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RESEARCH ARTICLE

# Antidiarrheal Activity of *Amaranthus spinosus L.* Leaf Extract in Castor Oil Induced Diarrhea and Gastrointestinal Motility Models in Mice

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Abstract Diarrhea is a common digestive disorder characterized by frequent bowel movements and a shift in stool consistency toward a more liquid form. *Amaranthus spinosus* L., a plant known to contain tannins and flavonoids, is believed to have antidiarrheal potential due to its astringent properties, which may help reduce intestinal secretions. This study explored the antidiarrheal effect of the ethanolic extract of *A.spinosus* leaves in male mice. A total of 25 mice were divided into five groups: a negative control group receiving 0.5% CMC-Na suspension, a positive control group treated with Loperamide HCl (0.52 mg/kg BW), and three test groups receiving the *A.spinosus* extract at doses of 25, 50, and 100 mg/kg BW. Diarrhea was induced using castor oil (oleum ricini), and observations were made every 30 minutes over six hours, focusing on the onset of diarrhea, stool consistency, frequency of defecation, and overall duration of symptoms. The results showed that all doses of the extract had a measurable antidiarrheal effect, with higher doses producing stronger responses. Mice treated with the highest dose (100 mg/kg BW) experienced a delayed onset of diarrhea, fewer episodes, faster normalization of stool consistency, and shorter symptom duration. Statistically, the 100 mg/kg BW dose showed a significant improvement ($p < 0.05$) compared to the Loperamide HCl treated group in delaying diarrhea onset and reducing defecation frequency. These findings support the potential use of *A.spinosus* leaf extract as a natural antidiarrheal agent, which may be associated with the presence of tannins and flavonoids identified through phytochemical screening. In conclusion, the ethanolic extract of *A.spinosus* leaves demonstrated promising antidiarrheal activity in mice, especially at higher doses.

**Keywords**: Antidiarrheal, *Amaranthus spinosus* L., oleum ricini, defecation.

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## Introduction

Diarrhea is a pathological condition characterized by three or more bowel movements per day, accompanied by a change in stool consistency from pasty to watery. In more severe cases, when stools are accompanied by mucus or blood, the condition is referred to as dysentery, which is commonly associated with invasive intestinal infections such as bacterial or protozoal pathogens rather than functional or non-infectious causes. Globally, diarrhea remains a significant public health concern due to its high morbidity and mortality rates, widespread prevalence, challenges in treatment, and limited investment in related research [1], [2]. Globally, diarrhea remains a major public health concern due to its high morbidity and mortality, particularly among children under five years of age. According to the

World Health Organization (WHO), diarrhea is the second leading cause of death in this age group, accounting for approximately 525,000 child deaths annually worldwide. Despite being largely preventable and treatable, diarrhea continues to impose a substantial disease burden, especially in low- and middle-income countries, due to limitations in access to effective treatment, adverse drug reactions, contraindications, and the increasing emergence of antimicrobial resistance [3], [4]. In many parts of the world, medicinal plants serve as vital and often primary sources of treatment for diarrhea. These plants are culturally accepted, readily accessible, and relatively inexpensive compared to conventional medicine [3]. Traditional knowledge, passed down through generations, has led to using various native plants for diarrhea management. For instance, the cashew tree has demonstrated antidiarrheal activity through polysaccharides extracted from its gum [5], and terpenes such as alpha-terpineol, found in pineapple, bergamot, lemongrass, and eucalyptus, have also been shown to possess antidiarrheal properties [6].

*Amaranthus spinosus* L., a member of the *Amaranthaceae* family, is a well-known traditional medicinal herb used for managing inflammation, diabetes, depression, malaria, wounds, and pain [7]. Nutritionally, it is rich in protein (12.6–18.0%), fats (5–8%), saccharides (60–65%), and crude fiber (3–5%). The stem bark is a notable source of phenolic acids [8], and the plant contains a wide array of bioactive phytochemicals, including flavonoids, alkaloids, glycosides, phenolics, terpenoids, saponins, tannins, and carotenoids. It also contains unique compounds such as amaranthosides, amaricins, coumaroyl adenosine, and stigmasterol glycoside [9]. Previous studies have documented various pharmacological activities of *A.spinosus*, including anti-diabetic, anti-tumor, antimicrobial, anti-inflammatory, analgesic, spasmolytic, bronchodilator, hepatoprotective, antifertility, antimalarial, and antioxidant properties [10]. Many of the bioactive compounds identified in *A.spinosus*, particularly flavonoids, tannins, alkaloids, and saponins, have been reported to influence gastrointestinal function through multiple mechanisms. Flavonoids are known to reduce intestinal motility and inhibit prostaglandin-mediated secretion, thereby decreasing fluid accumulation in the intestinal lumen. Tannins exhibit astringent properties that can reduce intestinal secretion and form protective layers on the mucosal surface, helping to limit irritation and inflammation. In addition, alkaloids and saponins have been associated with spasmolytic and anti-inflammatory effects, which may contribute to the regulation of intestinal peristalsis. Collectively, these mechanisms suggest a plausible pharmacological basis for the antidiarrheal potential of *A.spinosus* leaf extract.

