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Assessment of the Potential Mechanisms of Anti-diarrhoeal Activities of Ethanol Extract of *Monoon longifolium* Leave in Wistar Rat

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Authors' contributions

This work was carried out in collaboration among all authors. Authors UOC and ACA designed the study, performed the statistical analysis and wrote the protocol. Author UOC wrote the first draft of the manuscript. Authors AFK and AIJ managed the analysis. Author UCC and ACL managed the literature searches. Authors OCG and NCK manage the analysis of the study. Authors UCD and OCG managed the analysis of the study. All authors read and approved the final manuscript.

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ABSTRACT

Background: Numerous diseases related to diarrhea are the primary cause of morbidity and mortality in underdeveloped countries, accounting for hundreds of thousands of deaths annually. *Polyalthia longifolia*, the false ashoka, commonly referred to as Monoon longifolium is a tree species from Asia. It is commonly found in southern india, Sri Lanka and Nigeria. Polyalthia longifolia has been utilized for decades to treat an extensive variety of illnesses, including diarrhea.
Method: The plant was extracted with ethanol using cold maceration. Preliminary qualitative and quantitative phytochemical screening of ethanol extract of Polyalthia longifolia leaf was conducted using the techniquees of Harbone (1998); Trease and Evans (1978). The Lorke (1983) approach was used to calculate the Median Lethal Dose (LD50). Diarrhea caused by castor oil was produced using the Awouter et al., 1973 method. The testing for gastrointestinal transit was conducted using the Mascolo et al. (1996) technique. The Tietz (1994) method was used to conduct the electrolyte levels assay. The assay for kidney function indicators was conducted using Bartels and Bohmer's (1972) methodology.
Results: The results of phytochemical screening showed that alkaloids and tannins occurred in high concentration, flavonoids and steroids occurred in moderate concentration, whereas terpenoids, glycosides and phenolic acid occurred in lowest concentration. The acute toxicity study revealed that the plant extract was not toxic even at the dose of 5000 mg/kg. Groups treated with 200,400, and 600 mg/kg b.w of extract showed significant ($P < 0.05$) inhibition in the frequency of defecation of wet feces and total fecal output compared with positive control. Similarly, Groups treated with 200, 400, and 600 mg/kg body weight of extract demonstrated significant ($P < 0.05$) antimotility activity when compared with the positive control. However, groups treated with 200,400 and 600mg/kg body weight showed a significant ($P < 0.05$) increase in chlorine and sodium with corresponding decrease in bicarbonate and potassium level when compared with the positive control. The results from kidney function markers showed that groups treated with 200, 400 and 600 mg/kg b.w in a dose dependent manner showed a significant ($p < 0.05$) reduction in creatine and urea when compared with positive control.
Conclusion: The ethanol leaf extract of *Polyalthia longifolia* has considerable antidiarrheal activity on castor oil-induced diarrhea and gastrointestinal motility models, confirming the reason for its wide use in traditional treatment of diarrheal conditions.

Keywords: Diarrhoea; electrolytes; gastrointestinal motility; polyanltha logifolia; ethanol extract.

1. INTRODUCTION

"Diarrheal illnesses are a major source of morbidity and mortality in developing nations, accounting for thousands of deaths annually" [1]. "A 2015 estimate states that diarrhea accounts for 9% of all pediatric fatalities worldwide, making it one of the primary causes of death for children" [2]. "According to this data, sub-Saharan Africa and southern Asia have the greatest rates of child fatalities from diarrhea [3]. Diarrheal disease is a serious public health concern in Ethiopia, one of the top 15 countries where it causes almost three-fourths of child fatalities"[3]. "Overall, the prevalence of diarrheal illness is still high despite the best efforts of multiple governments and international organizations to reduce it" [4]. "In developing countries, 80% of people still receive their daily medical care from traditional healers and medicinal plants"[5]. "In a comparable manner Nigeria has long used plants as means of pharmaceuticals to treat a variety of

