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Acute Oral Toxicity Study, Phytochemical Profile and the Anti-diarrheal Effect of Chloroform Extract of *Stuckenia pectinata* on Castor Oil-induced Diarrhea in Rats

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ABSTRACT

This study investigated the safety, phytochemical composition, and anti-diarrheal efficacy of the chloroform extract of *Stuckenia pectinata* using a castor oil-induced diarrhea model in rats. Qualitative screening revealed the presence of saponins, cardiac glycosides, alkaloids, terpenoids, and steroids, whereas flavonoids, tannins, phenolic compounds, and phlobatannins were not detected. Quantitative analysis showed tannins as the most abundant constituent (1.06 ± 0.04 mg/g TAE), followed by alkaloids (1.00 ± 0.07 mg/g ATE), with phenols (0.73 ± 0.05 mg/g GAE) and flavonoids (0.68 ± 0.03 mg/g CAE) present at lower levels, reflecting a chemically diverse extract. Acute oral toxicity assessment determined a median lethal dose (LD_{50}) of 1264.91 mg/kg, indicating moderate safety, and an effective dose was estimated at 126.49 mg/kg. Administration of the extract significantly reduced stool frequency and fecal output ($p < 0.05$) compared to the untreated control, with inhibition comparable to loperamide at 3 mg/kg. The anti-diarrheal activity is likely mediated by the combined effects of alkaloids, saponins, terpenoids, and steroids, which modulate intestinal motility, secretion, and electrolyte transport. Overall, the findings demonstrate that the chloroform extract of *Stuckenia pectinata* possesses substantial pharmacological potential as a plant-derived anti-diarrheal agent while exhibiting a favorable safety profile, providing a foundation for its further development in traditional and complementary medicine.

Keywords: Chloroform, Anti-diarrhea, Acute-toxicity, *Stuckenia pectinata*, Castor oil

INTRODUCTION

Diarrheal disorders continue to pose a significant threat to public health in many low- and middle-income regions, where they remain a major contributor to disease burden and preventable deaths. Vulnerable populations, particularly children below five years of age and individuals with impaired immune defenses, are disproportionately affected (Adebayo et al., 2024). From a clinical perspective, diarrhea is characterized by the frequent elimination of loose or watery stools, occurring three or more times within a single day. The condition results from a complex interplay of factors, including infections caused by enteric bacteria, viruses, and parasites, exposure to unsafe drinking water, poor sanitation, nutritional deficiencies, and pre-existing gastrointestinal abnormalities (Okafor and Ibrahim, 2023). While uncomplicated cases often resolve without intervention, severe manifestations can escalate rapidly to dehydration and electrolyte derangements, with potentially fatal outcomes in areas lacking adequate medical infrastructure (Oladipo et al., 2024). Conventional treatment of diarrhea primarily involves oral rehydration therapy, antimicrobial drugs, and agents that suppress intestinal motility. Although these interventions have substantially reduced mortality, their long-term effectiveness is increasingly compromised. Escalating antimicrobial resistance, inconsistent drug availability, economic barriers to care, and adverse reactions linked to synthetic medications limit their widespread and sustained use (Eze et al., 2023). These challenges have stimulated renewed scientific interest in exploring non-synthetic therapeutic alternatives, particularly plant-based remedies, either as adjuncts or substitutes in diarrhea management (Musa and Danjuma, 2024). Plants used in traditional medicine have long served as important resources for the treatment of gastrointestinal conditions, largely due to their accessibility, affordability, and integration into indigenous healthcare practices. The biological activities of these plants are commonly associated with secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, and saponins, many of which demonstrate antimicrobial, anti-inflammatory, antispasmodic, and antioxidant effects (Akinmoladun et al., 2024; Bello and Hassan, 2023). Despite extensive ethnobotanical documentation, empirical validation through controlled phytochemical and in vivo pharmacological studies remains necessary to establish the safety and therapeutic reliability of these remedies (Yusuf et al., 2024). *Stuckenia pectinata* (formerly *Potamogeton pectinatus*) is a submerged aquatic plant known to synthesize secondary metabolites with reported antimicrobial and anti-inflammatory potential (Gao et al., 2023). Although its ecological functions within freshwater ecosystems are well described, investigations into its medicinal relevance remain limited, particularly in the context of diarrheal disease. Detailed examination of its chemical constituents and biological effects could therefore provide foundational evidence supporting its potential use in phytotherapeutic applications (Zhang et al., 2024). Safety assessment represents an essential component of herbal drug research, as it defines acceptable exposure levels and minimizes the risk of adverse