Numerous traditionally claimed therapeutic effects of *A.spinosus* leaf extracts such as antidiabetic [11], anti-inflammatory [12], antimalarial [13], antibacterial [14], anthelmintic [15] , and hepatoprotective [5] activities have been validated through in vitro and in vivo studies. However, despite its widespread traditional use, the antidiarrheal potential of *A.spinosus* has not been adequately evaluated in vivo. Therefore, this study aimed to investigate the antidiarrheal activity of *A.spinosus* leaf ethanol extract using castor oil- induced diarrhea models in mice. The scientific validation of this traditional claim is essential, given the plants rich phytochemical profile and broad therapeutic potential.

# Materials and Methods

## Animals

The use of experimental animals in this study was approved by the Animal Research Ethics Committee of Universitas Sumatera Utara (approval number 0195/KEPH-FMIPA/2019). Adult male Swiss albino mice (*Mus musculus* L.), weighing 25–30 g, were used in this study to minimize hormonal variations that may influence gastrointestinal motility. The mice were randomly assigned into five experimental groups, each consisting of five animals. Prior to the experiment, all animals were acclimatized for 7–14 days under standard laboratory conditions, including a controlled 12-hour light/dark cycle, with free access to food and water [16]. The animals were allocated into experimental groups using simple randomization to ensure equal distribution and reduce selection bias. Each group consisted of five animals (n = 5), a sample size commonly employed in preliminary and exploratory in vivo pharmacological studies and in accordance with ethical guidelines aimed at minimizing animal use while maintaining statistical reliability.

## Materials

Ethanol (96%, analytical grade), sodium carboxymethyl cellulose (CMC-Na), castor oil (oleum ricini), Loperamide HCl, Chinese ink, distilled water, and all reagents used for phytochemical screening were of analytical grade and obtained from Merck (Darmstadt, Germany). Nutrient agar and other microbiological-grade. All chemicals and reagents were used without further purification.

## Tools

The main equipment used in this study included an analytical balance (Shimadzu, Japan), rotary evaporator (Buchi, Switzerland), percolator, blender, hot air oven, desiccator, water bath, glassware,

electric blender (Philips, Indonesia), animal cages and oral gavage needles. All instruments were calibrated prior to use.

## Plant materials Collection and Authentication

The plant material used in this study was *Amaranthus spinosus* L., collected from Medan City, North Sumatera Province, Indonesia. The identification and authentication of the plant specimen were conducted at the Medanense Herbarium, Universitas Sumatera Utara, and verified under the specimen number 2528/MEDA/2019.

## Evaluation of Simplicia

The evaluation of *A.spinosus* simplicia characteristics was carried out through several standard quality parameters. These included the determination of moisture content, water- soluble extractive value, ethanol- soluble extractive value, total ash content, and acid- insoluble ash content [17].

## Preparation of Ethanolic Leaf Extract of *Amaranthus spinosus* L.

Fresh leaves of *A.spinosus* were collected, and the stems were removed. The leaves were thoroughly washed under running water to eliminate dirt and impurities, then drained and weighed to obtain the fresh weight. Subsequently, the leaves were air-dried at room temperature until a constant dry weight was achieved. The dried leaves were pulverized into a fine powder using an electric blender to obtain simplicia. A total of 500 g of the powdered simplicia was moistened with ethanol 96% and allowed to stand for 3 h. The material was then transferred into a percolator, and additional ethanol was added until the powder was completely submerged. The percolator was covered with aluminum foil and left to macerate for 24 h. After maceration, percolation was initiated by opening the stopcock, allowing the extract to drip at a constant flow rate of approximately 1 mL/min. The process was continued until the percolate no longer reacted with the test reagent, indicating complete extraction of the soluble constituents. The collected extract was subsequently concentrated using a rotary evaporator at a temperature not exceeding 50°C to obtain a thick, viscous ethanolic extract. Evaporation and drying were performed at controlled temperatures to prevent thermal degradation of thermolabile bioactive compounds, particularly flavonoids and tannins, which are known to be sensitive to prolonged exposure to high heat.