ailments that affect both people and their animals" [6]. Despite significant advancements in medical technology. Herbal therapy is growing more and more popular in both developed and developing nations due to its natural nature and reduced adverse effects. Furthermore, natural products have been successful in the drug-development process; at the moment, more than half of the most widely used prescription drugs are made from herbal remedies [7]. Consequently, relying on conventional medical practice, the World Health Organisation (WHO) promoted research for the prevention and treatment of diarrheal disorders [8]. Through its Diarrhoea Control Programme, the World Health Organization has utilized traditional medicine to combat the impact of diarrhoea [9]. The side effects associated with opioid-like antimotility drugs have limited their use, prompting researchers to seek new antidiarrheal agents with diverse chemical compositions. As a result, more scientists are turning to traditional medicine to broaden the range of treatments available for diarrheal diseases [10]. *Polyalthia longifolia*, also known as the false ashoka or by its synonym *Monoon longifolium*, is a tree species native to Asia and belongs to the Annonaceae family. It is commonly found in southern India and Sri Lanka [11]. The bark and leaves of this plant have demonstrated significant antimicrobial, cytotoxic, antiulcer, hypoglycemic, and hypotensive properties [12]. Traditional healers have used this plant to treat ulcers, inflammation, pain, and diarrhea, with reports of notable success. This suggests the plant's potential as a source for antidiarrheal agents. Consequently, this study was conducted to evaluate the antidiarrheal activity of the ethanol extract of *Polyalthia longifolia* leaves in rats.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant materials

The leaves of *Polyalthia longifolia* used in this study were collected from Oba, located in Nsukka Local Government Area of Enugu State. They were identified by Mr. Alfred Ozioko from the Bioresource Development and Conservation Programme (BDCP) research center in Nsukka, Enugu State.

2.1.2 Animals

Thirty adult male Albino rats, weighing between 170 and 220 grams, were used for the antidiarrheal studies, while eighteen adult male Albino mice, weighing between 16 and 26 grams, were used for the median lethal dose (LD50) study. All the animals were sourced from the animal house of the Department of Veterinary Medicine at the University of Nigeria, Nsukka. The rats were provided with water and standard Growers mash rat pellets (Grand Cereals LTD, Enugu).

2.1.3 Instruments/Equipment

The equipment used were obtained from the Department of Biochemistry, University of Nigeria Nsukka, Bishop Shanahan Hospital, Nsukka and other scientific shops in Nsukka. They include the following: Centrifuge (PAC, Pacific), Beakers (Pyrex), Conical flask (Pyrex), Filter paper (Whatman), Micro pipette (Perfect), Refrigerator (Haier thermocool), Spatula (Pyrex), Colorimeter (Techmel and Techmel), Spectrophotometer (E312 Model, Jenway, UK), Syringe (Life Scan), Grinding mechine, Incubator, Thermometer (Zeal), Water bath(Gallenkamp, London), Weighing balance (Metler HAS), Hand gloves, Metre rule, Razor.

2.1.4 Chemicals/reagents

The chemicals and reagents used for this research work were of analytical grade.

2.2. Methods

2.2.1 Preparation of plant material

The fresh leaves of *Polyalthia longifolia* were air-dried and ground into a coarse powder. A total of 1000 grams of this powdered leaf material was soaked in 5 liters of ethanol for 72 hours. The resulting suspension was first filtered using china white cloth and then with Whatman No. 1 filter paper. The filtrate was then concentrated at 64°C using a water bath, and the extract was evaporated to a slurry consistency. The percentage yield of the extract was then calculated.

$$\text{Percentage yield} = \frac{\text{weight of the crude extract}}{\text{Weight of the pulverized extract}} \times 100$$

2.2.2 Determination of median lethal dose

The acute toxicity test for the ethanol leaf extract of *Polyalthia longifolia* was carried out following the method outlined by [13]. The study was performed in two phases, involving a total of 18 mice. In the first phase, nine mice were divided into three groups, each consisting of three mice. The animals in Groups 1, 2, and 3 were administered the extract at doses of 10, 100, and 1000 mg/kg body weight (b.w.), respectively. Clinical signs of toxicity and mortality were monitored over a 24-hour period. In the second phase, 9 mice were divided into 3 groups of 3 mice each. Three groups of three (3) mice each were treated with 1600, 2900, and 5000 mg/kg b.w of the extract respectively. The extract was dissolved in normal saline and the route of administrate was oral (p.o).

Determination of qualitative and quantitative phytochemical screening of ethanol leaf extract of *Polyalthia longifolia* was carried out by method of [14-15] respectively.

