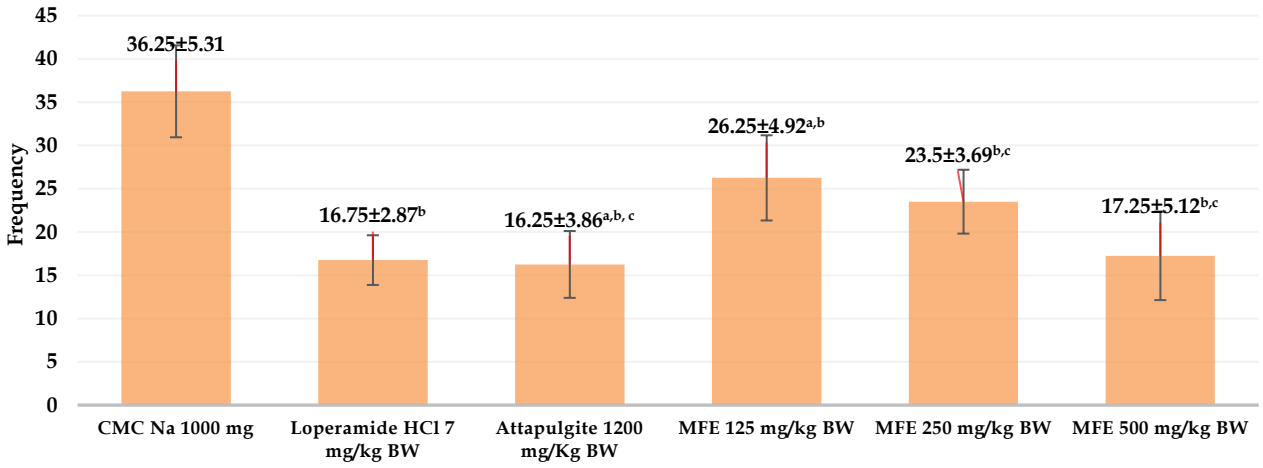

Figure 3. Frequency of diarrhea of each test group. **a**: There is a significant difference p <0.05; **b,c:** There is no significant difference p >0.05. MFE: *C. cujete* fruit extract.

The duration of diarrhea, determined by the time interval between the onset and resolution of liquid stool, was observed across all dose groups (**Figure 4**). Interestingly, the 250 and 500 mg/kg BW dose groups exhibited similar diarrhea durations, comparable to the positive control. In contrast, the 125 mg/kg BW group experienced a notably longer duration of diarrhea. These findings suggest that increasing the dose beyond 250 mg/kg BW may not significantly reduce diarrhea duration but could potentially enhance therapeutic efficacy.