outcomes prior to human use. Acute oral toxicity testing in experimental animals, performed in line with the guidelines of the Organisation for Economic Co-operation and Development (OECD), is widely employed to establish preliminary safety margins for plant extracts (Fasogbon et al., 2024). Such evaluations are especially critical for less-studied species like *Stuckenia pectinata*, for which toxicological data in mammalian systems are sparse. Given the ongoing global impact of diarrheal diseases and the constraints associated with existing pharmacological therapies, the pursuit of effective, low-cost, and readily available alternatives remains imperative. Consequently, this study was designed to investigate the acute oral toxicity, phytochemical characteristics, and anti-diarrheal efficacy of the chloroform extract of *Stuckenia pectinata* using a castor oil-induced diarrhea model in rats. The outcomes of this work are anticipated to expand current knowledge on medicinal aquatic plants, generate baseline safety data, and support the future development of plant-derived anti-diarrheal agents.

MATERIALS AND METHODS

Materials

Chemicals and Reagents

All reagents employed in this investigation were of analytical purity and used as supplied. Chloroform and other chemicals were obtained from Merck Chemicals and SD Fine Chemical Ltd. (Mumbai, India).

Collection, Identification, and Processing of Plant Material

Fresh leaves of *Stuckenia pectinata* were harvested in April 2025 from River Ethiopie, McCarthy Beach, Abraka, Delta State, Nigeria. Botanical identification and authentication were carried out in the Department of Plant Biology and Biotechnology, University of Benin, by Prof. Akinnibosun Henry Adewale. A voucher specimen (UBH-S548) was prepared and deposited in the departmental herbarium for future reference. The collected leaves were rinsed thoroughly with clean water to remove adhering debris, air-dried under shade with intermittent turning to promote even drying, and subsequently milled into a fine powder using a mechanical grinder.

Preparation of Chloroform Extract

One hundred grams of the powdered leaf material were soaked in 500 mL of chloroform (98.9% v/v) and allowed to stand for 48 h at ambient temperature with occasional agitation. The mixture was initially filtered through muslin cloth and further clarified using Whatman No. 1 filter paper. Concentration of the filtrate was achieved on a water bath maintained at 50°C, yielding a semi-solid extract, which was stored at 4°C until required.

The percentage yield of the extract was calculated using the formula:

$$\text{Percentage (\% Yield)} = \frac{\text{Weight of extract}}{\text{Weight of plant material}} \times \frac{1000}{1}$$

Phytochemical Screening

Qualitative Analysis

Preliminary screening of the chloroform leaf extract was conducted to detect major classes of secondary metabolites

using established phytochemical procedures. Tests were performed for saponins, phlobatannins, cardiac glycosides, flavonoids, tannins, phenolic compounds, steroids, and terpenoids based on characteristic color changes or precipitate formation, as described in standard phytochemical protocols.

Quantitative Phytochemical Determination

Determination of Total Phenolic and Tannin Contents

Total phenolic and tannin concentrations were determined using the Folin-Ciocalteu colorimetric assay. Briefly, 1.0 mL of the extract was reacted with Folin-Ciocalteu reagent, followed by the addition of saturated sodium carbonate solution. The reaction mixture was incubated in the dark for 90 min, after which absorbance was measured at 725 nm using a spectrophotometer. Calibration curves were prepared using gallic acid and tannic acid (20–100 µg/mL). Results were expressed as milligrams of gallic acid equivalents (GAE)/g extract and tannic acid equivalents (TAE)/g extract, respectively.

Determination of Total Flavonoid Content

Flavonoid content was quantified by the aluminium chloride colorimetric method. The extract was sequentially treated with sodium nitrite, aluminium chloride, and sodium hydroxide solutions, and absorbance was recorded at 510 nm. Catechin (20–100 µg/mL) was used as the reference compound, and flavonoid levels were expressed as milligrams of catechin equivalents (CAE)/g extract.

Determination of Alkaloid Content

Total alkaloids were estimated using the bromocresol green complexation method. The extract was mixed with phosphate buffer (pH 4.7) and bromocresol green reagent, followed by extraction with chloroform. The absorbance of the chloroform phase was measured at 470 nm. Alkaloid content was calculated from an atropine standard curve (40–120 µg/mL) and expressed as atropine equivalents.

Acute Oral Toxicity Study

The acute toxicity profile of the chloroform extract was determined according to the method of Lorke (1983) following a two-phase procedure. Eighteen Wistar albino mice were used for the study. In the first phase, groups of animals received oral doses of 10, 100, and 1000 mg/kg b.w. of the extract. Based on the outcome, higher doses of 1600, 2900, and 5000 mg/kg b.w. were administered in the second phase. Animals were observed for behavioral alterations, signs of toxicity within the first 24 h, and mortality over a 48 h period.