## Preliminary Phytochemical Screening

Phytochemical screening was conducted on both the simplicia and the ethanol extract of Amaranthus spinosus to identify the presence of major secondary metabolites. The analysis included qualitative tests for flavonoids, alkaloids, saponins, tannins, glycosides, steroids/triterpenoids, and anthraquinone glycosides [18].

## Animal grouping and Dose Administration

The dose of the ethanolic extract of *Amaranthus spinosus* (ASEE) was determined based on a preliminary orientation study in experimental animals. Based on this assessment, doses of 25, 50, and 100 mg/kg body weight (BW) were selected for further evaluation. A total of 25 male mice were randomly allocated into five groups (n = 5 per group). The negative control group received 0.5% sodium carboxymethyl cellulose (CMC-Na) suspension (50 mg/kg BW), while the positive control group was administered loperamide HCl suspension (0.52 mg/kg BW). The treatment groups received ASEE at doses of 25, 50, and 100 mg/kg BW, respectively. Throughout the experimental period, all animals were housed individually in observation cages under controlled environmental conditions. Animals were monitored for general behavioral changes, physical condition, food and water intake, and signs of discomfort or adverse effects following treatment administration. Specific observations related to gastrointestinal activity, including defecation pattern and stool characteristics, were recorded during the subsequent antidiarrheal evaluation.

## Antidiarrheal Activity Examination Castor oil-induced Diarrhea

The antidiarrheal activity induced by castor oil was evaluated using the method described by Husein *et al* [16] with minor modifications. The doses of *A.spinosus* ethanolic extract (25, 50, and 100 mg/kg BW) were selected based on a preliminary orientation study and supported by dose ranges previously reported for pharmacological evaluation of *A.spinosus* leaf extracts [15]. Prior to the experiment, mice were fasted for 18 h with free access to water. Following treatment administration, each mouse received 0.5 mL of castor oil orally one hour later to induce diarrhea. The negative control group received 0.5% Na-CMC suspension, while the positive control group was treated with loperamide HCl (0.52 mg/kg BW). The treatment groups received *A.spinosus* ethanolic extract at doses of 25, 50, and 100 mg/kg BW. Animals were individually placed in cages lined with pre-weighed absorbent paper, and observations

were recorded every 30 min for 6 h. Parameters evaluated included onset of diarrhea, frequency of defecation, stool consistency and weight, and duration of diarrheal symptoms. The percentage of diarrheal inhibition and reduction in total fecal output were calculated using standard formulas as previously described [19], [20]. To quantify the extract effectiveness, the percentage of diarrheal inhibition and reduction in total fecal output were calculated using standard formulas [21].

$$Percentage\ of\ diarrheal\ inhibition = \frac{\text{mean number of wet stools (control group} - \text{treated group}}{\text{mean number of wet stools of the control group}} x\ 100$$

## Castor Oil-induced Intestinal Transit

The antimotility activity of the extract was evaluated using the intestinal transit method as described by Husein *et al*. [16]. Castor oil was administered to induce intestinal hypermotility through its active metabolite, ricinoleic acid, which stimulates prostaglandin release and enhances intestinal peristalsis. This model allows assessment of the ability of test substances to attenuate pathologically increased gastrointestinal motility. Experimental animals were divided into negative control, positive control, and treatment groups. Prior to the procedure, all mice were fasted for 18 h with free access to water. Each mouse was administered 0.5 mL of castor oil orally. One hour later, the respective treatments were given orally: the negative control group received 0.5% Na-CMC suspension, the positive control group received loperamide HCl (0.52 mg/kg BW), and the treatment groups received *A.spinosus* ethanolic extract at doses of 25, 50, and 100 mg/kg BW. One hour after treatment administration, each mouse was given 1 mL of Chinese ink orally as a non-absorbable marker. Chinese ink was used to visually track the progression of intestinal contents, enabling quantitative measurement of intestinal transit. One hour after marker administration, all animals were euthanized by cervical dislocation. The small intestine was excised from the pylorus to the ileocecal junction, and the distance traveled by the marker was measured relative to the total intestinal length. The peristaltic index and percentage inhibition of intestinal transit were calculated as previously described [19], [20].

