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Research Article

PHYTOCHEMICAL AND ANTI-DIARRHOEAL PROPERTIES OF METHANOL ROOT EXTRACT OF *GUIERA SENEGALENSIS* J.F. GMEL

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ABSTRACT

The study investigated the anti-diarrhoeal properties and phytochemical constituents of the methanol root extract of *Guiera senegalensis*. The phytochemical components of the extract were evaluated. White Wister strain albino rats weighing between 150-200 g were used to investigate the acute toxicity, the castor oil-induced diarrhoea, intestinal fluid accumulation and transit of charcoal meal of the extract. The extract was safe at doses up to 5000 mg/kg. Phytochemical analysis revealed the presence of carbohydrates, reducing and combined sugars, tannins, alkaloids, saponins, flavonoids, cardiac glycosides, terpenoids and cardenolides. The results of the present study showed that the extract of the root of *G.senegalensis* produced a statistically significant ($p<0.05$) reduction in the frequency of diarrhoea produced by castor oil, castor oil-induced intestinal fluid accumulation and transit of charcoal meal.

The methanol root extract of *G. senegalensis* exhibited a significant inhibition of castor oil-induced diarrhea, though not dose-dependent. The highest dose of 800 mg/kg exhibited a lower reduction of 32.81±0.58 % when compared to the other two doses of 200 and 400 mg/kg which gave 45.31 and 57.89 % inhibition respectively. The loperamide (5 mg/kg) treated group gave a 100±0.00 % inhibition. The extract also slowed down the propulsion of charcoal meal through the GIT dose-dependently. The atropine-treated group gave 82.59±0.42 % inhibition. There was also a marked reduction in the weight and volume of intestinal contents which was dose-dependent.

The anti-diarrhoeal activity exhibited by the methanol extract could be due to the presence or solubility of most of the bioactive compounds in higher amounts or concentrations.

This study provided a scientific basis for the use of *G. senegalensis* root extract in the treatment of diarrhoea.

Keywords: *G. senegalensis,* Methanol extract, anti-diarrhoeal effect, acute toxicity and phytochemistry.

INTRODUCTION

Diarrhoea is a major health problem especially in developing countries. In Africa, diarrhoea remains a major contributor to the high rate of child mortality. In Nigeria, diarrhoeal infections remain the number one killer disease among children under 5 years, with 7–12 months old babies remaining the most susceptible[1]. The incidence of diarrhoeal diseases still remains high despite the intervention of government agencies and international organizations to halt the trend. The use of herbal drugs in the treatment of diarrhoea is a common practice in many African countries[2].

The World Health Organization has encouraged studies into traditional medical practice with the aim of improving treatment and prevention of diarrhoea diseases[3].The focus on plant research has increased all over the world in recent time, a large body of evidences has been collected to show immense potential of medicinal plants used in various traditional system[4]. Medicinal plants have been reported to be a promising source of anti-diarrhoeal agents[5].

The plant *Guiera senegalensis* J. F. Gmel is a member of the family Combrataceae[6]. It is a small shrub with green leaves. It is called "sabara" in Hausa and "kashishi" in Kanuri. The plant is widely distributed in Nigeria, Senegal, Gambia, Mali, Niger and Burkina Faso[7.8] stated that the macerated leaves of the plant were used orally for the treatment of febrifuge as well as for hyperglycaemia and hypertension whereas the roots are used mainly as antileprotic agents.[9] claimed that the plant is used by Fulani traditional healers to treat several disorders including veneral diseases. The root concoction is used to cure diarrhoea, dysentery and microbial infections. The plant continues to be one of the plants used by local livestock farmers, traditional healers and Fulani herdsmen in the treatment of snake bite in northern Nigeria[10]. Phytomedicine derived from plants have shown great promise in the treatment of intractable infectious disease including opportunistic acquired immune deficiency syndrome (AIDS) infections[11]. About 80 % of the rural population in developing countries, Nigeria in particular depends on it as an alternative to primary health care. This represents a potential pharmaceutical market and is an incentive for research into new drugs. Thus, in this study, the root of *Guiera senegalensis* partitioned in methanol was tested for its anti-diarrhoeal potentials.

MATERIALS AND METHODS

The plant material (root) of *G. senegalensis* was collected in Jere Local Government Area of Borno State, Nigeria. It was identified and authenticated by a plant taxonomist from the Department of Biological Sciences, University of Maiduguri. A voucher specimen with number BCH GRI was deposited at the herbarium of the Biochemistry Department, University of Maiduguri, Nigeria. Fresh root of *G. senegalensis* was dried in the open air and ground to powder form and kept in cellophane bag at 4°C before extraction.

