



# Anti-Diarrheal Studies of Aqueous Leaf Extract of *Chrozophora Senegalensis* in Albino Rats



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## Abstract

Diarrhea has a very high morbidity and mortality, especially in children and in developing countries. *Chrozophora senegalensis* is used in its management among North-Western Nigeria traditional healers. This claim was scientifically investigated using some diarrhea models. The leaves were also subjected to both phytochemical and proximate analyses, for possible explanations. Phytochemical studies of the leaves revealed the presence of Alkaloid (1.20%), Tannins (0.92%) and Flavonoids (0.029%) while proximate composition showed carbohydrates (73.3%), crude fibre (8.5%), crude protein (6.48%), ash (10.0%), moisture (4.0%) and crude fat (2.0%). The acute oral toxicity studies showed no mortality at 5000 mg/kg body weight. The anti-diarrheal activity of the plant using Activated Charcoal Model showed increase in average latent period and decrease in purging index with increase in dose of the extract. Conclusively, *Chrozophora senegalensis* could be a potential source of anti-diarrheal drug.

**Keywords:** Albino rats; Aqueous leaf extract; Anti diarrheal; *Chrozophora senegalensis*; Phytochemical studies

## Introduction

Diarrhea is a GIT clinical condition characterized by bowel hypermotility with an increase in both the volume and the frequency of stooling [1]. Diarrhea is not a disease per se, but a symptom of some other diseases. The condition may be caused by consumption of food or water contaminated with microbes or toxins or food that is difficult to digest [2]. Stress could also cause diarrhea and some medications also have it as a side effect. Acute diarrhea is a common cause of death in developing countries and the second most common cause of infant mortality worldwide [3]. It is most commonly caused in humans by gastrointestinal infections which kill about 2.2 million people, mostly children, globally in developing countries each year [4]. UNICEF earlier reported that some 1.5 million children die of diarrhea related diseases annually [5]. WHO also reported earlier that some 50 million children suffering from diarrhea have been successfully managed using oral rehydration salts for about 30 years now. This is probably due to the cheapness of these ORT salts [5]. In Nigeria, more than 315,000 deaths of pre-school age children are recorded annually as a result of diarrheal diseases [6,7].

Although much less has been understood about both the pathogenesis and the management of this condition, it still remains an important deadly disease of children. This is largely because the etiology and the pathogenesis of its persistent forms are mostly multi-factorial and mostly cannot be identified with ease [8]. Causes of diarrhea include pathogens such as parasites,

bacteria and viruses in outbreaks of infant diarrheal diseases [9]. For this, international organizations including the World Health Organization (WHO) encourage studies on the treatment, management and prevention of diarrheal diseases using traditional medical practices that are safe and effective [10]. Some traditional medicinal herbs that have scientifically been evaluated for anti-diarrhea activity include *Khaya senegalensis*, *Xylocarpus muculensis*, *Jatropha curcus* and *Adansonia digitata* [11].

The present plant of study is *Chrozophora senegalensis* and is locally known as Damaigi or Bauren Kiyashi in Hausa (Northwest Nigeria). It is a shrub with deep red flowers and violet tinged capsules. It belongs to the family Euphorbiaceae and it is commonly found in dried up sandy riverbeds. This shrub is traditionally used in the management of diarrhea and treatment of boils and to manage both. It has also been reported to alleviate abdominal pain, conjunctivitis and syphilis [12]. The present investigation seeks to scientifically evaluate the anti-diarrheal activity of this shrub in experimental animal models so as to verify both its safety and efficacy by acceptable standards.

## Materials and Methods

### Plant material

The whole plant of *C. senegalensis* was collected from the wild of Sifawa area (Sokoto state, Nigeria). The plant was

identified and authenticated at the herbarium, Biological Sciences Department, Usmanu Danfodiyo University, Sokoto. A voucher specimen was deposited for further reference and a voucher number of UDUH/ANS/0005 *Chrozophora senegalensis* was issued.

### Preparation of plant material

The leaves of *C. senegalensis* were cleaned of to remove extraneous matter by dipping in cold water and air-dried at room temperature for 5 days. They were then pulverized into fine powder. The powder was mixed with distilled water (100g of powder in 1Litre of distilled water) for 24 hours with intermittent stirring. The mixture was filtered using Whatman® filter paper (No. 1). The filtrate was evaporated to dryness at 450C in a drying cabinet electrothermal oven, DHG Series 9030A model.

### Animals

A total of 37 Wista albino rats of both sexes were used for this study and they were acquired from the Zoological garden of Dept of Biological Sciences, U. D. U. Sokoto. The protocol for screening the anti-diarrhea activity in animals was cleared by the ethical committee of Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria. The rats were housed under standard laboratory environmental conditions for acclimatization for a period of 14 days prior to the commencement of experiments. The proximate analysis was carried out to determine protein, fats, carbohydrates, ash and moisture contents of *C. senegalensis* according to the procedure earlier described by [13] while the phytochemical analysis was evaluated using standard procedures of [14,15].

