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Prophylactic Anti-diarrhoea Property of *Chrysobalanus icaco* Fruit Methanol Extract in Swiss Mice

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ABSTRACT: Diarrhoea is one of the major health issues in the developing world due to poor water and sanitation. The methanol extract of the fruit of *Chrysobalanus icaco* (Coco plum) was evaluated for the prophylactic anti-diarrhoeal activity against castor oil-induced diarrhoea in Swiss mice. Diarrhoea was induced in the mice experimentally by castor oil administration, and the anti-diarrhoeal activity of various doses of the fruit extract (25, 50, and 100 mg/kg) was assessed against a control group and a reference group treated with loperamide (3 mg/kg). The results showed that the extract decreased the severity of diarrhoea in a dose-dependent manner, with the highest dose (100 mg/kg) showing 58.6% inhibition of diarrhoeal stools compared to the control group. The extract also delayed the onset of diarrhoea and reduced total stool weight. A charcoal meal gastrointestinal motility test demonstrated that the extract significantly delayed intestinal transit, substantiating its ability to modulate gut motility. The anti-diarrhoeal activity observed could be due to the presence of phytochemicals such as flavonoids, tannins, and anthocyanins. These findings provide a scientific rationale for the folkloric use of *Chrysobalanus icaco* in the treatment of diarrhoeal conditions and indicate its prospects as a plant-derived, natural alternative to synthetic anti-diarrhoeal medications.

Keywords: Anti-diarrhoeal, *Chrysobalanus icaco*, Methanol, Swiss mice

Introduction

Diarrhoea is a common symptom of gastrointestinal infections caused by various bacterial, viral, or parasitic organisms (Peek *et al*., 2018; Njunguna, 2017). Diarrhoea diseases are a major cause of morbidity and mortality, particularly in developing countries where access to clean water and sanitation tends to be limited. Diarrhoea leads to significant dehydration and electrolyte imbalances, especially in young children and vulnerable populations (Amadus *et al*., 2023). Prevention strategies, such as improved hygiene practices, safe drinking water, and vaccination, have proven effective in reducing the incidence of diarrhoeal diseases (Troeger *et al*., 2018). As a leading cause of morbidity and mortality, the World Health Organisation (WHO) estimated that approximately 1.6 million people died annually due to diarrhoeal diseases, with children under five being disproportionately affected (WHO, 2023). While various pharmaceutical treatments were available, there was growing interest in traditional medicine as an alternative and complementary approach to managing diarrhoea. Traditional medicine, according to WHO, encompasses a diverse body of knowledge and practices derived from cultural traditions, often including the use of plants, animals, and minerals to maintain health and treat illness

(WHO, 2019). One plant of interest in this regard is *Chrysobalanus icaco* (coco plum), a fruit-bearing shrub widely used in ethnomedicine for its purported anti-diarrhoeal properties (Onilude *et al*., 2021).

Approximately 65-80% of the world's population relied on traditional medicine as their primary source of healthcare, especially in rural areas of Africa, Asia, and Latin America (Kim *et al*., 2010). This reliance stemmed from several factors, including accessibility, affordability, and the deep-rooted cultural significance of traditional remedies. In Africa, for instance, traditional medicines were often used alongside modern pharmaceuticals to complement healthcare (Asakitikpi, 2022). This implies that the diversity of traditional practices in Africa was reflected in the wide variety of plants utilised for medicinal purposes, with *Chrysobalanus icaco* being one such example (Arce-ortiz *et al*., 2024).

The *Chrysobalanus icaco* plant, also known as the coco plum, is a small tree commonly noticed in most tropical regions such as Central and South America, the Caribbean, and parts of Africa. The plant had been utilised for both culinary and medicinal purposes, with its fruits, leaves, and stems traditionally used to treat ailments such as diabetes, inflammation, and gastrointestinal disorders (Oganezi *et al*., 2024). It thrives in tropical regions, coastal vegetation, and mangroves, especially in sandy soils with high salinity. The plant typically grows to 1.5-7 meters tall, with bright green, leathery, orbicular leaves. Its fruit is a drupe, 2 to 5 cm in length, with soft, edible white pulp surrounding one or two seeds. The fruits ripen from green to shades of pink, purple, and white, making them available from spring to summer (Smith, 2010).