Plant Extraction

A 200 g portion of the weighed, powdered dried root sample was partitioned using methanol. The sample was put into 1 litre separating funnel. This was covered, shaken every 30 min for 6 h and then allowed to stand for about 48 h The solution was subsequently shaken and filtered using Whatman filter paper No.1. The filtrate was evaporated to dryness using a rotary evaporator at a temp. range of 40-45°c. The extract was then stored below ambient temperature.

Phytochemical Screening

Phytochemical analysis of the extract was carried out according to the methods of [12], [13].

Experimental Animals

White Wistar strain albino rats of both sexes weighing between 150–200g were acquired from the animal house of Department of Biochemistry, University of Maiduguri,

Nigeria. All animals were used after 1 week of acclimatization, they had free access to water and food. The experiments reported here comply with ethical procedures with investigated animals[14].

Acute Toxicity Test

The method described by[15] with a slight modification in the number of animals was used to determine the safety of the root extract of *G. senegalensis*. Apparently healthy albino rats of Wistar strain weighing between (150–200 g) were divided into groups of three in each cage. The extract was dissolved in normal saline and administered via the oral route. The first batch of rats consisting of three groups received (10, 100, and 1000 mg/kg) of the extract. Similarly, the second batch was given with (1600, 2900 and 5000 mg/kg). The general behaviour of rats was observed continuously for 1 h after the treatment and then intermittently for 4 h and thereafter over a period of 24 h. The rats were further observed for up to 14 days following treatment for any sign of toxicity and mortality.

Evaluation of Anti-diarrhoeal Activities

The anti-diarrhoeal effect of the methanol root extract of *G. senegalensis* was tested in normal healthy rats in three different set of experiments.

Effect of Methanol Root Extract of *G. senegalensis* on Castor Oil-induced Diarrhoea in Rats

Five groups of five rats were housed in separate cages having paper placed below for collection of faecal matter. Diarrhoea was induced by oral administration of castor oil 1ml/rat[16]. Group 1 rats which served as control were given normal saline (2 ml/kg) (negative control). The second group received loperamide (5 mg/kg) as standard anti-diarrhoeal drug and served as the (positive control) group. Groups 3–5 were given the test extract (200, 400 and 800 mg/kg body weight). 1 h after extract administration, castor oil was given (1ml). The number of both dry and wet faecal droppings was counted each hour for 6h and the paper was changed every hour after castor oil administration; absence of which was regarded as protection[17].

Effect of Methanol Root Extract of *G. senegalensis* on Small Intestinal Transit Time of Charcoal Meal in Rats

The effect of the extract on intestinal propulsion in rats was tested using the charcoal meal method of[18,16]. The rats were fasted for 18 h prior to the experiment but allowed free access to water. They were randomized and placed in five cages of five rats per cage. Group 1 was administered normal saline orally. Group 2 was given atropine sulphate 3 mg/kg intraperitoneally (i.p) which served as negative control and positive control groups respectively. Groups 3–5 were pretreated with 200, 400 and 800 mg/kg body weight with *G. senegalensis* root extract respectively. After 1 h, each rat was administered with 1m of charcoal meal (CM) (10% activated charcoal suspended in 5% gum Arabic) orally. The rats were humanely sacrificed 30 min later by cervical dislocation and bled and the small intestine was rapidly dissected out and placed on a clean surface. The small intestine was carefully inspected and the distance traversed or travelled by the CM from the pylorus to the ileacaecal junction was measured. The length of the whole small intestine was also measured. The distance traversed by the CM from the pylorus was expressed as a percentage of the distance from the pylorus to the ileocaecal junction.

$$\text{Intestinal propulsion} = \frac{\text{Distance moved by the suspended CM}}{\text{Whole length of small intestine}} \times 100$$

Effect of Methanol Root Extract of *G. senegalensis* on Castor Oil-induced Fluid Accumulation (Enteropooling) in Rats

This was determined according to the method of [19,20.] The rats were fasted for 24 h but allowed free access to water. The rats were randomized and placed into five cages of five rats each. Group 1 received normal saline 2 ml/kg orally served as a control, group 2 received atropine sulphate 3 mg/kg intraperitoneally and groups 3, 4 and 5 received the extract 200, 400 and 800 mg/kg body weight orally respectively 1 h before the oral administration of castor oil. Two hours later the rats were sacrificed, the small intestine was removed after tying the ends with thread and weighed. The intestinal contents were collected by milking into a graduated tube and their volume was measured. The intestine was reweighed and the difference between full and empty intestines was calculated.