2.2.3 Experimental design

Thirty adult male albino rats, previously fed a standard Pfizer diet with free access to water, were used for the study. After fasting for 18 hours with continued access to water, the rats were divided into six groups of five rats each and treated using appropriate stomach tubes as follows:

- **Group 1:** Received 0.2 ml of normal saline (control).
- **Group 2:** Received 1 ml of castor oil (positive control).
- **Group 3:** Administered 2 mg/kg of the standard drug, loperamide, prior to castor oil administration.
- **Group 4:** Treated with 200 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia* before castor oil administration.
- **Group 5:** Treated with 400 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia* before castor oil administration.
- **Group 6:** Treated with 600 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia* before castor oil administration.

2.3 Antidiarrheal Studies

The effect of the ethanol leaf extract of *Polyalthia longifolia* on castor oil-induced diarrhea was assessed in rats using the method described by [16]. Thirty adult male albino rats, previously fed a standard Pfizer diet with free access to water, were used in the study. After fasting for 18 hours with access to water, the rats were divided into six groups of five rats each and treated using appropriate mouth gauge needles as follows:

- **Group 1:** Received 0.2 ml of normal saline (control).
- **Group 2:** Received 1 ml of castor oil (positive control).
- **Group 3:** Administered 2 mg/kg of the standard drug, loperamide.
- **Group 4:** Treated with 200 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.
- **Group 5:** Treated with 400 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.
- **Group 6:** Treated with 600 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.

One hour after the treatments, each rat in groups 3 to 6 received 1 ml of castor oil orally and was then placed in its own individual metabolic cage. The rats were monitored for the consistency of their fecal output and the frequency of defecation. The number of both wet and dry droppings for each rat was recorded every hour for 5 hours, with the paper in the cages being changed periodically for each evaluation. The count of wet and dry feces was used to assess the degree of wetness and the frequency of defecation.

2.3.1 Determination of gastro intestinal motility

The effect of the ethanol leaf extract of *Polyalthia longifolia* on gastrointestinal motility was assessed in rats using the method described by [17]. Thirty adult male albino rats, previously fed a standard Pfizer diet with free access to water, were used in the study. After fasting for 18 hours with continued access to water, the rats were divided into six groups of five rats each and treated with the following:

- **Group 1:** Received 0.2 ml of normal saline (control).
- **Group 2:** Received 1 ml of castor oil (positive control).
- **Group 3:** Administered 2 mg/kg of the standard drug, loperamide.
- **Group 4:** Treated with 200 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.

- **Group 5:** Treated with 400 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.
- **Group 6:** Treated with 600 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.

One hour after these treatments, each rat in groups 2 to 6 received 0.2 ml of charcoal meal (10% activated charcoal suspended in 5 g of gum acacia). After an additional hour, the rats were anesthetized with chloroform, sacrificed by abdominal incision, and their small intestines were carefully separated from the mesentery to avoid stretching. The length of the intestine from the pyloric sphincter (pylorus) to the ileo-caecal junction (caecum) was measured, as well as the distance traveled by the charcoal meal. Gastrointestinal transit for each rat was calculated as the percentage of the distance traveled by the charcoal meal relative to the total length of the intestine.

2.4 Electrolytes Test

The impact of the ethanol leaf extract of Polyalthia longifolia on the electrolyte concentration in intestinal fluid was evaluated in rats using the method described by [18]. Thirty adult male albino rats, previously fed a standard Pfizer diet with free access to water, were used. The rats were divided into six groups of five rats each and fasted for 18 hours. Each rat was then given 1 ml of castor oil orally using appropriate intubation tubes. After one hour, the rats in different groups were treated as follows:

- **Group 1:** Received 0.2 ml of normal saline (control).
- **Group 2:** Received 1 ml of castor oil (positive control).
- **Group 3:** Administered 2 mg/kg of the standard drug, loperamide.
- **Group 4:** Treated with 200 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.
- **Group 5:** Treated with 400 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.
- **Group 6:** Treated with 600 mg/kg body weight of ethanol leaf extract of *Polyalthia longifolia*.

One hour after these treatments, each rat in groups 3 to 6 received an additional 1 ml of castor oil orally. Two hours post-treatment, the rats were anesthetized with chloroform, sacrificed, and their small intestines were isolated and tied at the pyloric sphincter and ileo-caecal junction. The small intestines were then removed, and the contents were collected into a test tube. The intestinal effluents (serosal solution) were centrifuged at 3000 G for 30 minutes. The supernatants were analyzed for concentrations of Na+, K+, HCO3-, and Cl-.

