ORIGINAL ARTICLE

# *Cassia singueana* Root Extracts Ameliorates Induced Diarrhea and Enteropooling in vivo

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**ABSTRACT**

**Introduction:** Infective diarrhea is a leading cause of death in many developing countries. Exploration of anti-diarrheal natural products from medicinal plants is increasingly recognized as a viable and safe alternative to conventional anti-diarrheal drugs. **Objective:** This study investigates the folkloric claim of *Cassia singueana* as herbal remedy for diarrhoeal related gastroenteritis. **Materials and Methods:** *Cassia singueana* root was extracted with methanol (CSR) then fractionated with saturated butanol fraction (SBF) and ethylacetate fraction (EAF). In-vivo castor oil induced-diarrheal model was used to evaluate the antidiarrheal, anti-enteropooling and intestinal transit effects of the crude extract and fractions. The control group was administered 5 mg/kg Loperamide standard drug. Protein precipitation and antibacterial susceptibility tests were performed against *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*. Data were analyzed using descriptive or one-way analysis of variance and Tukey post-hoc test with significance determined at $P<0.05$. **Results:** Inhibition of defecation was 89.5%, 78.7% and 92% following administration of 150 mg/kg of SBF, EAF and 5 mg/kg loperamide drug treatments. SBF exhibited significant anti-enteropooling effects ($P<0.05$) at 150 mg/kg with 78% reduction in intestinal volume compared to CSR (47.87%). Charcoal transit times were reduced ($P<0.05$) in groups that received 150 mg/kg of CSR (60%), SBF (68.94%) and EAF (73.80%) compared to the loperamide control group (38.8%). Protein precipitation rate was 75.58% and 71.46% for SBF and EAF while 60 mg/ml SBF had zones of inhibition ranging between 23.50±2.40–24.00 ± 3.19 mm. **Conclusion:** Pharmacological activities of SBF and EAF were dose dependent and sub-fractionation increased the overall activities of CSR. These findings underscore the antidiarrheal folkloric claims of *Cassia singueana*.
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**Keywords:** *Cassia singueana*, anti-diarrhea, anti-enteropooling, enteropathogenic bacteria, loperamide

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## INTRODUCTION

Diarrhoea can be defined as the passage of three or more loose or liquid stools per day or more frequent passage than is normal for the individual (1). Diarrhea has devastating consequences, particularly among children in low-income countries, leading to poor growth, delayed development, and, in severe cases, mortality (2). In 2019, diarrhea caused around 1.5 million deaths including half a million among children (3). Despite being preventable, factors such as inadequate sanitation, lack of access to safe drinking water among others exacerbate the severity of diarrheal cases especially in sub-Saharan Africa. In 2008, Nigeria, India, Congo, Pakistan and China together accounted for over 4 Million cases (4).

Clinically, infectious diarrhea may present as acute, watery diarrhea lasting several hours to days, commonly associated with cholera, or as acute bloody diarrhea (dysentery). Diarrhea can also manifest as persistent diarrhea, lasting 14 days or more. Non infectious diarrhea may arise from gastrointestinal hormone imbalance and malabsorption, gut motility, inflammatory mediators, hypersensitivity and chemical irritation (5, 6). Loss of body fluid electrolytes and nutrients in almost all types of diarrhea induces redistribution of body water in response to dehydration and hypovolaemia which necessitates oral rehydration intervention measures. This may however, not be adequate in restoring normal body metabolic processes as the root or underlying causes of the disease may persist in the individual. The use of antibiotics may further disrupt gut microbiota, induce nauseating side effects and predispose to the risk of drug resistance. Socio-economic factors such as poverty, high cost of drugs, difficult to reach communities and displaced populations are immediate causes of diarrheal complications especially among children.

Rural populations driven by necessity or traditional beliefs incline towards herbal remedies or other medicinal plant-based formulations for treatment of various diseases including diarrhea. Globally, there is an increasing reliance on products of traditional and complementary medicine (T&CM). The growing accessibility, affordability and acceptance among local populations position traditional medicine as a valuable tool in advancing universal health coverage (7, 8). The global herbal products market is valued at over $83 billion, with Europe accounting for more than 50% of the total share (9). Several evidence based studies have been conducted on traditional medicinal treatment for diarrhea in sub-Saharan Africa (10, 11) and elsewhere (12, 13). The plant *Cassia singueana* (Delile) (synonym Senna singueana) and locally called rumfu in Hausa is a promising medicinal plant traditionally used to treat a range of non-infectious and infectious diseases including cancer, ulcer, epilepsy, typhoid, malaria, gonorrhea and bilharzia, (14). Furthermore, *C. singueana* root is employed for treatment of diarrhoea among locals in Northern part of Nigeria but with little or no scientific validation. It is probable that while the extractives of the plant act as an antimicrobial, the phytochemical components may also act to suppress enteropooling effects common in diarrheal related gastroenteritis. This paper aims to investigate the folkloric claim of *C. singueana* as a potential herbal remedy for diarrhoeal-related gastroenteritis.