Statistical Analysis

The results were expressed as means ± S.D. Differences between means were analyzed using one-way analysis of variance (ANOVA) and Tukey Kramer Comparison Test using INstat statistical software. Values of ($P<0.05$) were considered statistically significant.

RESULTS

Phytochemical Screening

The phytochemical analysis of the extract of *G. senegalensis (*Table 1) revealed the presence of carbohydrate, reducing and combined sugars respectively, tannins, cardiac glycoside, terpenoids, saponins, flavonoids, cardenolides,ketose and alkaloids.

Acute Toxicity Test

The result indicated that the methanol/water root extract of *G. senegalensis* on acute treatment of the rats via the oral route at doses of 10, 100 and 1000 mg/kg and second dose levels of 1600, 2900 and 5000 mg/kg did not produce any sign of toxicity or death in rats during the 14 days of observation. Therefore, the LD_{50} could not be calculated and it is possibly higher than 5000 mg/kg body weight.

Effect of Methanol Root Extract of *G. senegalensis* on Castor Oil -Induced Diarrhoea in Rats

G. senegalensis methanol root extract significantly ($P<0.05$) inhibited the mean number of faeces when compared to the saline group by 7.00, 5.40 and 8.60 at the oral doses of 200, 400 and 800 mg/kg respectively relative to the saline group which had a mean number of faeces of 12.80±1.09 (Table 2). The rats that were given loperamide were completely protected from diarrhoea with a 100±0.00 % inhibition. The percentage inhibition of all the three doses were significantly different ($P<0.05$) from that of the control though not dose dependent (45.31±0.13, 57.81±0.55 and 32.81±0.58 % for all the doses of 200,400 and 800 mg/kg.

Table 1: Phytochemical Components of the Methanol Root Extract of *G. senegalensis*

S/No	Constituents / Test	
1	Carbohydrates i. General test (Mollisch's test)	MA +
	ii. Test for monosaccharide (Barfoed's test)	-
	iii. Reducing sugar (fehling's)	+
	Combined sugar (fehling's)	+
	v. Ketoses (saliwanoff's)	+
	vi. pentoses	-
	vii. Soluble starch	-
2.	Tannins	
	i. Ferric chloride	+
	ii. Lead acetate	+
3.	Phlobatannins	-
4.	Free anthraquinones (Borntrager's tests) Combined anthraquinones (Borntrager's tests)	- -
5.	Cardiac glycoside	
	i. Salkowski tests	+
	ii. Leiberman-Burchard	+
6.	Terpenoids	+
7.	Saponin glycoside i. Frothing test	 +
	ii. Fehling test	+
8.	Flavonoids	
	i. Shinoda's test	+
	ii. ferric chloride	+
	iii. sodium hydroxide	-
	iv. Lead acetate	+
9.	Cardenolides	
	i. Legal test	+
	ii. keller-killiani	+
10	Alkaloids	
	i. Dragendorff's	+
	ii. Mayer's reagent	+

Key: (+) present (-) absent
MA – methanol / water

Table 2: Effect of Methanol Root Extract of *G. senegalensis* on Castor Oil-induced Diarrhoea in Rats

Treatment control	Dose (mg/kg)	Total number of faeces	Percentage inhibition (%)
(Normal saline)	-	12.80±1.09	0.00
Extract 2	200	7.00 ± 1.22^{a}	45.31 ± 0.13^{a}
Extract 3	400	5.40 ± 0.54^{b}	57.81 ± 0.55^{b}
Extract 4	800	8.60 ± 1.67^{c}	32.81 ± 0.58^{c}
Control drug (Loperamide)	5	0.00^{d}	100±0.00

Values are means ± SD (n=5) Values with different superscripts alphabets are statistically significantly different ($p<0.05$) relative to normal control.