The fruit was particularly noted for its use in treating diarrhoea, a condition that can cause severe dehydration and electrolyte imbalance if not managed effectively. Diarrhoea is typically characterized by the frequent passage of loose or watery stools and is often caused by infections, toxins, or malabsorption syndromes (Sheikh *et al*., 2018; Siciliano *et al*., 2020). In conventional medicine, anti-diarrhoeal drugs such as loperamide were used to reduce gastrointestinal motility and increase intestinal fluid and electrolyte absorption (Baird *et al*., 1997). However, concerns about drug side effects and the emergence of drug-resistant pathogens led to a resurgence of interest in plant-based treatments (Ugboko *et al*., 2020). Natural remedies, especially those derived from plants like *Chrysobalanus icaco*, were viewed as safer alternatives with fewer side effects, offering a potential source of novel therapeutic compounds. Moreover, studies have shown that many plant extracts possess anti-diarrhoeal properties, often attributed to their ability to modulate intestinal motility, reduce fluid secretion, and restore electrolyte balance (Na'allah *et al*., 2024).

The pharmacological activity of *Chrysobalanus icaco* was linked to its rich phytochemical profile. The plant was known to contain a variety of bioactive compounds, including flavonoids, tannins, and anthocyanins, which had been demonstrated to possess anti-inflammatory, antioxidant, and antimicrobial properties (Araujo-Filho *et al*., 2016). Flavonoids, in particular, were believed to contribute to the anti-diarrhoeal activity of many plants by reducing intestinal motility and fluid secretion, thereby normalising stool consistency and frequency (Tan *et al*., 2017). Additionally, tannins were thought to exert astringent effects on the intestinal lining, helping to reduce excessive fluid loss and improve stool formation (Molino *et al*., 2022). Anthocyanins, another class of compounds found in *Chrysobalanus icaco*, have been reported to enhance gut health by modulating the gut microbiota and inhibiting the growth of pathogenic bacteria (Verediano *et al*., 2021).

Despite its traditional use in treating diarrhoea, there remained a paucity of scientific data supporting its efficacy. Thus, this assessment sought to systematically evaluate the anti-diarrhoeal effects of *the methanol extract of Chrysobalanus icaco* fruit in an experimental model using Swiss mice. The castor oil-induced diarrhoeal method was used to induce diarrhoea in experimental animals. Castor oil was known to stimulate gastrointestinal motility and secretion, mimicking the symptoms of diarrhoea in humans (Mariott *et al*., 2022). By comparing defecation frequency, stool consistency, and faecal weight between the control and treatment groups, the study aimed to highlight the potential therapeutic benefits of *Chrysobalanus icaco* extract. Furthermore, the study examined the prophylactic effects of the extract on gastrointestinal motility and intestinal transit time using the charcoal meal test, a standard method for assessing intestinal motility (Atta *et al*., 2019).

Materials and methods

Collection and verification of plant material: Ripe fruits of *Chrysobalanus icaco* were obtained from Uselu Market, located within Egor Local Government Area of Benin City, Edo State, Nigeria. The specimen was initially identified by Professor Odaro Timothy of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin. Further confirmation and authentication were carried out by Professor H.A. Akinnibosun at the Herbarium Unit of the same department, where a voucher specimen was catalogued under the reference code UBH-C201.

Plant preparation: Fresh *Chrysobalanus icaco* fruits were rinsed with distilled water, and the plant material was first dried by placing it in a shaded, hygienic area at room temperature. It was then subjected to controlled oven drying at 40 °C for 24 hours. Pulverisation was carried out using a British mechanical grinder (Model PG 501), after which 1,800 g of the powdered fruits was extracted with 4,000 ml of absolute methanol (capable of isolating polar phytoconstituents implicated in antidiarrhoea) using the maceration technique. The filtrate obtained was concentrated to a semi-solid consistency using an HH-S water bath (Search Tech Instruments) maintained at 45 °C. The procedure yielded an extract with a 16.67% yield.

Preliminary Phytochemical Screening: The n-hexane crude extract from the leaves and roots of *Jatropha gossypiifolia* was screened using a standard procedure. The extracts were phytochemically screened for alkaloids, flavonoids, tannins, terpenoids, saponins, phenolic compounds, and glycosides.

Anti-diarrhoeal activity assessment

Experimental animals and housing conditions: A total of twenty-five adult Swiss mice weighing between 28 and 32 g were employed in this experiment. The animals were housed in groups of five in wooden cages and kept under standardised environmental conditions, including a temperature of 25 ± 1 °C, a relative humidity of 45–55%, and a 12-hour alternating light/dark cycle. Before experimentation, they were allowed a one-week acclimatisation period with free access to a standard diet and water. All experimental protocols were approved by the Institutional Animal Ethical Committee and conducted in accordance with CPCSEA guidelines. Ethical number issue by Life Sciences animal house.