## MATERIALS AND METHODS

### Plant Material

The root of *C. singueana* plant was collected from Morei village in Talata Mafara Local Government Area, Zamfara State, Nigeria. The plant was authenticated taxonomically by Mallam Auwalu Muhammad Umaru at the herbarium, Botany unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto with the voucher specimen number UDUH/ANS/0072. The root was pulverized, shade dried and preserved in a plastic container for further processing.

### Chemicals and Reagents

Organic analytical-grade solvents including methanol, n-hexane, ethylacetate and n-butanol (BDH Chemical Limited, Poole, England) were used for extraction of the plant material. Castor oil (Bell Sons & Co (DRUGGIST) Ltd, Southport PR9 9AL, England) was donated by the Department of Biochemistry and Molecular Biology, Usmanu Danfodiyo University, Sokoto. Activated Charcoal (Qualikems Batch number: CCW170811), Loperamide (Imodium)® (JANSSEN-CILAG Ltd, 50-100 Holmers farm way, High Wycombe, Bucks HP124EG, UK, Batch number: BFB2000), Muller- Hinton nutrient agar, and Ciprofloxacin standard (supplier Divine Essential Formulations Km. 10, Lasu-Ojo Road, Lagos, Nigeria, Batch number: 018SN) were obtained commercially.

### Experimental Animals

Albino wistar rats of both sexes weighing between 140-200 g were used for this study. They were housed in standard rat cages in the Animal House of the Department of Biochemistry and Molecular Biology, Usmanu Danfodiyo University, Sokoto and were allowed access to clean water and feed ad libitum for two weeks to acclimatize before the commencement of the experiments. Ethical clearance for the study was initially approved by the Usmanu Danfodiyo University, Sokoto Ethical clearance Committee after inspection on adherence to Organization for Economic Cooperation and Development (15) recommendations for handling of experimental animals.

### Bacterial Organisms

Clinical isolates of *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* were collected from the Microbiology Laboratory at Usmanu Danfodiyo University Teaching Hospital Sokoto, Nigeria. The isolates were confirmed through standard biochemical tests then sub-cultured on Nutrient Agar plates and incubated for 18 hours prior to use (16).

### Extraction of *C. singueana* Root

Exactly 550 g of the pulverized *C. singueana* root sample was macerated with 5000 ml of methanol for 72 hours at room temperature then filtered using a white muslin cloth and then re-filtered again with Whatman filter paper No 1. A rotavapour set at 45$^{o}$C was used to concentrate the filtrate then dried to a constant weight using a drying cabinet to obtain the methanol root extract (CSR). A portion of the dried extract was sub-fractionated using the organic solvents ethylacetate (EAF), and saturated butanol (SBF). The sub-fractions were concentrated and dried as described.

### Experimental Design

### Induction of Diarrhea in Rats

A total of 24 rats were fasted for 18 hours and were divided into 6 groups of 4 rats per group as follows:
Group 1: Orally received distilled water only and served as Negative control group; Group 2 received orally 5 mg/kg of loperamide and served as positive control. Groups 3, 4, 5 and 6 (treatment groups) received 1 ml of 25, 50, 75 and 150 mg/kg of the CSR respectively. After one hour, each rat was administered 1 ml of castor oil. Feces were retrieved and diarrhea severity was assessed every hour for 6 hours (17). Percent inhibition of defecation as described by Malik et al (18) was determined using the relationship:

$$\% \text{ inhibition} = (Mo–M)/Mo \times 100$$

Where, Mo = Mean defecation of control group taken as 100%; M = Mean defecation of treatment group.