Experimental Animals

Adult male Wistar albino rats weighing between 100 and 120 g were used for the antidiarrheal experiments. Animals were housed under standard laboratory conditions with controlled temperature and a 12 h light/dark cycle, and were provided free access to feed and water. All experimental procedures complied with institutional guidelines for animal care and use.

Experimental Design and Treatment

Twenty rats were randomly allocated into four groups (n = 5 per group):

Group I: Castor oil only (1 mL/kg body weight)

Group II: Loperamide hydrochloride (3 mg/kg body weight) followed by castor oil

Group III: *S. pectinata* chloroform extract (25 mg/kg body weight) followed by castor oil

Group IV: *S. pectinata* chloroform extract (50 mg/kg body weight) followed by castor oil

Induction of Diarrhea and Assessment

Animals were fasted overnight prior to the experiment while water was allowed ad libitum. Thirty minutes after administration of the extract or reference drug, diarrhea was induced by oral administration of castor oil (1 mL per animal). The number of diarrheal feces was recorded, and the percentage inhibition of defecation was calculated as:

$$\text{Inhibition (\%)} = \frac{A-B}{A} \times 100$$

where *A* represents the mean number of diarrheal feces in the control group and *B* represents the mean number in the treated groups.

Enteropooling Assay

The effect of the extract on intestinal fluid accumulation was evaluated using the castor oil-induced enteropooling model. Animals were divided into four groups (n = 6). The control group received normal saline, the negative control received castor oil alone, while the treatment groups received the extract at doses of 300 and 600 mg/kg body weight one hour prior to castor oil administration. Two hours later, animals were anesthetized and euthanized. The small intestine was excised, and the intestinal contents were collected and measured. Fluid accumulation was determined by calculating the difference between the weights of the filled and emptied intestines.

Statistical Analysis

Experimental data were analyzed using one-way analysis of variance (ANOVA). Results were expressed as mean ± standard deviation (SD), and intergroup comparisons were performed using Tukey's post-hoc test. Statistical significance was accepted at *p* < 0.05.

RESULTS

Percentage Yield of Chloroform Extract of *Stuckenia pectinata* Leaves

The chloroform extraction of *Stuckenia pectinata* leaves produced a dark green semi-solid extract weighing 12.326 g, corresponding to a percentage yield of 0.51% w/w. This relatively low yield is consistent with expectations for non-polar solvents, which tend to extract concentrated lipophilic phytochemicals rather than large quantities of material.

Qualitative Phytochemical Profile of Chloroform Extract of *Stuckenia pectinata* Leaves

Preliminary screening of the chloroform leaf extract revealed the presence of several bioactive compounds (Table 1). Saponins, cardiac glycosides, alkaloids, terpenes, and steroids were detected, whereas flavonoids, tannins, phenols, and phlobatannins were absent.

Table 1: Qualitative phytochemical profile of chloroform extract of *Stuckenia pectinata* leaves

| Phytochemicals | Presences |
|---------------------|-----------|
| Saponins | + |
| Cardiac glycosides | + |
| Flavonoids | - |
| Tannins | - |
| Alkaloids | + |
| Terpenes/terpenoids | + |
| Phenols | - |
| Steroids | + |
| Phlobatanin | - |

Key: + Present, - Absent

Quantitative Phytochemical Evaluation

Quantitative analysis showed variation in phytochemical concentrations (Table 2). Tannins were the most abundant (1.06 ± 0.04 mg/g TAE), while flavonoids were the least (0.68 ± 0.03 mg/g CAE).

Table 2: Quantitative phytochemical composition of chloroform extract of *Stuckenia pectinata* leaves

| Phytochemicals | <i>Stuckenia pectinata</i> |
|-----------------------|----------------------------|
| Phenols (mg/g GAE) | 0.73 ± 0.05 |
| Flavonoids (mg/g CAE) | 0.68 ± 0.03 |
| Tannin (mg/g TAE) | 1.06 ± 0.04 |
| Alkaloids (mg/g ATE) | 1.00 ± 0.07 |

Values are means \pm SD of triplicate determinations. GAE = Gallic acid equivalent; CAE = Catechin equivalent; TAE = Tannic acid equivalent; ATE = Atropine equivalent.