Table 3: Effect of Methanol Root Extract of *G. senegalensis* on Small Intestinal Transit of Charcoal Meal in Rats

Treatment	Dose (mg/kg)	Mean intestinal length (cm)	Mean distance traveled by charcoal meal(CM)	Percentage inhibition traveled by charcoal meal (%)
Normal control(1) normal saline	-	121.10±2.81	97.52±2.71	19.47±0.03
Extract 2	200	104.84±4.11	75.10 ± 3.50^{a}	28.37 ± 0.14^{a}
Extract 3	400	115.7±3.12	73.64 ± 2.89^{b}	36.35 ± 0.07^{b}
Extract 4	800	104.38±3.81	66.38 ± 2.21^{c}	36.41 ± 0.41^{c}
Control drug (atropine sulphate)	3	112.36±2.70	19.56 ± 1.56^{d}	82.59 ± 0.42^{d}

Values are means ± SD of five replicates. Values with different superscripts (alphabets) are statistically significantly different ($p<0.05$) relative to normal control.

Table 4: Effect of Methanol Water Root Extract of *G. senegalensis* on Castor Oil-induced Intestinal Fluid Accumulation in Rats

Treatment	Dose (mg/kg)	Mean weight of intestinal content (g)	Mean volume of intestinal fluid (ml)	Percentage inhibition of intestinal fluid (%)
Normal control Normal (1) saline	-	1.58±0.41	1.78±0.04	0.00±0.00
Extract 2	200	1.53 ± 0.15^{a}	1.56 ± 0.15^{a}	12.36
Extract 3	400	1.34 ± 0.04^{a}	1.34 ± 0.05^{b}	24.16
Extract 4	800	1.06 ± 0.06^{b}	1.06 ± 0.05^{c}	40.45
Control drug (atropine sulphate)	3	0.21 ± 0.10^{c}	0.22 ± 0.10^{d}	87.64

Values are means ± SD of five replicates. Values with different superscripts ($p<0.05$) (alphabets) are statistically significantly different relative to normal control.

Effect of Methanol Root Extract of *G. senegalensis* on Small Intestinal Transit of Charcoal Meal in Rats

G. senegalensis extract significantly (P<0.05) decreased the distance travelled by the charcoal meal and consequently the percentage inhibition of intestinal transit in a dose-dependent manner (Table 3). The three doses of the extract (200, 400 and 800 mg/kg) produced 28.37±0.14, 36.35±0.07 and 36.41±0.41 % intestinal transit induced by castor oil respectively. The mean distance travelled by the charcoal meal was 75.10±3.50, 73.64±2.89 and 66.38±2.21 cm respectively for the three doses. The mean distance travelled by charcoal mean in the atropine treated rats was 19.56±1.56 cm and the percentage inhibition was 82.59±0.42 which was significant (P<0.05) when compared to the control and extract treated rats.

Effect of Methanol Root Extract of *G. senegalensis* on Castor Oil-Induced Intestinal Fluid Accumulation in Rats

G. senegalensis methanol / water extract (200, 400 and 800 mg/kg body weight) reduced the intestinal fluid accumulation by 12.36, 24.16 and 40.45 % inhibition with a mean volume of intestinal fluid of 1.56±0.15, 1.34±0.05 and 1.06±0.05 ml respectively (Table 4). Both the mean volume of intestinal fluid and percentage inhibition were dose dependent and significantly different at (P<0.05) relative to the control rats which were given only normal saline with a mean volume of intestinal fluid of 1.78±0.04 ml without any inhibition. The atropine sulphate treated rats had a mean volume of intestinal fluid of 0.22±0.10 ml and a percentage inhibition of 87.64.

DISCUSSION

The root extract of *G. senegalensis* did not show any toxic effects because doses 5000 mg/kg did not cause any death or alter the behaviour of normal animals. According to[15] any substance that is not toxic at 5000 mg/kg is considered relatively safe. The plant extract was therefore considered to be safe at doses ≤5000 mg/kg.

Phytochemical analysis of the extract of *G. senegalensis* revealed the presence of carbohydrates, reducing and combined sugars, tannins, cardiac glycosides, terpenoids, saponins, flavonoids,cardenolides and alkaloids. [21] reported the presence of alkaloids, tannins, flavonoids, anthracene derivatives and sterols and triterpenes in different parts of *G. senegalensis* i.e. leaves, fruits, root and stem. Alkaloids, cardiac glycosides, coumarines, saponins and tannins have also been reported by[22] to be present in the root of *G. senegalensis*. The bioactive compounds in the root extract of *G. senegalensis* were responsible for the antidiarrhoeal effects recorded in the extract. Flavonoids and sugars obtained from selected traditional medicinal plants in Bangladesh and some parts of the world were reported by[23,24] respectively and were shown to exhibit antidiarrhoeal properties. Flavonoids have been shown to attenuate contraction of guinea pig ileum induced by some spasmogens[25] and inhibit small intestinal transit [26].