**Gastrointestinal Motility Test**

A total of 24 wistar rats divided into 6 groups of 4 rats were fasted for 18 hours. Treatment was conducted as follows: Group 1 received distilled water orally and served as negative control. Group 2 was given 5 mg/kg of loperamide serving as the positive control group while Groups 3, 4, 5 and 6 were administered 1 ml of 25, 50, 75 and 150 mg/kg of CSR respectively. After 30 min, each rat was then orally administered 1 ml of charcoal meal (prepared as 10% activated charcoal in distilled water). All animals were humanely sacrificed after 30 min. Motility distance of the charcoal meal from the pylorus to the caecum of the intestine was measured. Gastrointestinal motility was then determined using the formula as described (19-21):

% Inhibition = (Dc - Dt )/Dc x 100

Where Dc = Charcoal distance travelled in the control group; Dt = Charcoal distance travelled in Treatment Group

**Castor Oil Induced Enteropooling**

The methods of Robert et al (22) and Dicarlo et al (23) were used to determine intraluminal fluid accumulation. After grouping 24 wistar rats into 6 groups of 4 rats per group and fasting for 18 hours, treatment was administered as follows: The negative control (Group 1) served received distilled water orally; while the positive control (Group 2) was administered 5 mg/kg loperamide; The remaining groups 3, 4, 5 and 6 received 1 ml of 25, 50, 75 and 150 mg/kg of CSR respectively. After one hour, each rat was given 1 ml castor oil then anaesthetized using chloroform 1 h later. Small intestine dissection from the pylorus to caecum was done and the fluid volume measured using a measuring cylinder (24). The average volume for each group was then calculated:

Percentage Reduction (%) = (Vc - Vt )/Vc x 100

where, Vc = Intestinal content mean volume of the control group and Vt = Intestinal content mean volume of the treatment group.

Similar experiments were conducted using EAF and SBF as treatment.

**Protein Precipitation of Total Tannins**

For the crude and sub-fractions of the extract, 0.2, 0.4, 0.6, 0.8, 1.2, 1.4 and 1.6 ml of 100 mg/ml extracted total tannins (TT) solution were added to the test tubes containing 2 ml of 2 mg/ml bovine serum albumin (BSA) solution. The volume was then made up to 4 ml with distilled water to obtain 1 mg/ml final concentration of BSA and 0.5, 10, 15, 20, 25, 30, 35 and 40 mg/ml total tannins concentration respectively for each tube. After mixing thoroughly, each test tube was kept in dark at 4°C for 12 hours. The protein content of the supernatant from each tube was determined by Coomassie brilliant blue kit. The BSA precipitation rate was calculated following methods described by Yi et al (25). The procedure was conducted in triplicate for each tube.

Precipitation rate (%) = (Cs - Cn)/Cs x 100

Where Cs = absorbance of supernatant before protein precipitation; Cn = absorbance of supernatant after protein precipitation.

**Antibacterial susceptibility Test**

The agar well diffusion method was used to determine bacterial susceptibility to CSR, EAF and SBF. Briefly, Pure culture of the organism were inoculated onto prepared Muller - Hinton nutrient broth, incubated for 24 hour at 37°C after which the broth was diluted to obtain Mc-Farland standard equivalent to $9 \times 10^8$ cfu/ml. The suspension was used to streak the surface of prepared Muller-Hinton agar plates. Thereafter, a cork borer was used to create equally spaced wells into which 20 mg/ml of the ciprofloxacin antibiotic (control) and 10, 20, 40 and 60 mg/ml CSR were respectively dispensed. The plates were incubated at 37°C for 24 h after which the zones of inhibition was determined by measuring the diameter in mm. The procedure was conducted in triplicate and repeated for each sub-fraction for the respective organisms.

**Data Analysis**

The data were presented as mean ± standard deviation where procedures were done in replicates and results analyzed by one-way analysis of variance (ANOVA) followed by Tukey posthoc and values were considered statistically significant at $P<0.05$. Graph Pad Instat® software (San Diego, U.S.A) was employed for the analysis. Descriptive statistics was also used to present formula derived results in percentages.

**RESULTS**

Presented in Table I is the result of anti-diarrheal properties of the various fractions of *C. singueana* root extract. The SBF sub-fraction at a concentration of 150 mg/kg significantly reduced diarrhea in the rat models up to 89.00% suggesting the anti-diarrheal effect was dose dependent. The sub-fraction may also contain bioactive components that may have preferentially partitioned in the fraction. Table II shows the effects of *C. singueana* root extracts administration on intestinal transit properties of experimental rats fed charcoal diet. Charcoal transit times were significantly reduced ($P<0.05$) in groups administered 150 mg/kg of CSR (60.00%), SBF (68.94%) and (EAF) (73.80%) compared to the loperamide control group (38.80%).