### Drugs and chemicals

The drugs and chemicals used are atropine sulphate, Diphenoxylate, castor oil, normal saline (0.9% NaCl) and charcoal meal (10% activated charcoal in 100ml of 5% aqueous gum acacia) and they were of pharmacological grade. All other reagents and solvents used were of analytical grade.

### Determination of LD50

Aqueous extract of *C. senegalensis* (5000mg/kg body weight) was administered in a single oral dose to five (5) rats, one after the other at a grace observation period of 48 hours. The observation was done every 15 minutes in the first one hour, then hourly for the next six hours and continued to the next 48 hours. All observations were recorded up to 14<sup>th</sup> day after administration in each group [16]. This is the limit dose test of Up and Down method of LD50 determination.

### Gastrointestinal motility test

Twenty rats were divided into five groups of four rats each and fasted for 18 hours, but water was provided ad libitum. The first group (control group) received normal saline (5mL/kg body weight) orally, while the second, third and fourth groups

were administered the plant extract in oral doses at 200,400 and 600mg/kg body weight respectively. The fourth group was administered atropine sulphate, intraperitoneally (3mg/kg body weight). Thirty (30) minutes later, each rat was administered 10% charcoal meal (10% activated charcoal in 5% gum acacia) orally at 5ml/kg body weight as a watery diet using a 5ml syringe without needle. All the rats were sacrificed 30 minutes after treatment, and the distance left uncovered by the charcoal meal in the intestine from the pylorus to the caecum was measured and expressed as a percentage of the distance from the pylorus to the caecum [17]. This uncovered distance is the motility inhibitory effect of the treatment.

### Castor oil-Induced diarrhea in rats

Twenty (20) rats were fasted for 18 hours and divided into five groups (A-E) of four rats each. The plant extract (200, 400 and 600mg/kg) were administered orally to groups B, C and D respectively. Group A rats were administered normal saline (5mL/kg) and served as control, while the group E was administered diiphenoxylate, also serving as control (0.3mg/kg). An hour later, all the animals were administered castor oil orally (15mL/.kg). The animals were kept in separate metallic cages with transparent plastic containers beneath them to collect faeces. The severity of diarrhea was assessed each hour for 6 hours. The total number of faeces (both diarrheal and non-diarrheal) expelled were compared with the control group. The purging index (PI) for each group was then calculated using the formula [1].

### Statistical Analysis

The results of gastrointestinal motility and the castor oil induced diarrhea were analyzed statistically using one-way analysis of variance followed by Dunnett's 't' test of the Graph Pad INSTAT (San Diego, USA). The data are expressed as mean ± S.E.M (Standard Error of the Mean). P ≤ 0.05 was significant.

$$PI = \frac{\text{Respondent percentage} * \text{average number of stools}}{\text{Average latent period}}$$

### Results

#### Chemical analysis

**Table 1:** Proximate Composition of *C. senegalensis* leaves.

Proximate composition of aqueous extract of <i>C. senegalensis</i>	
Parameter	Percentage composition (%)
Ash	10
Crude fibre	8.5
Crude fat	2
Carbohydrate	73.03
Crude protein	6.48
Moisture	4

The percentage yield of the extracts of *C. senegalensis* was 10.9% while the proximate composition is represented in Table

1. The phytochemical analysis of the plant extract showed the presence of Alkaloids (1.20%), Tannins (0.029%) and Saponins (0.92%) (Table 2).

**Table 2:** Phytochemical analysis of *C. senegalensis* aqueous leaf extract.

S/No.	Secondary Metabolite	Qualitative analysis	Quantitative analysis (%)
1	Alkaloids	+	1.2
2	Hydrolysable Tannins	+	0.029
3	Saponins	+	0.92
4	Flavonoids	-	-
5	Flavonols	-	-
6	Flavonoid Aglycone	-	-
7	Glycosides	-	-
8	Cardiac Glycosides	-	-
9	Flavonoid Glycosides	-	-
10	Saponins Glycosides	-	-
11	Anthraquinone Glycosides	-	-

Detected (+), Not detected (-)

### Acute toxicity test (LD<sub>50</sub>)

It was observed that oral administration of the aqueous leaf extract of *C. senegalensis* to 5 consecutive rats at 5000mg/kg body weight neither gave mortality nor produced any apparent signs of toxicity in the rats within 48 hours and up to 14 days after dosing. Thus, the mean lethal dose of the aqueous leaf extract of *C. senegalensis* is greater than 5000mg/kg body weight, orally in rats.

### Effect of *C. senegalensis* aqueous leaf extract on Gastro-intestinal transit of charcoal meal

The extract of *C. senegalensis* decreased propulsion of charcoal meal in the rat's gastrointestinal tract at oral doses of 200, 400 and 600mg/kg, compared with the control group that was administered normal saline (5mg/kg). A similar reduction in the gastrointestinal transit of charcoal meal in rat was achieved with atropine sulphate (3mg/kg) in comparison with the control group of normal saline. The results are shown in Table 3.