Evaluation Using Castor Oil-Induced Diarrhoeal Model: The mice were fasted for 18 hours before the test, during which they were allowed unrestricted water intake. Randomisation placed the animals into five groups of five (per the advice of the animal ethics committee, Life Science, University of Benin, and CPCSEA guidelines, which recommend a maximum sample size of 5). Three groups were treated orally with the fruit extract at doses of 25, 50, and 100 mg/kg (the animals weight were obtained to calculate the dose in volume using an established stock solution, the weighed extracted was properly dissolved in distilled water), while the negative control group received distilled water (0.2 ml/kg) and the positive control group was given loperamide (3 mg/kg). After a one-hour interval, 0.5 ml/kg body weight of castor oil was administered to each animal by oral gavage. The mice were then transferred individually into clear cages lined with absorbent filter paper. The onset time, frequency, and consistency of stools were observed for 4 hours. Diarrhoeal output in the control group was set at 100%, and the percentage inhibition for each treated group was calculated according to the method of Tan *et al.* (2017).

Data analysis was performed using GraphPad Prism (version 6). Results are presented as mean values accompanied by the standard error of the mean (SEM). Group comparisons were made using one-way ANOVA, and Dunnett's multiple comparison test was applied for post hoc evaluation. A probability value of less than 0.05 was regarded as statistically significant.

Results

Table 1 showed the qualitative phytochemical screening results of the methanol fruit crude extract of *Chrysobalanus icaco*, indicating the presence of alkaloids, flavonoids, tannins, phlobatannins, saponins, and terpenoids.

Table 1: Qualitative phytochemical screening results of the methanol fruit crude extract of *Chrysobalanus Icaco*

Phytochemicals	Fruit Extract
Alkaloids	+
Flavonoids	++
Tannins	++
Phlobatannins	++
Saponnins	+
Terpenoids	++
Cardenolides	-

+ present, – not detected

The methanol extract of *C. icaco* fruits demonstrated significant anti-diarrhoea activity in mice, as evidenced by a dose-related suppression of diarrhoea episodes in the castor oil model. Compared with the group that did not receive treatment, all extract-treated groups showed marked inhibition of diarrhoea, with the differences reaching statistical significance ($p < 0.05$).

Anti-diarrhoea effect of Chrysobalanus icaco fruit methanolic extract in castor oil-induced diarrhoea in mice: Findings from the castor oil-induced diarrhoea model are presented in Table 2, illustrating the inhibitory activity of the fruit extract against diarrhoeal episodes in mice.

Table 2. Anti-diarrhoea effect of *Chrysobalanus icaco* fruit methanol extract in castor oil-induced diarrhoea in mice

Treatment	Dose mg/kg	Onset of stool (sec)	Total number of stools	Number of diarrhoea	Weight of stool (g)
Control	DW	18.67±0.95	7.67±0.23[a]	7.33±0.76[a]	0.80±0.11
Loperamide	3	72.67±5.71	3.00±0.15[c]	3.00±0.55[c]	0.57±0.03
CIFME	25	52.67±5.84	5.67±0.33[b]	4.33±0.67[b]	0.57±0.14
CIFME	50	57.00±3.00	4.33±0.88[b]	2.00±0.15[b]	0.37±0.02
CIFME	100	35.00±1.53	6.67±0.22[b]	5.00±1.00[b]	0.80±0.06

Result values are reported as mean ± SEM, with a threshold for statistical significance defined as $p < 0.05$. DW (distilled water); CIFME (*Chrysobalanus icaco* fruit methanol extract)

Percentage inhibition of Chrysobalanus icaco fruit methanolic extract in castor oil-induced diarrhoea in mice: Figure 1 illustrates the percentage inhibition of diarrhoea following treatment with the methanol extract of *C. icaco* fruit in the castor oil-induced model. The extract at 50 and 100 mg/kg produced a significant ($p < 0.05$) reduction in diarrhoeal activity compared with the control group. Thereby, the % inhibition of diarrhoea was higher at 50 and 100 mg/kg than in the control groups. Loperamide is the positive control used in the castor oil-induced diarrhoea model to inhibit prostaglandin release and control nonspecific diarrhoea, and to compare its % inhibitory effect with that of the tested extract.