Results of *C. singueana* root extract enteropooling effects are presented in Table III. Loperamide standard treatment group demonstrated highest intestinal fluid reduction (84.00%) followed SBF (78.00%) and EAF

(75.00%) treatments at 150 mg/kg.

**Table I: Anti-diarrheal Effects of *Cassia singueana* Root Extract in Rats Induced with Castor oil Diarrhea Values with different superscript are significantly different at P<0.05. CSR = *C. singueana* root methanol extract; EAF = ethylacetate subfraction; SBF = saturated butanol subfraction**

| Treatment | Dose mg/kg | Diarrhea stools | Inhibition % |
|---|---|---|---|
| Water | - | 8.75±1.16[b] | - |
| Loperamide | 5 | 0.13±0.13[a] | 97.3 |
| CSR | 25 | 5.40±0.51[b] | 26.3 |
| | 50 | 4.40±1.03[b] | 30.5 |
| | 75 | 4.40±0.68[b] | 32.6 |
| | 150 | 3.20±0.86[e] | 41.1 |
| EAF | 25 | 2.00±0.91[d] | 57.9 |
| | 50 | 1.75±0.63 | 63.2 |
| | 75 | 1.65±1.18 | 65.7 |
| | 150 | 1.00±0.71[c] | 78.9 |
| SBF | 25 | 1.63 ± 0.62 | 65.6 |
| | 50 | 1.50±0.65 | 68.4 |
| | 75 | 1.25 ± 0.48[c] | 73.7 |
| | 150 | 0.50 ± 0.29[a] | 89.5 |

**Table II: Small Intestinal Transit Properties in Rats Administered Root Extracts of *C. singueana* IL: Intestinal Length, CML: Charcoal Meal Length, IT: Intestinal transit.**

| Fractions/ Drug | Dose (mg/kg) | IL (cm) | CML (cm) | IT % | Inhibition % |
|---|---|---|---|---|---|
| Water | - | 106.20 ± 8.80 | 106.20 ± 8.80 | 100.00 | - |
| Lopera-mide | 5 | 111.20 ± 4.10 | 69.20 ± 5.70 | 62.00 | 38.00 |
| CSR | 25 | 90.20 ± 3.90 | 55.20 ± 2.00 | 61.00 | 39.00 |
| | 50 | 101.6 ± 6.20 | 49.40 ± 4.60 | 49.00 | 51.00 |
| | 75 | 97.50 ± 2.50 | 56.50 ± 2.90 | 58.00 | 42.00 |
| | 150 | 111.10 ± 2.60 | 44.50 ± 1.40 | 40.00 | 60.00 |
| EAF | 25 | 100.90 ± 3.80 | 70.30 ± 3.00 | 69.67 | 30.33 |
| | 50 | 107.50 ± 5.50 | 62.20 ± 4.60 | 57.90 | 42.10 |
| | 75 | 102.30 ± 8.00 | 58.10 ± 1.60 | 56.80 | 43.20 |
| | 150 | 108.30 ± 5.10 | 8.40±8.50 | 26.22 | 73.78 |
| SBF | 25 | 97.18 ± 5.91 | 71.83 ± 6.89 | 73.91 | 26.09 |
| | 50 | 98.10 ± 9.53 | 61.20 ± 1.79 | 62.39 | 37.61 |
| | 75 | 97.00 ± 7.46 | 47.28 ± 4.17 | 48.74 | 51.26 |
| | 150 | 97.80 ± 7.89 | 30.38 ± 8.83 | 31.06 | 68.94 |

**Table III: Enteropooling Properties of *Cassia singueana* Root Extract in Rat models of Castor Oil induced Diarrhea**
**Superscript * indicate no significant difference (P>0.05) compared with the loperamide positive control group**

| Fractions/ Drug | Dose (mg/kg) | Mean volume of Intestinal content (ml) | Reduction % |
|---|---|---|---|
| (mg/kg) | - | 4.38 ± 0.77 | - |
| Loperamide | 5 | 0.70 ± 0.32* | 84.00 |
| CSR | 25 | 3.24 ± 0.09 | 13.83 |
| | 50 | 2.74 ± 0.06 | 27.13 |
| | 75 | 2.00 ± 0.13 | 46.81 |
| | 150 | 1.96 ± 0.09 | 47.87 |
| EAF | 25 | 2.28 ± 0.25 | 48.00 |
| | 50 | 2.23 ± 0.14 | 49.00 |
| | 75 | 2.03 ± 0.17 | 54.00 |
| | 150 | 1.08 ± 0.28* | 75.00 |
| SBF | 25 | 2.00 ± 0.24 | 54.00 |
| | 50 | 1.85 ± 0.16 | 57.00 |
| | 75 | 1.83 ± 0.19 | 58.00 |
| | 150 | 0.98 ± 0.09* | 78.00 |