2.4.1 Determination of sodium Ion (Na) concentration (Teco diagnostic kit)

The determination of sodium ion concentration was performed using the method described by [19], where sodium is precipitated as sodium magnesium uracyl acetate, and any excess uranium reacts with ferrocyanide to produce a colored chromophore. The absorbance of this chromophore inversely correlates with the sodium concentration in the test sample. Test tubes were labeled as standard (S), control (C), test (T), and blank (B). A 1.0 ml aliquot of the filtrate reagent was added to each test tube. Then, 50 µl of the extract was added to the test, standard, and control tubes, while distilled water was added to the blank tube. The tubes were shaken vigorously and mixed for 3 minutes. They were then centrifuged at 1500 G for 10 minutes. Following centrifugation, 1.0 ml of acid reagent was added to each tube during the color development stage. The supernatant (50 µl) was added to each respective tube and mixed thoroughly. Next, 50 µl of the color reagent was added to all the test tubes and mixed thoroughly. After blanking the spectrophotometer with distilled water, the absorbance of each tube was measured at 550 nm. The sodium ion concentration in milliequivalents per liter (mEq/L) was then calculated using the formula:

$$\text{Concentration of } Na^{+} = \frac{\text{Abs of blank-Abs of sample x concentration of standard}}{\text{Abs of blank-Abs of standard}}$$

2.4.2 Determination of potassium ion (K^{+}) Concentration (Teco diagnostic kit)

Potassium concentration was determined using the method described by [20]. In this method, potassium is quantified by forming a colloidal suspension with sodium tetraphenylboron in a specially prepared mixture. The turbidity of the suspension, which is proportional to the potassium concentration in the test sample, is

measured. Test tubes were labeled as standard, control, and test, with an additional blank provided. A 1.0 ml volume of potassium reagent was added to each test tube, and 0.01 ml (10 µl) of the sample was also added to the respective tubes and thoroughly mixed. The mixtures were left to stand at room temperature for 3 minutes. Afterward, the spectrophotometer was set to 500 nm and blanked. The absorbance of each solution in the test tubes was measured at this wavelength. The concentration of potassium ions in milliequivalents per liter (mEq/L) was then calculated using the formula:

$$\text{Concentration of } K^+ = \frac{\text{Abs of blank-Abs of sample x concentration of standard}}{\text{Abs of blank-Abs of standard}}$$

2.4.3 Determination of bicarbonate ion (HCO_3^-) concentration (Teco diagonistic Kit)

Carbon dioxide in serum or plasma exists primarily as dissolved CO_2 and bicarbonate anion (HCO_3). The CO_2 reagent measures CO_2 content enzymatically and the procedure is a modification of the method of [21].

Principle:

$$\text{Phosphoenol pyruvate} + HCO_3^- \xrightarrow{\text{PEPC}} \text{Oxalate} + H_2PO_4$$

$$\text{Oxalate} \xrightarrow{MDH} \text{Malate} + \text{NAD}$$

Phosphoenolpyruvate carboxylase (PEPC) catalyzes the reaction between phosphoenolpyruvate and carbon dioxide (bicarbonate) to produce oxaloacetate and a phosphate ion. Oxaloacetate is then converted to malate while simultaneously oxidizing an equal amount of reduced nicotinamide adenine dinucleotide (NADH) to NAD. This reaction is facilitated by malate dehydrogenase (MDH). The result is a decrease in absorbance at 340 nm, which is directly proportional to the CO2 concentration in the sample.

Procedure: CO2 reagent was prepared according to the specified method. Test tubes were labeled as blank, standard, control, and patients. A 1 ml aliquot of the CO2 reagent was added to each tube, which were then incubated for 3 minutes at 37°C. The spectrophotometer was adjusted to a wavelength of 340 nm and a temperature of 37°C. A 0.005 ml sample of water, standard, and patient samples were pipetted into cuvettes labeled blank, standard, and patients, respectively. The contents were mixed gently by inversion and incubated for 5 minutes. The absorbance of each cuvette was then measured and recorded at 340 nm.