$$The\ Peristalsis\ Index\ (PI) = \frac{\text{distance travelled by the Chinese ink}}{\text{total length of small intestine}} x\ 100$$

$$Percentage\ of\ inhibition = \frac{\text{PI of negative control} - \text{PI of drug or extract}}{\text{PI of negative control}} x\ 100$$

## Statistical Analysis

The data are presented as mean values accompanied by the *standard error of the mean* (SEM). To determine differences among groups, a *one-way analysis of variance* (ANOVA) was applied, followed by Tukey's post hoc test for detailed pairwise comparisons. All analyses were carried out using SPSS software version 25, with the level of significance set at 95% confidence. Results were considered statistically significant when the p-value was below 0.05.

# Results and Discussion

## Simplicia Characterization

The results of the characterization of simplicia powder and ethanol extract of *A.spinosus* show the characteristics as shown in Table 1 below:

**Table 1**. Characterization of *A.spinosus* L.

| No | Characterization | Observed value (%) | Reference standard (%) |
|---|---|---|---|
| 1 | Water content | 6,67 | < 10,00 |
| 2 | Water soluble essence | 23,00 | > 7,00 |
| 3 | Ethanol soluble essence | 10,67 | > 2,50 |
| 4 | Total ash content | 8,27 | < 10,00 |
| 5 | Acid insoluble ash | 0,87 | < 1,00 |

The quality of *A.spinosus* simplicia was evaluated based on physicochemical parameters, including moisture content, extractive values, and ash content, and the results were compared with the acceptance limits established by Materia Medika Indonesia (MMI). All observed values fell within the specified standard ranges. Moisture content and ash values were below the maximum permissible limits, while water- and ethanol-soluble extractive values exceeded the minimum required levels, indicating adequate content of extractable constituents. Since these parameters were assessed for quality control purposes, no statistical comparison was performed. Compliance with the established standards confirms that the simplicia used in this study met the required quality criteria and was suitable for further extraction and pharmacological evaluation [16].

## Phytochemical Screening

The results of phytochemical screening of simplicia powder and ethanol extract of *A.spinosus* leaves show the chemical compound groups as shown in Table 2 below:

**Table 2**. Phytochemical screening of simplicia powder and *A.spinosus* L. ethanol extract

| No | Secondary metabolites | Result | |
|---|---|---|---|
| | | Simplicia powder | Crude extract |
| 1 | Alkaloids | + | + |
| 2 | Glycosides | + | + |
| 3 | Steroids | + | + |
| 4 | Flavonoids | + | + |
| 5 | Tannins | + | + |
| 6 | Saponins | + | + |
| 7 | Anthraquinones | + | + |

(+) : present (-) : absent

The phytochemical screening results of *A.spinosus* revealed the presence of several secondary metabolites, including flavonoids, tannins, saponins, alkaloids, glycosides, steroids, and anthraquinones, in both the simplicia powder and the ethanolic extract (Table 2). These classes of compounds have been widely reported in the literature to possess antidiarrheal activity through various mechanisms. Flavonoids have been shown to exert antidiarrheal effects by reducing intestinal motility and inhibiting prostaglandin-mediated intestinal secretion, leading to decreased fluid accumulation in the intestinal lumen [15], [19], [22]. Tannins are known for their astringent properties, which enable them to precipitate proteins on the intestinal mucosa, thereby reducing intestinal secretion and enhancing water reabsorption [22], [23]. Saponins and alkaloids have also been reported to exhibit antispasmodic and anti-inflammatory activities that contribute to the regulation of intestinal peristalsis [24], [25]. The presence of these bioactive constituents in the ethanolic extract of *A.spinosus* provides a plausible pharmacological explanation for the antidiarrheal and antimotility effects observed in the present study, particularly at higher doses. Although the present investigation employed qualitative phytochemical screening, the findings are consistent with previous reports demonstrating the antidiarrheal potential of these secondary metabolites.

## Antidiarrheal Effect Results Castor oil induced Diarrhea

In the evaluation of antidiarrheal activity induced by castor oil, the observed parameters included the onset time of diarrhea, frequency of defecation, duration of diarrheal episodes, and stool consistency. In this model, castor oil was administered to all experimental groups to induce diarrhea. Therefore, a separate castor oil only group was not included in Table 3. The negative control group (0.5% Na-CMC) represents the diarrheal response induced solely by castor oil in the absence of any antidiarrheal treatment.