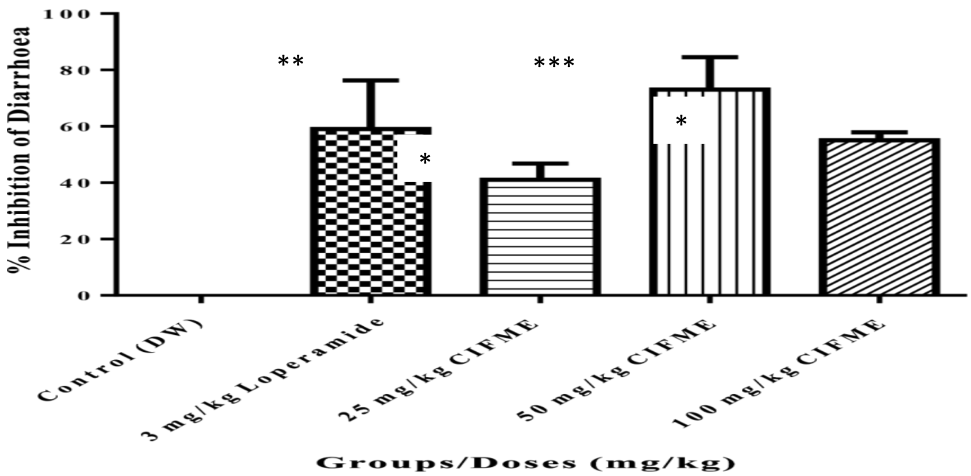

Figure 1. Percentage inhibition of diarrhoea in mice treated with the methanol extract of *Chrysobalanus icaco* fruit in the castor oil-induced model. Data are expressed as mean ± standard error of the mean (SEM), with statistical significance set at $p < 0.05$. DW = distilled water; CIFME = *Chrysobalanus icaco* fruit methanolic extract. Percentage inhibition of diarrhoea was calculated using the formula = Control - Treatment/Control x 100

Anti-diarrhoea effect of Chrysobalanus icaco fruit methanolic extract in the charcoal meal-induced diarrhoea model in mice: Table 3 summarises the effect of the methanolic extract on gastrointestinal motility parameters in mice subjected to the charcoal meal-induced diarrhoea assay. A statistically significant inhibitory effect was observed, with the 25 mg/kg dose showing the highest percentage inhibition. These results highlight the extract's ability to reduce intestinal transit and to mitigate the onset of diarrhoea.

Table 3: Anti-diarrhoea effect of *Chrysobalanus icaco* fruit methanol extract in charcoal meal-induced diarrhoea in mice.

Treatment	Dose mg/kg	Total length of Intestine (cm)	Length travel by charcoal meal (cm)	Weight of intestine (g)	Peristalsis index
Control	DW	33.33±6.68	33.00±4.04[a]	1.50±0.29	99.00±6.07[a]
Atropine	5	44.83±5.18	13.33±1.83[c]	1.27±0.63	29.74±1.46[c]
CIFME	25	45.00±2.75	8.67±0.78[c]	1.47±0.75	19.27±4.28[c]
CIFME	50	39.00±2.52	17.17±1.59[c]	1.03±0.52	44.03±2.22[b]
CIFME	100	44.00±1.53	19.50±3.18[c]	1.27±0.64	44.32±1.62[b]

The Percentage (%) Peristalsis Index (PI) was calculated as:

$$PI = \frac{LM}{LSI}$$

where **LM** = length of charcoal meal travelled and **LSI** = total length of the small intestine. Data are expressed as mean ± standard error of the mean (SEM), with statistical significance defined at **p < 0.05**. DW = distilled water; CIFME = *Chrysobalanus icaco* fruit methanolic extract.

The effectiveness of Chrysobalanus icaco fruit methanol extract as an anti-diarrhoea agent was evaluated on charcoal meal-induced diarrhoea in mice: Figure 2 illustrates the influence of the methanol fruit extract on gastrointestinal motility in mice, assessed using the charcoal meal method. Across all doses tested (25, 50, and 100 mg/kg), the extract produced a significant reduction in the distance travelled by the charcoal marker compared to controls, indicating suppression of intestinal propulsion. The effect was dose-dependent, with the highest inhibition observed at 100 mg/kg. Interestingly, the lowest dose (25 mg/kg) also markedly delayed the onset of diarrhoea stools relative to the untreated. Atropine is the positive control used in the charcoal meal-induced diarrhoea model to inhibit acetylcholine release, thereby reducing gastrointestinal tract motility, acting as an acetylcholine antagonist, and allowing comparison of its % inhibitory effect with the tested extract.