Calculation:

$$Co_2 \text{ content of sample (mmol)} = \frac{\text{Abs of blank-Abs of sample x conc. of standard}}{\text{Abs of blank-Abs of standard}}$$

2.4.4 Determination of chloride ion

The concentration of chloride ions was measured using the method described by [22] and detailed in the Teco kit.

Principle: Chloride ions form a soluble, non-ionized compound. When these ions interact with mercuric ions, they displace thiocyanate ions from non-ionized mercuric thiocyanate. The displaced thiocyanate ions then react with ferric ions to create a colored complex that absorbs light at 480 nm. The intensity of the color produced is directly proportional to the concentration of chloride ions.

$$Hg(SCN)_2 + 2Cl^- \rightarrow HgCl_2 + 2SCN^- \rightarrow 3SCN^- + Fe^{3+} \rightarrow 4Fe(SCN)_3 \text{ red complex}$$

Procedure

Two test tubes were labeled blank and calibrator

One and half milliliter (1.5 ml) of chloride reagent (Mercuric Nitrate 0.058 mM, Mercuric Thiocyanate 1.75 mM, Mercuric Chloride 0.74 mM and Ferric Nitrate 22.3 mM in dilute acid and methanol) was pipetted to each tube. This was followed by the addition of 10 µl of calibrator/sample to respective tubes and then mixed. They were incubated at room temperature for at least five minutes and absorbance read at 480 nm. Chloride ion concentration (mEq/L) was calculated thus:

$$\text{Concentration (mEq/L)} = \frac{\text{Abs of Unknown x conc. of standard}}{\text{Abs of Calibrator}}$$

2.5 Determination of Urea

2.5.1 Urea: Determination of urea concentration as described by (Randox kits).

The concentration of urea was measured following the method outlined in reference [23], as detailed in the Randox kit.

Principle: Urea in the serum is hydrolyzed into ammonia by urease. The resulting ammonia is then quantified using spectrophotometry.

Urease:

$$\text{Urea} + H_2O \longrightarrow 2NH_3 + CO_2$$

$$NH_3 + \text{Hypochlorite} + \text{Phenol} \longrightarrow \text{Indophenol (blue compound)}$$

Reagents (R): R1: EDTA (116 mmol/L), Sodium Nitroprusside (6mmol/L),
Urease (1g/L)
R2: Phenol (120 mmol/L)
R3: Sodium hypochlorite (27 mmol/L), Sodium (0.14 N)

Procedure: Ten microliters (10 µl) of distilled water (as a blank), a standard calibrator (urea), and the sample were each added to three separate test tubes. Then, 100 µl of reagent was added to each tube. The mixtures were thoroughly mixed and incubated at 37°C for 10 minutes. The absorbance of the sample (A sample) and the standard (A standard) was then measured at 546 nm, with the blank serving as the reference.

Calculation:

$$\text{Urea Conc} = \frac{\text{Abs of sample x conc. of standard (mmol/L or mg/dl)}}{\text{Abs of standard}}$$

1 mg of urea correspond to 0.467 mg of urea nitrogen.

2.5.2 Determination of creatinine

The serum creatinine was determined using the method of [24] as outlined in Randox kit.

Principle: Creatinine reacts with picric acid in an alkaline solution to produce a colored complex. The intensity of the color formed is directly proportional to the concentration of creatinine.

Procedure: Two milliliters (2 ml) of the working reagent were combined with 1 ml of the creatinine standard and incubated for 30 seconds. The same procedure was followed for the blood sample. The initial absorbance (A1) of both the sample and the standard was measured at 492 nm. After exactly 2 minutes, the absorbance (A2) of the sample and standard was measured again. The serum creatinine was then calculated as follows:

$$\text{Serum Creatinine Conc.} = \frac{\Delta\text{Abs of Sample x standard conc. (mg/dl)}}{\Delta\text{Abs of standard}}$$

A1 = absorbance 1
A2 = absorbance 2
$\Delta A = A2 - A1$ = change in absorbance (ΔA sample or ΔA standard)
This could either be in mg/dl or µ mol/L

2.6 Statistical Analysis

The data were presented as mean ± SD and analyzed using Statistical Product and Service Solutions (SPSS) version 20. Statistical

significance was assessed through both one-way and two-way Analysis of Variance (ANOVA). P values < 0.05 were regarded as statistically significant.