**Table 3**. Activity of the extract of *A.spinosus* L. on diarrhea induced by castor oil

| No | | | Frequency (times) | Duration (minute) | Number of wet stool | Number of total stool | % Diarrheal Inhibiton |
|---|---|---|---|---|---|---|---|
| 1 | Na-CMC 0,5% | 65.77 ± 0.170$^{+}$ | 6.00 ± 0.447$^{+}$ | 280.70 ± 0.307$^{+}$ | 8.04 ± 0.118$^{+}$ | 11.39 ± 0.118$^{+}$ | - |
| 2 | Loperamide 0,52 mg/kgBW | 132.24 ± 0.482$^{*}$ | 3.60 ± 0.244$^{*}$ | 129.86 ± 0.288$^{*}$ | 2.93 ± 0.229$^{*}$ | 5.94 ± 0.258$^{*}$ | 63,92 |
| 3 | *A.spinosus* extract 25 mg/kgBW | 74.19 ± 0.274$^{+*}$ | 5.40 ± 0.244$^{+}$ | 229.97 ± 0.267$^{+*}$ | 6.72 ± 0.238$^{+*}$ | 10.52 ± 0.117$^{+*}$ | 10,30 |
| 4 | *A.spinosus* extract 50 mg/kgBW | 97.12 ± 0.450$^{+*}$ | 4.40 ± 0.244$^{*}$ | 191.15 ± 0.345$^{+*}$ | 5.75 ± 0.262$^{+*}$ | 10.07 ± 0.041$^{+*}$ | 22,16 |
| 5 | *A.spinosus* extract 100 mg/kgBW | 102.38 ± 0.500$^{+*}$ | 3.80 ± 0.200$^{*}$ | 134.60 ± 0.241$^{+*}$ | 4.03 ± 0.110$^{+*}$ | 9.51 ± 0.196$^{+*}$ | 50,17 |

Results are reported as mean ± SEM (n = 5). Statistical significance was assessed using one-way ANOVA with Tukey's post hoc test. $p < 0.05$ denotes a significant difference from the negative control (0.5% Na-CMC), and $^{+}p < 0.05$ indicates significance compared to the positive control (loperamide 0.52 mg/kg BW). Diarrheal inhibition (%) represents the percentage reduction in wet stool frequency compared to the negative control group, which reflects castor oil–induced diarrhea without treatment.

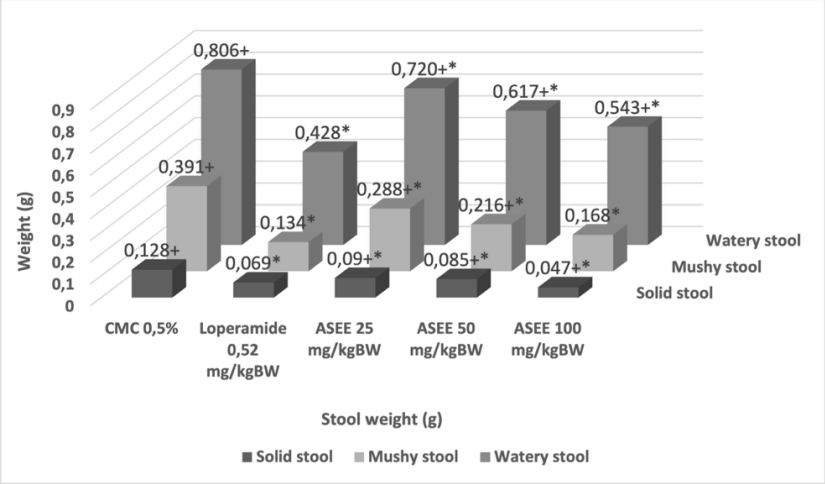


**Figure 1.** Effect of *A.spinosus* L. ethanolic extract on stool consistency in castor oil–induced diarrhea in mice. Stool weight (g) was measured and classified according to consistency into solid, mushy, and watery stools. Data are presented as mean ± SEM (n = 5). *$p < 0.05$ indicates a significant difference compared with the negative control group (0.5% Na-CMC), and $^{+}p < 0.05$ indicates a significant difference compared with the positive control group (loperamide 0.52 mg/kg BW). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