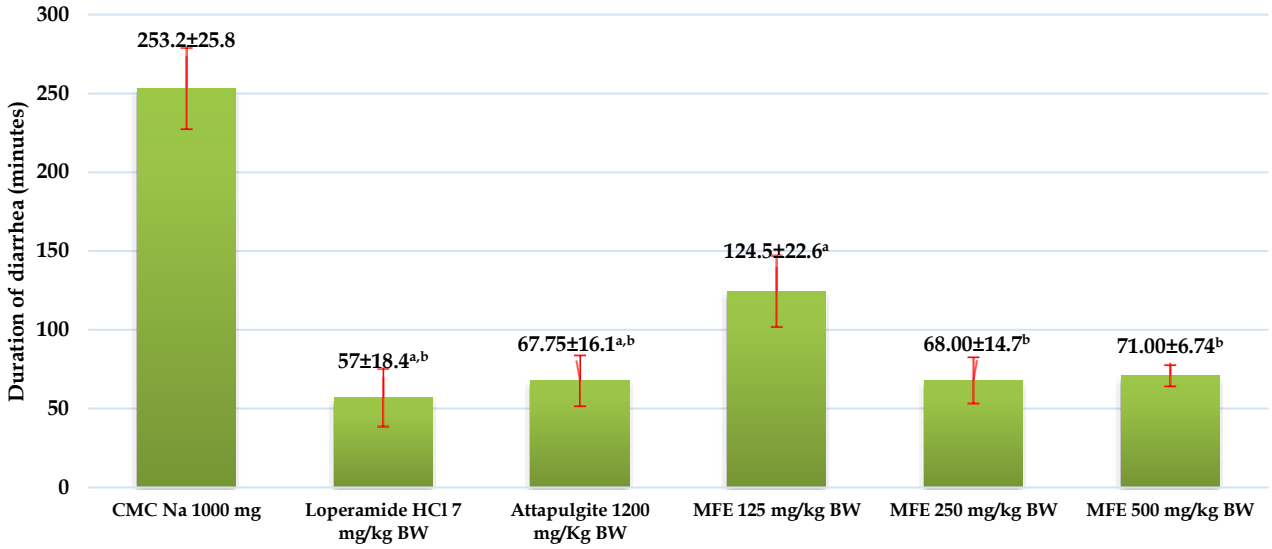

Figure 4. Duration of diarrhea of each test group. **a**: There is a significant difference $p < 0.05$; **b,c**: There is no significant difference $p > 0.05$. MFE: *C. cujete* fruit extract.

Table IV presents the average onset of action for the *C. cujete* fruit extract, attapulgite, and loperamide. Notably, the *C. cujete* fruit extract exhibited comparable anti-diarrheal activity to attapulgite and loperamide at significantly lower doses (500 mg/kg BW compared to 1200 mg/kg BW and 4 mg/kg BW, respectively). These findings suggest that *C. cujete* fruit extract could be a promising natural alternative to conventional anti-diarrheal medications. However, further research is needed to optimize the dosage and formulation of *C. cujete* extract for optimal therapeutic efficacy. Additionally, investigating the underlying mechanisms of action of the active compounds in *C. cujete* fruit extract could provide valuable insights for developing novel anti-diarrheal therapies[28].

Table IV. Phytochemical screening of *C. cujete* fruit extract.

Group	Treatment - dose administrated (per oral)	Onset (minutes±SD)
I	1% CMC-Na 1000 mg	-
II	Loperamide 7 mg/kg BW	124±33.37
III	Attapulgite 1200 mg/kg BW	130±36.34
IV	*Crescentia cujete* fruit extract 125 mg/kg BW	162±97.58
V	*Crescentia cujete* fruit extract 250 mg/kg BW	131±36.85
VI	*Crescentia cujete* fruit extract 500 mg/kg BW	130±36.34

This study's findings corroborate previous research[29,30], which has shown that tannin-rich plants like *C. cujete* possess chelating properties and spasmolytic effects. Tannins interact with proteins in mucus and epithelial cells, forming cross-links that lead to astringency. This mechanism can reduce intestinal motility by inhibiting the release of acetylcholine, a neurotransmitter that regulates smooth muscle contractions[31].

However, further research is necessary to fully elucidate the antidiarrheal properties of *C. cujete*. Future studies should explore different extraction methods, solvents, and pharmaceutical dosage forms. Additionally, comprehensive safety assessments, including acute and chronic toxicity studies, are crucial before clinical trials can be conducted to confirm the safety and efficacy of *C. cujete* as an antidiarrheal agent.

CONCLUSION

The most potent antidiarrheal effect was observed with the 500 mg/kg BW dose of *C. cujete* fruit extract, which was comparable to the standard antidiarrheal medications, loperamide and attapulgite. At this dose, the extract normalized fecal consistency within 210 minutes, indicating significant antidiarrheal activity.

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AUTHORS' CONTRIBUTION

Conceptualization: Teodhora
Data curation: Rosario Trijuliamos Manalu
Formal analysis: Rosario Trijuliamos Manalu
Funding acquisition: -
Investigation: Teodhora
Methodology: Teodhora
Project administration: Teodhora
Resources: -
Software: -
Supervision: Rosario Trijuliamos Manalu
Validation: Rosario Trijuliamos Manalu
Visualization: Teodhora
Writing - original draft: Richi Andika Saputra
Writing - review & editing: Teodhora, Rosario Trijuliamos Manalu

DATA AVAILABILITY

None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this study.

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