3. RESULTS

3.1 Percentage Yield of Ethanol Leaf Extract of *Polyalthia longifolia*

One thousand gram (1000g) of dried crude sample of *Polyalthia longifolia* leaf gave a percentage yield of 3.36%

3.2 Preliminary Phytochemical Screening

The results presented in Table 1 indicate that alkaloids and tannins were found in high concentrations, flavonoids and steroids were present in moderate concentrations, while terpenoids, glycosides, and phenolic acid were detected in the lowest concentrations.

3.3 Median Lethal Dose (LD_{50}) of the Ethanol Extract leaf *Polyalthia longifolia*

The ethanol extract of *Polyaltha logifolia* leaf (EPLL) showed no signs of toxicity or caused any deaths during the 24-hour observation period following oral administration at doses up to 5000 mg/kg. The lack of mortality and absence of toxic effects indicate that EPLL has a wide safety margin, suggesting that the plant extract is non-toxic.

3.4 Effect of Ethanol Leaf Extracts of *Polyalthia longifolia* on Castor Oil-Induced Diarrhea in Rat Model

The results in Table 3 revealed a significant ($p < 0.05$) decrease in fecal droppings in groups treated with 200, 400, and 600 mg/kg body weight of the extract compared to the positive control. Additionally, groups treated with 200 and 400 mg/kg body weight of the extract showed a significant ($p < 0.05$) reduction in fecal droppings at 5 hours compared to the 2, 3, and 4-hour marks. In terms of protection, the groups treated with 200, 400, and 600 mg/kg body weight of the extract exhibited percentage inhibitions of 67.60, 76.63, and 84.73, respectively, compared to the standard drug, which had a percentage inhibition of 40.18.

Mean values having different upper case letters(ABC) as superscripts are considered significant ($p < 0.05$) down the group.

Table 1. Quantitative and Qualitative phytochemical screening of ethanol leaf extracts of Polyalthia longifolia

Phytoconstituents	Qualitative remarks	Concentration mg/100g
Alkaloids	+++	774.99±6.92
Flavanoids	++	646.25±10.44
Tannins	+++	879.62±0.58
Steroids	++	543.72±1.53
Terpenoids	+	36.38±2.02
Glycosides	+	36.48±0.48
Reducing sugar	+	94.83±0.52
Phenolic acid	+	33.45±0.55

Key: +++ = Present in high concentration; ++ = Present in moderate concentration; + = Present in lower concentration

Table 2. Median lethal dose of ethanol extract of *Polyalthia longifolia* leaf

Phase I	Dose (mg/kg b.w)	Mortality Rate
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
PHASE II		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

Table 3. Effect of ethanol leaf extracts of *Polyalthia longifolia* on Castor oil-induced diarrhea in rats

Treated groups	Wet feacal droppings at different time interval					Mean number of feacal droppings in 5hr	% Inhibition
	1hr	2hr	3hr	4hr	5hr		
Group 1 Normal control	7.80 ± 0.84^{Ba}	7.2±0.44^{Cd}	4.40 ± .89^{Cc}	3.00 ± 0.71^{Cc}	2.40±0.54^{Cc}	4.96±1.08	61.37
Group 2 Positive control	17.60 ± 1.34^{Ec}	15.00 ±1.58^{Ee}	12.60± 1.14^{Ee}	10.40 ± 0.51^{Ee}	8.60±1.51^{Dd}	12.84±2.62	_
Group 3 Standard control	12.80± .58^{Db}	10.60±1.14^{Dd}	8.40± 0.54^{Dd}	4.40 ± 0.54^{Dd}	2.20±0.83^{ACbc}	7.68±0.72	40.18
Group 4 200mg/kg	9.80 ± 0.83^{Cc}	5.20 ± 0.37^{Bd}	3.00± 0.71^{Bd}	1.60 ± 0.24^{Bb}	1.20±0.44^{ABab}	4.16±0.51	67.60
Group 5 400mg/kg	7.20±0.34^{Bb}	3.60±0.54^{Ac}	2.00±0.31^{ABab}	1.40±0.54^{ABab}	0.80±0.37^{Aa}	3.00±0.42	76.63
Group 6 600mg/kg	4.60±0.89^{Ad}	2.60±0.24^{Ac}	1.60±0.54^{Aa}	0.60±0.24^{Aa}	0.40±0.24^{Aa}	1.96±0.43	84.73

Results are expressed in means ± SD. (n = 5)

Mean values having different lower case letters(abc) as superscripts are considered significant ($p < 0.05$) across the period.