The consistency and weight of feces were assessed by directly weighing the excreted stool and classifying it into three categories: solid, mushy, and watery. Treatment with the ethanol extract of *A.spinosus* resulted in a dose-dependent reduction in total stool weight and a shift toward firmer stool consistency. Notably, the group receiving 100 mg/kg BW of the extract demonstrated an effect comparable to the positive control, loperamide ($p > 0.05$), indicating significant antidiarrheal activity at this dose level. These findings are illustrated in Figure 1. The presence of flavonoids and tannins in *A.spinosus* extract may significantly contribute to its observed antidiarrheal activity. Flavonoids have been reported to reduce intestinal hypersecretion and inhibit prostaglandin-mediated fluid accumulation in the intestinal lumen, thereby improving stool consistency and decreasing diarrheal severity. Recent studies indicate that flavonoids also modulate intestinal motility through interactions with calcium channels and inflammatory mediators, supporting their role in regulating gastrointestinal function [26], [27]. Tannins contribute to antidiarrheal activity through their astringent properties, which promote protein precipitation on intestinal mucosa, forming a protective layer that reduces intestinal secretion and enhances fluid reabsorption. Contemporary pharmacological evaluations confirm that tannin-rich plant extracts can stabilize intestinal epithelial integrity and inhibit enterotoxin activity, thereby reducing stool frequency and fluid loss [28], [29]. In addition, alkaloids and steroidal compounds identified in *A.spinosus* have been associated with antispasmodic and anti-inflammatory properties. These compounds are known to influence smooth muscle relaxation and inhibit inflammatory mediators involved in intestinal motility disorders. Recent experimental evidence

suggests that alkaloids may modulate cholinergic pathways and reduce intestinal hypermotility, supporting their involvement in diarrhea management [30]. Furthermore, secondary metabolites such as saponins and glycosides have demonstrated antimicrobial and mucosal protective activities, which may help prevent diarrhea caused by infectious agents. Studies published in recent years emphasize that plant-derived saponins can inhibit pathogenic bacterial adhesion and reduce gastrointestinal inflammation, thereby contributing to overall antidiarrheal effects [31]. Collectively, the presence of these bioactive compounds provides a pharmacological basis for the dose-dependent antidiarrheal activity observed in this study and is consistent with recent scientific findings demonstrating the therapeutic potential of plant secondary metabolites in gastrointestinal disorders.

## Castor Oil-induced intestinal Transit

The effect of *A.spinosus* on intestinal motility was evaluated using the intestinal transit method, with Chinese ink employed as a marker to assess the inhibition of intestinal peristalsis. As illustrated in Figure 2, *A.spinosus* ethanol extract demonstrated a dose- dependent reduction in intestinal transit in mice induced with castor oil (oleum ricini). Statistical analysis revealed that the extract at a dose of 100 mg/kg BW (25.22 ± 0.172) exhibited intestinal transit inhibition comparable to that of the positive control, loperamide at 0.52 mg/kg BW (22.77 ± 0.215), with no significant difference observed ($p > 0.05$). The percentage inhibition of intestinal motility across the treatment groups is presented in Figure 3.

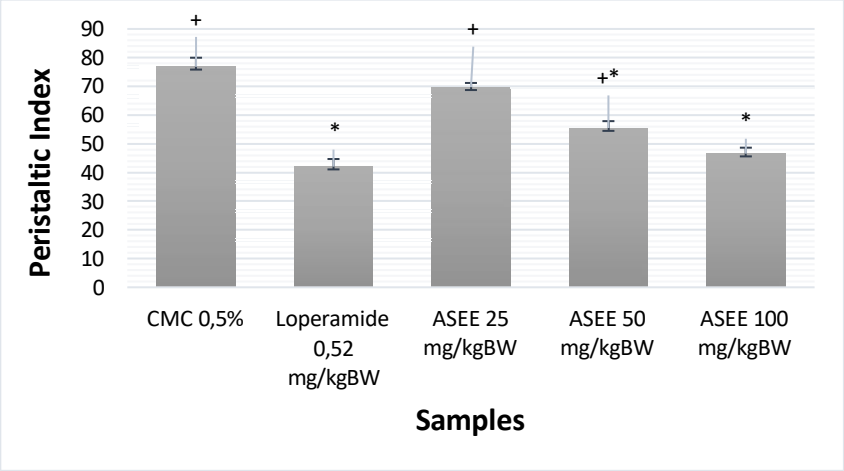


**Figure 2.** The peristaltic index of the *A.spinosus* ethanol extract activity on the intestinal transit test of mice administered with Chinese ink. Data presented as mean + SEM, n = 5. *p< 0.05 compared to negative control (Na CMC 0.5%), +p < 0.05 compared to positive control as loperamide 0.52 mg/kgBW (One-way ANOVA followed by Tukey post hoc tests)