### Effect of *C. senegalensis* aqueous leaf extract on castor oil-induced diarrhea

In the castor oil induced diarrhea experiment, the aqueous leaf extract of *C. senegalensis* produced a marked anti-diarrhea effect in the rats, as shown in Table 3. At doses of 200, 400 and 600mg/kg, the extract significantly ( $p < 0.05$ ) decreased the total number of wet faeces produced upon administration of castor oil (12.20  $\pm$  1.06 at 250mg/kg, 7.00  $\pm$  0.94 at 500mg/kg and 6.20  $\pm$  0.58 at 1000mg/kg) compared to the control group (18.6  $\pm$  0.74). The effect of the highest dose of the extract was similar to that of the standard drug, diphenoxylate (0.3mg/kg) as presented in Table 3.

**Table 3:** Effects of aqueous extract of *C. senegalensis* on the intestinal transit.

Treatment	Number of faeces induced by castor oil after treatment	Inhibition % of GIT motility after treatment
Normal saline (5mL/kg)	45.76 $\pm$ 3.29	8.25
Diphenoxylate (3mg/kg)	12.03 $\pm$ 1.08**	73.71
<i>C. senegalensis</i> (200mg/kg)	38.76 $\pm$ 6.65*	15.3
<i>C. senegalensis</i> (400mg/kg)	18.90 $\pm$ 2.75**	58.7
<i>C. senegalensis</i> (600mg/kg)	13.02 $\pm$ 2.25	69.45

Values are expressed as mean  $\pm$  S.E.M (n = 4) \*P < 0.05, when compared to the control

### Discussion

The low percentage yield of 10.9% obtained from cold aqueous extraction of the leaves of *C. senegalensis* suggests that it contains a lot of fibres. However, the yield can be improved by changing the solvent and the temperature of extraction. The experiment can also be repeated using the resulting extract otherwise more of the plant is required to have more yield. The LD50 of the extract was found to  $\geq 5000$ mg/kg. This makes it safe for medicinal purposes by WHO standard [18].

The phytochemical analysis of the aqueous leaf extract of *C. senegalensis* revealed that it contains tannin, alkaloids and saponins (Table 2). Alkaloids have been reported to have antibacterial, antifungal and some other pharmacological activities [19]. It has also been reported that bacteria such as *Campylobacter jejuni*, *Salmonella* spp, *Clostridium* spp etc are some of the causes of diarrhea [20]. It will therefore not be out of place to suggest that the alkaloid content of the extract of this plant could reduce the intensity of diarrhea if it is of bacteria origin.

Tannins react with proteins to form stable water insoluble components. Since bacteria cell wall contains proteins, by tannins precipitating these proteins, there is inhibition of the growth of these bacteria. If these bacteria are the causes of diarrhea then the diarrhea can be controlled. Also, the precipitation of the proteins of the intestinal mucosa by tannins in the extract also helps reduce the secretion into the bowel thereby reducing the intensity of diarrhea [21]. Saponins have also been reported to have antibacterial activity, its presence in the extract could therefore also account for its anti-diarrheal activity [21]. The proximate analysis of the extract revealed that it mainly contains organic extract (Table 1). This suggests that it is high in nutritive value. It should therefore be compatible with the body system to a large extent. However, the presence of secondary metabolites like tannins, saponins and alkaloids poses some anti-nutritional potential. Although these anti-nutritional factors give the plant its medicinal properties [21]

Castor oil is a laxative and induces diarrhea by increasing both the motility and the secretions of the GIT [22] This is through the activities of both autocoids and prostaglandins [23].

These cause increase in the permeability and the motility of the GIT, resulting in diarrhea. In this study, the three doses of the extract used all significantly and dose dependently reduced the purging index in castor oil diarrhea model, in comparison with control group (Table 3). This suggests that the extract has inhibitory effects on autocooids and prostaglandin production and hence, on secretion and motility of the GIT. By this, it can be deduced that one of the mechanisms of action of this extract is by inhibition of both motility and secretion.

In the charcoal meal model of this study (Table 3), the three doses of the extract significantly inhibited the motility of the GIT. This also buttresses the earlier postulation in the castor oil model that this plant extract reduces diarrhea by inhibition of both motility and secretion. However, antimicrobial activity of this plant extract be investigated to see its likely application in diarrhea of infective origin.

### Conclusion and Recommendations

The results of this investigation revealed that the crude aqueous leaf extract of *C. senegalensis* contains pharmacologically active substances with dose dependent anti-diarrheal properties. These attributes may provide the rationale for the use of *C. senegalensis* leaf extract in diarrhea management by traditional healers in humans and animals. It is recommended that antibacterial studies of this extract be carried out on clinical isolates causing diarrhea as antibacterial action may be part of the observed anti-diarrheal effect, since treatment may also depend on the type and cause of the GIT erosion [24]. Further studies with purified constituents are needed to completely understand the mechanism of anti-diarrheal action of aqueous extract of *C. senegalensis* leaves. Tannins (0.029%) and Saponins (0.92%) (Table 2) [25,26].

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