**Superscript * indicate no significant difference (P>0.05) compared with the loperamide positive control group**

**Fig. 1** shows the tannin protein precipitation rate of CSR, EAF and SBF. The protein precipitation rate increases as the concentration of tannins increase with SBF and EAF having the highest percentage precipitation rate of 75.58% and 71.48% respectively at 1.6 mg/ml. Protein precipitation surpasses 50.00% even at low concentrations of 0.2 mg/ml of *C. singueana* root extracts suggesting significant protein-tannin interaction.

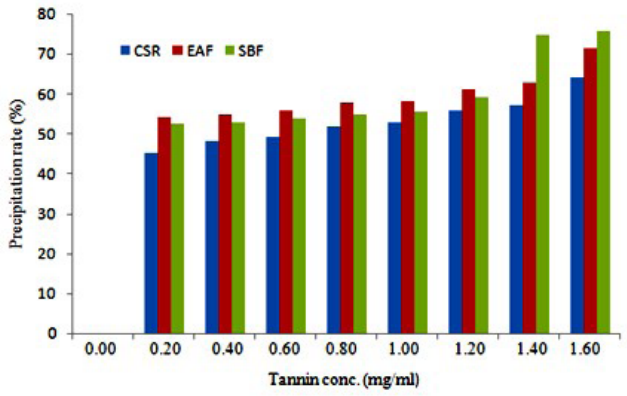


**Fig. 1: Protein Precipitation Test of Total Tannins of Crude Methanol Root Extract of *Cassia singueana***
**CSR = CSR = *C. singueana* root methanol extract; EAF = ethylacetate subfraction; SBF = saturated butanol subfraction**

Zones of inhibition as presented in **Table IV** suggest *P. aeruginosa* to be the most susceptible organism tested ranging from 21.00±1.56 mm to 25.00±1.73 mm for the various extract fractions tested. Comparatively at 10 mg/ml, SBF is the most active against all three organisms followed by EAF.

**Table IV: Antibacterial Activity of *C. singueana* Root Extract on Some Selected Bacteria**
**Superscripts across the row for each dose indicate significant difference at P<0.05. CSR = CSR = *C. singueana* root methanol extract; EAF = ethylacetate subfraction; SBF = saturated butanol subfraction**

| | Conc. (mg/ml) | Zones of Inhibition (mm) | | |
|---|---|---|---|---|
| | | *E. coli* | *P. aerugi-nosa* | *S. aureus* |
| **Water** | - | - | - | - |
| **Ciprosan** | 20 | 32.00±1.18 | 31.69±1.02 | 32.00±1.16 |
| **CSR** | 10 | 9.00±0.16 | 17.67±1.86 [a] | 11.00±0.56 |
| | 20 | 11.67±2.31 | 16.00±1.64[b] | 12.10±1.14 |
| | 40 | 13.30±2.11 | 20.63±2.27[c] | 13.00±1.30 |
| | 60 | 16.23±1.46 | 21.00±1.56[d] | 14.33±1.40 |
| **EAF** | 10 | 12.00±0.16 | 20.67±1.76[a] | 14.00±0.58 |
| | 20 | 13.67±2.40 | 22.00±1.73[b] | 14.30±1.16 |
| | 40 | 16.33±2.19 | 22.67±2.40[c] | 15.00±1.10 |
| | 60 | 19.33±1.76 | 25.00±1.73[d] | 18.33±1.20 |
| **SBF** | 10 | 14.00±1.08 | 17.00±0.91 | 20.75±0.48 [a] |
| | 20 | 18.25±4.66 | 21.75±1.25 | 20.75±2.87 |
| | 40 | 22.75±2.48 | 22.00±2.19 | 23.25±3.64 |
| | 60 | 24.00±3.19 | 24.50±2.36 | 23.50±2.40 |