Acute Oral Toxicity (LD₅₀)

The oral administration of the chloroform extract to Wistar mice revealed dose-dependent effects on behavior and survival (Table 3). Doses up to 1000 mg/kg did not cause mortality or behavioral changes. However, higher doses (1600–5000

mg/kg) resulted in decreased activity and 100% mortality. The median lethal dose (LD₅₀) was calculated as 1264.91 mg/kg, and the effective safe dose for subsequent experiments was 126.49 mg/kg.

Table 3: Acute oral toxicity of chloroform extract of *Stuckenia pectinata* leaves

| Phase | Dose (mg/kg b.w) | Mortality | Behavioural changes |
|-----------------|------------------|-----------|----------------------------|
| Phase I | | | |
| Group 1 | 10 | 0/3 | Normal physical activities |
| Group 2 | 100 | 0/3 | Normal physical activities |
| Group 3 | 1000 | 0/3 | Normal physical activities |
| Phase II | | | |
| Group 1 | 1600 | 3/3 | Reduced activity/death |
| Group 2 | 2900 | 3/3 | Reduced activity/death |
| Group 3 | 5000 | 3/3 | Reduced physical/death |

Anti-Diarrheal Activity

The chloroform extract demonstrated significant anti-diarrheal activity in castor oil-induced diarrhea in Wistar rats (Table 4). Both 25 and 50 mg/kg b.w. reduced total fecal output and inhibited defecation compared to the diarrhea control group. The 25 mg/kg dose showed comparable efficacy to loperamide (3 mg/kg)

Table 4. Anti-diarrheal effects of chloroform extract of *Stuckenia pectinata* leaves

| Groups | Treatment | Inhibition of defecation (%) | Total fecal output (%) |
|--------|---|------------------------------|------------------------|
| I | Diarrhea control (castor oil only) | - | - |
| II | Loperamide (3 mg/kg) + castor oil | $61.74 \pm 0.68a$ | $114 \pm 0.38a$ |
| III | <i>S. pectinata</i> 25 mg/kg + castor oil | $85.30 \pm 0.49b$ | $120 \pm 0.86b$ |
| IV | <i>S. pectinata</i> 50 mg/kg + castor oil | $41.17 \pm 0.45c$ | $116 \pm 0.43c$ |

Values are means \pm SD of triplicate determinations. Different superscripts within the same column indicate significant differences ($p < 0.05$).

DISCUSSION

This study evaluated the acute oral toxicity, phytochemical profile, and anti-diarrheal effects of the chloroform extract of *Stuckenia pectinata* in Wistar rats. The percentage yield of 0.51% w/w, producing a dark green residue, reflects the non-polar nature of chloroform, which typically extracts lipophilic compounds in moderate amounts. This aligns with findings in other studies where non-polar solvents yielded smaller quantities but concentrated bioactive constituents. The LD₅₀ of 1264.91 mg/kg indicates that the extract is moderately safe. The selected experimental dose (126.49 mg/kg) falls well within the

safe range, confirming the extract’s suitability for pharmacological testing. Phytochemical analyses revealed saponins, cardiac glycosides, alkaloids, terpenes, and steroids as the major constituents. These compounds are known to influence gastrointestinal motility and secretion, supporting their likely contribution to the observed anti-diarrheal effects. The presence of alkaloids and terpenoids is particularly noteworthy, as these have been associated with reduced intestinal smooth muscle contractions and inhibition of prostaglandin-mediated secretion. The anti-diarrheal study confirmed that the extract significantly reduced fecal output and

inhibited defecation. The dose of 25 mg/kg b.w. exhibited effects comparable to loperamide, a standard anti-diarrheal drug. This efficacy is likely mediated by the synergistic action of the identified phytochemicals, which can reduce intestinal hypermotility and fluid accumulation. Overall, the findings indicate that chloroform leaf extract of *Stuckenia pectinata* possesses considerable anti-diarrheal potential, with a moderate safety profile and bioactive constituents responsible for its pharmacological effects. These results support the traditional use of the plant for gastrointestinal ailments and provide a foundation for further pharmacological and mechanistic studies.

CONCLUSION

The study revealed that the chloroform leaf extract of *Stuckenia pectinata* is rich in bioactive compounds, including saponins, cardiac glycosides, alkaloids, terpenes, and steroids, which likely contribute to its potent anti-diarrheal effects. The extract showed a moderate safety profile, with an LD₅₀ of 1264.91 mg/kg, and effectively reduced castor oil-induced diarrhea in rats at a dose of 25 mg/kg, demonstrating efficacy comparable to that of loperamide.

Conflict of interest

No potential conflicts of interest were reported by the authors in connection with this work.

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Ethical approval

None.

Contribution of authors

We affirm that the research reported in this article was carried out by the authors named herein, who assume full responsibility for any content-related claims.

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