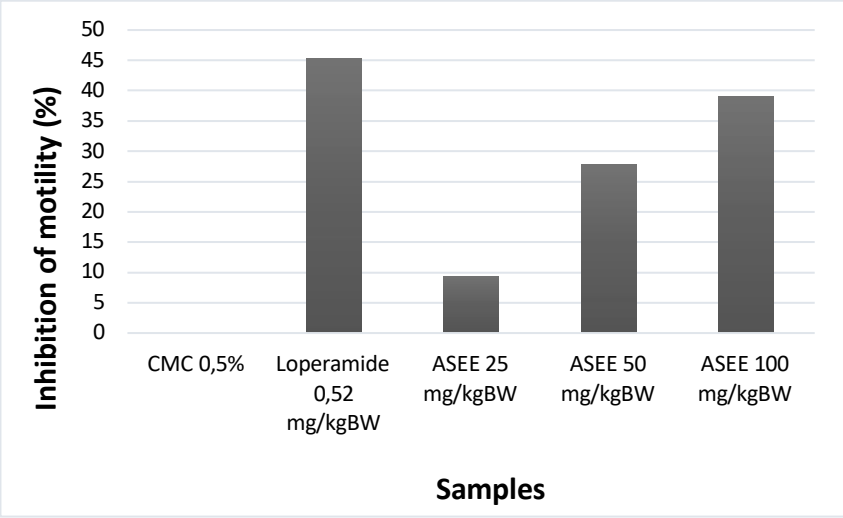


**Figure 3.** The inhibition of motility of the *A.spinosus* ethanol extract activity on the intestinal transit test of mice administered with Chinese ink

Castor oil induced diarrhea is a widely accepted experimental model for evaluating the antidiarrheal activity of medicinal plants [20]. In the present study, the in vivo antidiarrheal effect of *A.spinosus* ethanolic extract was assessed using castor oil induced models. The active component of castor oil, ricinoleic acid, exerts its effect by binding to and activating prostanoid EP3 receptors, which subsequently stimulate the release of endogenous prostaglandins derived from arachidonic acid in the intestinal mucosa. These prostaglandins increase gastrointestinal motility, produce a laxative effect, and disrupt water and electrolyte transport across the intestinal lumen, ultimately resulting in diarrhea [20], [32]. Clinical manifestations of diarrhea generally appear within 1–2 hours following oral administration of castor oil at doses ranging from 0.1 to 0.3 mL [33]. The antidiarrheal effects of medicinal plants are commonly attributed to their phytochemical constituents, including flavonoids, tannins, saponins, sterols, terpenes, and other polyphenolic compounds [34]. These compounds may counteract ricinoleic acid induced intestinal disturbances through multiple complementary mechanisms. Flavonoids have been reported to inhibit prostaglandin synthesis and reduce intestinal hypersecretion by modulating cyclooxygenase and lipoxygenase pathways. Additionally, flavonoids exhibit smooth muscle relaxant properties, which can reduce intestinal motility and prevent excessive peristalsis triggered by inflammatory mediators [24], [25]. Tannins contribute to antidiarrheal activity primarily through their protein-precipitating and mucosal protective effects. By forming complexes with mucosal proteins, tannins create a protective layer on the intestinal surface, thereby reducing intestinal secretion, enhancing water and electrolyte reabsorption, and minimizing mucosal irritation caused by ricinoleic acid exposure [35]. Furthermore, tannins possess anti-inflammatory and antimicrobial properties that may reduce intestinal inflammation and suppress pathogenic microorganisms that exacerbate diarrheal conditions [23]. In addition to flavonoids and tannins, other secondary metabolites present in *A.spinosus*, such as saponins and alkaloids, may further contribute to the observed pharmacological activity. Saponins are known to enhance mucosal barrier integrity and regulate intestinal permeability, whereas alkaloids may exert antispasmodic and anti-inflammatory effects that help stabilize intestinal motility. The synergistic interaction among these phytochemical constituents likely attenuates the physiological disturbances induced by ricinoleic acid, thereby supporting the antidiarrheal and antimotility effects observed in this study.