3.5 Effect of Ethanol Leaf Extract of *Polyalthia longifolia* on Gastrointestinal Motility in Rats Models

The motility results shown in Table 4 indicate that groups treated with 200, 400, and 600 mg/kg body weight of the extract experienced a significant ($p < 0.05$) reduction in gastrointestinal motility compared to the positive control. However, the groups treated with 200, 400, and 600 mg/kg of the extract exhibited percentage inhibitions of 25.48%, 19.44%, and 39.01%, respectively. In terms of protection, the standard drug, with a percentage inhibition of 51.84%, provided better protection than the extract, which had percentage inhibitions of 25.48%, 19.44%, and 39.01%, respectively.

Mean values with different letters as superscripts down the column (the groups) are considered significant at $p < 0.05$

3.6 Effect of Ethanol Leaf Extract of *Polyalthia longifolia* on Electrolytes Levels of Castor Oil Induced Diarrhea Rats

The results depicted in Table 5, indicated that groups treated with 200,400 and 600mg/kg body weight showed a significant ($P < 0.05$) increase in chlorine and sodium with corresponding decrease in bicarbonate and potassium level when compared with the positive control.

Mean values with different letters as superscripts down the group are considered significant at p
< 0.05

3.7 Effect of Ethanol Leaf Extract of *Polyalthia longifolia* on Kidney Function Markers of Castor Oil-Induced Diarrhea Rats

The results presented in Table 6 demonstrate that the ethanol leaf extract of Polyalthia longifolia significantly ($p < 0.05$) reduced creatinine and urea levels compared to the positive control. Furthermore, groups treated with 200, 400, and 600 mg/kg body weight showed a significant ($p < 0.05$) dose-dependent reduction in creatinine and urea levels when compared to the positive control.

Mean values with different letters as superscripts down the group are considered significant at $p < 0.05$.

4. DISCUSSION

There is currently no scientific evidence supporting the broad use of plant components for treating various illnesses, especially diarrheal diseases. However, several studies have examined the biological activity of plant extracts with antispasmodic properties, which are known to slow intestinal transit, decrease gut motility, and enhance water absorption, thus supporting the antidiarrheal effects of medicinal plants [25]. Research has shown that plants containing alkaloids, flavonoids, saponins, steroids, and tannins exhibit antidiarrheal activity due to their antisecretory and antispasmodic effects on the gastrointestinal tract [26-27]. Tannins work by precipitating proteins, which reduces secretion and peristaltic movements, while flavonoids can limit intestinal motility and hydroelectrolytic secretions [28]. Additionally, tannins can relax muscles by either stimulating calcium pumping mechanisms or decreasing intracellular Ca2+

Table 4. Effect of ethanol leaf extract of *Polyalthia longifolia* on Gastrointestinal Motility in Rats

Treatment Group	Gastrointestinal Transit	
	Peristaltic Index (%)	% Inhibition
Group 1 (Normal control)	72.66 ± 13.40ab	14.58%
Group 2 (Positive control)	85.06± 13.46^{c}	_
Group 3 (Standard control)	40.96 ± 10.15^{a}	51.84%
Group 4 (200mg/kg b.w)	63.38 ± 5.93^{abc}	25.48%
Group 5 (400mg/kg b.w)	68.52 ± 8.23^{bc}	19.44%
Group 6 (600mg/kg b.w)	51.87 ± 8.59^{ab}	39.01%

Results are expressed as Means ± SD (n = 5)

Table 5. Effect of ethanol leaf extract of *Polyalthia longifolia* on electrolytes levels of castor oil-induced diarrhea in rats

Groups	Electrolyte Concentration (mmol/l)			
	Sodium	Potassium	Bicarbonate	Chloride
Group 1 (Normal control)	135.13±0.46^{c}	2.58±0.58^{ab}	24.84±2.11^{b}	97.22±0.58^{ab}
Group 2 (Positive control)	129.13±0.37^{a}	3.34±0.36^{c}	28.34±2.75^{c}	80.82±0.96^{a}
Group3 (Standard control)	138.1±0.46^{d}	3.57±0.39^{c}	26.42±1.34^{bc}	92.26±0.6^{b}
Group 4 (200mg/kg b.w)	132.38±0.14^{b}	3.04±0.6^{bc}	24.71±0.67^{b}	93.98±0.54^{c}
Group 5 (400mg/kg b.w)	141.04±0.44^{e}	4.50±0.45^{d}	27.26±0.79^{d}	100.32±0.12^{e}
Group 6 (600mg/kg b.w)	143.20±0.44^{f}	2.38±0.28^{a}	22 ± 0.87^{a}	104.69±0.48^{f}