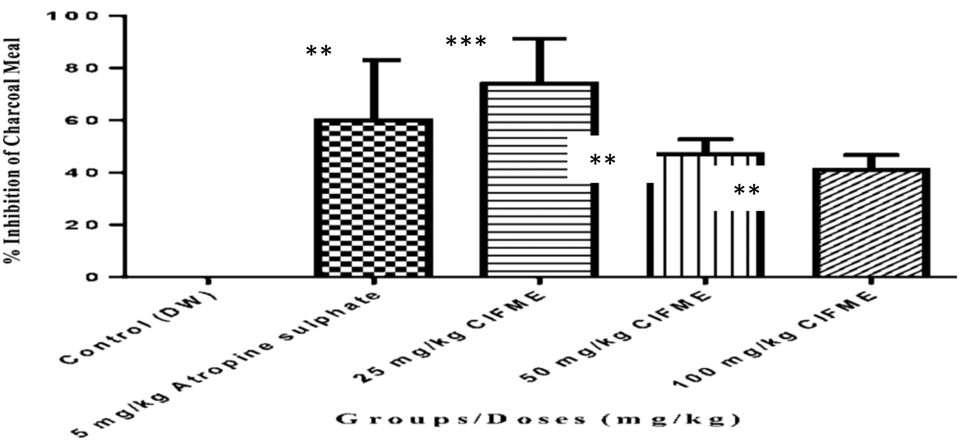

Figure 2: Percentage inhibition of *Chrysobalanus icaco* fruit methanol extract in charcoal meal induced-diarrhoea in mice. Values were expressed as Mean ± SEM, and the level of significance was determined as *p-value < 0.05.* DW (distilled water); CIFME (*Chrysobalanus icaco* fruit methanol extract).

Discussion

The amount of phytochemicals found in *Chrysobalanus icaco* was determined using the qualitative standard techniques. The plant extracts showed diverse phytoconstituents present, such as tannins, terpenes, flavonoids, and saponins at (1.470 and 0.877 mg/kg). The root extract showed the highest in carbohydrates and saponins at (2.680 and 1.661 mg/kg), followed by tannins and terpenes at (1.187 and 0.780 mg/g) (Table 1).

Diarrhoea is a common gastrointestinal disorder that can lead to dehydration and electrolyte imbalances if left untreated. Natural products have long been of interest in the quest for potent and safe remedies against diarrhoea. *Chrysobalanus icaco*, a plant known for its medicinal properties, was selected for the present research, prompted by its historical use in folk medicine to treat diarrhoea. Previous investigations of potential mechanisms of action of plant extracts with anti-diarrhoea activity revealed that they exert anti-diarrhoea effects through multiple pathways (Plaatjie *et al*., 2024). It has been noted that the castor oil-induced diarrhoea model is commonly used to evaluate the anti-diarrhoeal effects of medications (Mariott *et al*., 2022). Ricinoleic acid has been reported as the most active substance in castor oil (Nitbani *et al*., 2022). They further opined that the intestinal mucosa becomes inflamed and irritated when exposed to ricinoleic acid. The irritation alters the intestinal mucosa's electrolyte permeability by enhancing peristaltic activity in the small intestine. As a result of this cascade of events, the released prostaglandins promote motility, secretion, and reduce the absorption of sodium and potassium ions (Ogunro and Ofeniforo, 2021). In light of this, the current investigation found that the methanol fruit extract of *Chrysobalanus icaco*, administered at 25, 50, and 100 mg/kg, inhibited the severity of castor oil- and charcoal meal-induced diarrhoea (Table 2). In this study, CIFME exhibited dose-dependent anti-diarrhoeal activity, with significant effects observed at p < 0.05, particularly at the lower and moderate doses (25 and 50 mg/kg). These doses markedly delayed the onset of diarrhoea episodes, decreased overall stool output, lowered the frequency of diarrhoeal stools, and decreased stool weight compared with the control group. At the highest dose (100 mg/kg), however, the effect was less pronounced, as reflected by a shorter onset time, higher stool count, and stool weight comparable to that of the control group. This suggests that while CIFME is effective at moderate doses, its anti-diarrhoeal potential diminishes at higher concentrations. This could be associated with the presence of high amounts of flavonoids (quercetin, myricetin, and rutin) responsible for the extract to elicit an inhibitory effect in the gastrointestinal tract, thereby reducing peristaltic movement; tannins and triterpenes (oleanolic acids and pomolic acid) could be associated with the reabsorption rate of the extract, hence reducing the increase in GLT motility to inhibit diarrhoea. Further, the results were buttressed by the dose-dependent percentage inhibitory effect recorded at 50 and 100 mg/kg of the extracts, which had a significant inhibitory effect against diarrhoea, as shown in Figure 1. Comparable trends have been reported for the leaves of *Clerodendrum viscosum*, where 80% methanol extracts and their fractions were evaluated in mice using various diarrhoea models, including the castor oil-induced model. At a moderate dose of 200 mg/kg, the extract significantly delayed diarrhoea onset and reduced stool weight and frequency. However, similar to the present findings, the highest tested dose (400 mg/kg) was less effective than the moderate dose, reinforcing the conclusion that optimal anti-diarrhoeal efficacy is achieved at lower to moderate doses rather than at maximum concentrations. In contrast, a study on *Citrus aurantiifolia* (lime) revealed that higher doses exhibited substantial anti-diarrhoea properties, with no reduction in effectiveness even at higher concentrations (Isirima and Uahomo, 2023). In contrast to this investigation's findings, the research showed that lime extract decreased stool weight and frequency at various dosages, suggesting a distinct pharmacological profile. The results implied that some herbal extracts maintain their efficacy even at higher doses, contradicting the study’s observation of reduced effectiveness at maximum dosages.