The chemical compounds containing tannins and flavonoids in the ethanol extract of *A.spinosus* are likely to have an anti-diarrhea effect by inhibiting intestinal peristalsis, inhibiting prostaglandin production, and shrinking the intestinal mucous membrane, thereby helping to stop diarrhea. The anti-motility activity of medicinal plant extracts relates to phytochemicals such as tannins, flavonoids, and alkaloids [22]. The antidiarrheal and antimotility effects observed at the 100 mg/kg BW dose of *A.spinosus* extract suggest a sufficient concentration of active phytochemicals. In contrast, the lower doses (25 and 50 mg/kg BW) likely contained insufficient levels of these constituents, resulting in limited effect. Previous studies have also reported the antispasmodic activity of *A.spinosus* leaf extracts [36]. This is supported by results from the Chinese ink intestinal transit test, where higher doses of the extract significantly inhibited gastrointestinal motility, whereas lower doses did not. This confirms the dose-dependent antimotility effect of *A.spinosus*, consistent with the mechanism of conventional antidiarrheal drugs, which act by reducing gut motility and secretion. In models assessing gastrointestinal motility, commonly used antidiarrheal agents exert their effects through distinct mechanisms, primarily by decreasing intestinal peristalsis and inhibiting the secretion of luminal contents. The Chinese ink transit model is frequently employed to evaluate the impact of medicinal plants on gastrointestinal motility, with the ink functioning as a reliable marker to quantify intestinal transit [16]. The significant reduction in marker progression observed at the highest extract dose indicates inhibition of intestinal peristalsis. This mechanism is comparable to conventional antidiarrheal drugs such as loperamide, which decreases gastrointestinal motility and increases intestinal transit time, allowing greater fluid reabsorption [37]. The comparable effectiveness between the highest extract dose and the positive control suggests that *A.spinosus* possesses promising therapeutic potential as a natural antidiarrheal agent. At higher doses, the extract of *A.spinosus* was able to slow down the movement of Chinese ink through the gastrointestinal tract, indicating its potential to reduce stool frequency. On the other hand, the lower doses of the extract did not produce a statistically significant reduction in gastrointestinal motility, suggesting that its antimotility effect is minimal at smaller doses. Since cholinergic stimulation is known to trigger diarrhea by enhancing intestinal motility, these findings imply that *A.spinosus* may act in a way similar to anticholinergic agents, helping to counter excessive motility [38]. The intestinal transit test using Chinese ink further supported the antimotility effect of *A.spinosus* extract. The weaker activity observed at lower doses may be attributed to insufficient concentrations of bioactive metabolites required to achieve pharmacological efficacy. Phytochemical compounds often exhibit limited oral bioavailability due to metabolic transformation and poor intestinal absorption, which may reduce therapeutic activity at suboptimal concentrations [39]. Therefore, the dose-dependent response observed in this study highlights the importance of phytochemical concentration in achieving optimal therapeutic effects.

Overall, these findings provide experimental support for the traditional use of *A. spinosus* in diarrhea management and highlight its potential as a natural antidiarrheal agent. Compared with synthetic drugs, plant-based therapies may offer advantages such as fewer adverse effects and a lower risk of antimicrobial resistance [40]. Nevertheless, further studies focusing on quantitative phytochemical analysis, toxicity

evaluation, and molecular mechanism elucidation are required to establish the safety and therapeutic applicability of A. spinosus leaf extract.

## Conclusions

The present study demonstrated that the ethanolic extract of *A.spinosus* leaves exhibits significant antidiarrheal activity in castor oil induced diarrhea models in mice. The extract showed a clear dose-dependent effect, with the 100 mg/kg BW dose producing the most pronounced therapeutic response. At this dose, the extract significantly delayed the onset of diarrhea, reduced stool frequency and total fecal output, improved stool consistency, and decreased intestinal transit, with effects comparable to loperamide (0.52 mg/kg BW). Phytochemical screening confirmed the presence of flavonoids, tannins, alkaloids, glycosides, saponins, and steroids, which likely contribute to the observed antisecretory and antimotility effects. The inhibition of intestinal transit observed in the Chinese ink model further supports the extract's ability to modulate gastrointestinal motility disrupted by ricinoleic acid. These findings provide experimental validation for the traditional use of *A.spinosus* in the management of diarrhea and suggest its potential development as a phytotherapeutic candidate. Nevertheless, further studies are required to quantify the active constituents, clarify molecular mechanisms of action, and evaluate long-term safety and pharmacokinetic properties.

## Conflicts of Interest

There are no conflicts of interest to declare in relation to this article

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