Results are expressed as means ± SD (n = 5)

influx [29]. Other literature reports suggest that flavonoids inhibit intestinal secretion triggered by prostaglandin E2, saponins inhibit histamine release, terpenoids block prostaglandin release, phenols reduce intestinal secretion and transit, and tannins provide antispasmodic and muscle relaxant effects. Collectively, these activities contribute to the suppression of diarrhea by reducing intestinal secretion and motility [30]. Most of these secondary metabolites were found in the ethanol extract of P. longifolia leaves. Although the exact mechanism of action of the plant is not fully understood, these chemical components may contribute to its antidiarrheal activity, including its antimotility, antisecretory effects, and ability to delay the onset of diarrhea. The plant extract was deemed safe in the acute toxicity test, as no toxicity was observed at the maximum dose of 5000 mg/kg in rats. Diarrhea can be caused by various factors, but the main mechanisms include: (a) osmotic diarrhea, which results from increased intraluminal osmolarity and decreased water absorption; (b) secretory diarrhea, characterized by elevated electrolyte secretion; (c) altered intestinal motility, leading to reduced transit time [31]; and (d) inflammatory and infectious diarrhea, caused by disruptions to the intestinal epithelium due to bacterial, viral, or protozoal pathogens and the resulting immune response [32]. Antimotility and antisecretory drugs are considered fundamental in managing diarrhea because they address the underlying pathophysiological conditions [33]. Additionally, castor oil, which releases ricinoleic acid—a metabolite known to induce diarrhea—is commonly used in antidiarrheal research [34]. Inflammation of the gastrointestinal mucosa leads to the release of prostaglandins, which increase gastrointestinal motility and electrolyte secretion, similar to the mechanisms causing diarrhea. Ricinoleic acid induces diarrhea through this pathway [35]. Prostaglandins of the E series are known to induce diarrhea in both humans and animals, and thus, prostaglandin biosynthesis inhibitors are thought to delay diarrhea induced by castor oil [31]. On the other hand, loperamide, a typical antidiarrheal drug used as a positive control, is a synthetic opioid agonist that activates μ-opioid receptors in the

Table 6. Effect of ethanol leaf extract of *Polyalthia longifolia* on kidney function markers of castor oil-induced diarrhea rats

Treated group	Creatine (mg/dl)	Urea (mg/dl)
Group 1 (Normal control)	1.35 ± 0.12^{c}	3.13 ± 0.48^{b}
Group 2 (Positive control)	2.02 ± 0.52^{d}	4.96 ± 0.47^{d}
Group 3 (Standard control)	0.92 ± 0.06^{b}	2.38±0.73^{a}
Group 4 (200mg/kg b.w)	1.51 ± 0.07	4.34 ± 0.19^{c}
Group 5 (400mg/kg b.w)	0.78 ± 0.07^{ab}	2.27 ± 0.25^{ab}
Group 6 (600mg/kg b.w)	0.53 ± 0.09^{a}	2.18 ± 0.17^{cd}

Results are expressed as means ± SD (n = 5)

myenteric plexus of the large intestine [32]. This leads to increased intestinal transit time, enhanced colonic segmentation, and reduced peristalsis, while inhibiting acetylcholine-mediated secretion. As a result, loperamide decreases daily fecal volume, reduces electrolyte and fluid loss, and can increase stool bulk density and viscosity [36].

5. CONCLUSION

The ethanol extract of *P. longifolia* leaves exhibited antidiarrheal activity in Swiss albino rats. The extract significantly delayed the onset of diarrhea, reduced the frequency of wet feces, and showed notable antisecretory effects at all tested doses. Additionally, the plant extracts displayed antimotility effects at higher concentrations. Although further research with different antidiarrheal models and solvents is needed, the current study's results are promising.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author(s) attest that zero generative AI tools, including text-to-image generators and huge model languages such as (chatgpt, copilot, etc.), were used during the writing and modification of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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