Furthermore, the methanol fruit extract of *Chrysobalanus icaco* (CIFME) produced a dose-dependent reduction in diarrhoeal activity, with the 25 mg/kg dose exhibiting the greatest inhibition of intestinal motility and peristalsis. This was evidenced by the shorter distance travelled by the charcoal meal and a lower peristalsis index compared with other doses. Interestingly, higher doses (50 and 100 mg/kg) were less effective than 25 mg/kg. This suggests that the extract’s optimal activity occurs at lower concentrations. At $p < 0.05$, the 25 mg/kg dose produced the most pronounced anti-diarrhoeal effect, likely mediated through suppression of intestinal motility and reduced peristaltic movement. The possible effect could be tied to the presence of flavonoids (quercetin, myricetin, and rutin), tannins, and triterpenes (oleanolic acids and pomolic acid), which are responsible for the therapeutic effect in regulating peristaltic movement and increasing the reabsorption rate of the extract, thereby reducing diarrhoea. Also, the results shown in Figure 2 for the percentage inhibitory effect were dose-dependent, with a significant inhibitory effect against diarrhoea. A comparable pattern was reported for *Clerodendrum myricoides* in a castor oil-induced diarrhoeal model (Desta *et al.*, 2021). In that study, lower to moderate doses (100 and 200 mg/kg) significantly slowed the gastrointestinal transit of a charcoal meal, consistent with the present findings. The extract showed a clear dose-linked trend, with the 200 mg/kg dose yielding the strongest inhibition of intestinal motility. However, as with CIFME, the highest dose tested (400 mg/kg) showed reduced efficacy, reinforcing the notion that moderate doses achieve the most favourable anti-diarrhoeal outcomes.

Due to the presence of castor oil and the inhibition of prostaglandin release, it may be inferred that the extract has a preventive effect on lowering intestinal electrolyte permeability. Also, the extract's suppression of intestinal fluid accumulation suggests the cause of the inhibition of gastrointestinal function. However, previous studies showed that anti-dysenteric and anti-diarrhoeal properties of medicinal plants could be due to the presence of tannins, alkaloids, saponins, flavonoids, sterols, and triterpenes (Sahoo *et al*., 2014). The anti-diarrhoeal properties of the *C. icaco* fruit methanol extract could be attributed to flavonoids, which have been shown to possess antioxidant properties (Sheikh *et al*., 2018).

Conclusion

Taken together, the outcomes of this study show that the *Chrysobalanus icaco* fruit methanol extract possesses significant anti-diarrhoeal activity in the animal model, specifically at 100 mg/kg. The extract's ability to reduce stool frequency, improve stool consistency, and modulate gastrointestinal motility suggested its potential as a natural anti-diarrhoeal agent. However, additional investigations are needed to clarify the molecular pathways involved and to assess the extract’s long-term safety and therapeutic efficacy in humans. The present findings add to the expanding body of evidence supporting the medicinal potential of *Chrysobalanus icaco* and highlight its promise as a natural source for the development of innovative and effective anti-diarrhoeal agents.

Competing interests

The authors declare no conflict of interest

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