**Superscripts across the row for each dose indicate significant difference at P<0.05**

## DISCUSSION

Castor oil active metabolite ricinoleic acid induces diarrhea which triggers intestinal hypersecretory response. This response enhances peristaltic activity in the small intestine and alters electrolyte permeability in the intestinal mucosa (26-28). Furthermore, castor oil stimulates the release of endogenous prostaglandins E and F, leading to stomach cramps and diarrhea by influencing smooth muscle activity and secretion, likely via cAMP-mediated signaling (29-31). The findings suggest the extract may either inhibit prostaglandin biosynthesis, thereby preventing or delaying castor oil-induced diarrhea, or promote protein precipitation in the small intestine. This mechanism appears to exert a dual effect on gastrointestinal motility and the regulation of water and electrolyte transport (17).

### Effect of gastrointestinal motility test

The extracts slowed intestinal transit as indicated by the reduced distance traveled by charcoal meal. This suggests the extract inhibited charcoal meal propulsion thus enhancing water and electrolytes absorption. Moreover antimotility and antisecretory agents are central in the management of diarrhea. A similar pattern is observed in the anti-diarrheal effectiveness of *C. singueana* likely due to its bioactive phytochemicals including flavonoids, tannins, alkaloids, saponins, terpenes and steroids (32-35). Flavonoids in particular have been associated with antidiarrheal activity by inhibiting intestinal motility and hydro-electrolytic secretions, which are typically altered in diarrheal conditions (23, 36). Both in vitro and in vivo studies have demonstrated that flavonoids suppress intestinal secretory responses induced by prostaglandins E2 (37). Though not reported in this study, the flavonoid rich root of *C. singueana* may be involved in activating adrenoceptors (38) in absorptive gastrointestinal cells thereby promoting water and electrolyte absorption.

### *C. singueana* Root Extract on Castor oil-induced Enteropooling Effects

This study revealed the plant extract significantly reduced (P<0.05) the volume of intestinal content indicating inhibition of castor oil-induced intestinal enteropooling effects. This inhibitory effect was more pronounced in SBF (78.00%) then EAF (75.00%) both at 150 mg/kg treatment group and comparable to that observed in the loperamide positive control group (84.00%). The anti enteropooling effects of the extracts may result from either reduced mucosal secretion or enhanced mucosal absorption. By inhibiting intestinal transit, as observed in this study, the extracts slow the movement of gastrointestinal contents, allowing for desiccation of feces and further delaying their passage through the colon (39).

### Protein Precipitation Test of Total Tannins

Protein precipitation is a technique that relies on variations in protein solubility and precipitation as influenced by tannins in solution. (40) Tannins known for their protein precipitating properties, act as astringent on the intestinal wall. They deposit protein on the epithelial surface, forming a stable, protective film that mitigate effects of local irritants, reduce water exudation and restore normal intestinal hyper-peristalsis (41). Protein precipitation test in this study supports the dose dependent anti-diarrheal pharmacological properties of *C. singueana* extracts. This finding suggests further that fractionation of the extract increased the overall antidiarrheal and enteropooling properties of the root extract.

### Bacterial Susceptibility to *C. singueana* root extracts

Susceptibility of *S. aureus*, *P. aeruginosa* and *E. coli* to CSR, EAF and SBF were investigated in this study. The antibiotic ciprofloxacin was active against all the test bacteria. Secondary metabolites such as saponins, flavonoids, steroids and tannins are responsible for antibacterial properties of plant (42, 43). These bioactive compounds exert their antimicrobial effects by different mechanisms. Tannins for example interfere with microbial protein synthesis by binding to proline rich proteins (44). Other studies on *Senna singueana* such as reported by Ochieng Nyalo et al (45) showed that ethylacetate leaf extract of the plant significantly inhibited the growth of the enteropathogenic bacteria *E. coli*, *Staphylococcus aureus* and *Salmonella typhi.* The significant activity demonstrated by the fractions against clinical bacterial strains linked to diarrhea, along with the observed antidiarrheal properties, supports the ethnomedicinal use of this plant and provides a scientific

basis for its traditional applications

## CONCLUSION

The pharmacological activities of *Cassia singueana* root extracts were demonstrated to be dose-dependent, with SBF and EAF sub-fractionations significantly enhancing the overall antidiarrheal effects of the crude extract. This study provides scientific evidence supporting the traditional antidiarrheal uses of *C. singueana*, reinforcing its ethnomedicinal relevance.

## DECLARATION

The authors of this article declare no conflict of interest.

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