Diarrhoea is the frequent passage of liquid faeces and it involves both an increase in the motility of the gastrointestinal tract, along with increased secretion and decreased absorption of fluid and thus loss of electrolytes (particularly water and sodium)[27]. Hence to restore personal comfort and convenience, many patients require anti-diarrhoeal therapy and treatment is carried out to achieve, among other objectives, increased resistance to flow (segmental contraction, decreased propulsion and persistalsis) and increased mucosal absorption or decreased secretion[28].

In this study, the investigation of the anti-diarrhoeal effects of the methanol root extract of *G. senegalensis* was evaluated using various methods, which included castor oil-induced diarrhoea, intestinal transit time and intestinal fluid accumulation. The results of the present study showed that the extract of the root of *G. senegalensis* produced a statistically significant (p<0.05) reduction in the frequency of diarrhoea produced by castor oil. It was also noted that the extract significantly inhibited (p<0.05) castor oil-induced intestinal fluid accumulation and transit of charcoal meal.

The methanol root extract of *G. senegalensis* also exhibited a significant inhibition of castor oil-induced diarrhea, though not dose-dependent. The highest dose of 800 mg/kg exhibited a lower reduction of 32.81±0.58 % when compared to the other two doses of 200 and 400 mg/kg which gave 45.31 and 57.89 % inhibition respectively. The loperamide (5 mg/kg) treated group gave a 100±0.00 % inhibition.

The extract also slowed down the propulsion of charcoal meal through the GIT dose-dependently. The atropine-treated group gave 82.59±0.42 % inhibition. There was also a marked reduction in the weight and volume of intestinal contents which was dose-dependent.

The anti-diarrhoeal activity exhibited by the methanol extract could be due to the presence or solubility of most of the bioactive compounds in higher amounts or concentrations.

Castor oil (a prodrug) is reported to induce diarrhoea by increasing the volume of intestinal content by prevention of the re-absorption of water. This property of castor oil is due to its active metabolite, ricinoleic acid which is an irritant[29,30.]

The liberation of ricinoleic acid results in irritation of the intestinal mucosa, leading to release of prostaglandins which results in stimulation of secretion [31] thereby preventing the re-absorption of NaCl and Water [32.]

Probably the extract increased the re-absorption of water by decreasing intestinal motility observed by the decrease in intestinal transit of charcoal meal. The delay in faecal emptying by the extract allowed more time for fluid absorption and subsequently reduced fluid loss in the stool. The anti-diarrhoeal activities of the extract may also be due to the presence of denature proteins forming protein tannates, which make the intestinal mucosa more resistant and reduce secretion [33.] Secrertory diarrhoea is associated with an activation of Cl-channels causing Cl-efflux from the cell, the efflux of Cl-results in massive secretion of water into the intestinal lumen and profuse watery diarrhoea[34].

The extract might inhibit the secretion of water into the lumen by reverting this mechanism.

Earlier studies have shown that anti-dysentric and anti-diarrhoeal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterol and / or triterpenes and reducing sugars[35,33.] The phytochemical screening of the extract revealed the presence of tannins, saponins, flavonoids and alkaloids. Thus, tannins, saponins, alkaloids and flavonoids may be responsible for the mechanism of action of the methanol root extract of *G. senegalensis* anti-diarrhoeal activity.

Loperamide (a standard anti-diarrhoeal drug) is a synthetic opiate analogue developed specifically for use in diarrhoea. All opiate agonists have effects on intestinal smooth muscle. Loperamide regulates the gastrointestinal tract by inhibiting the propulsive motor activities, predominately in the jejunum, and this effect is partially inhibited by opiate antagonists. Other effects on intestinal motility may be mediated through inhibition of prostaglandin stimulation of gut motility and / or through calcium antagonist actions[36.] Apart from regulating

the gastrointestinal tract, loperamide is also reported to reduce colonic flow, and consequently increase colonic water absorption, but it does not have any effect on colonic motility[37].

Atropine produced a significant reduction in both the intestinal fluid accumulation and transit time, possibly due to its anticholinergic effect[38].

Castor oil is a suitable model of diarrhoea in rats, since it allows the observation of measurable changes in the number of stools, enteropooling (fluid accumulation) and intestinal transit[39]. The extract exhibited a marked reduction in the number of diarrhoeal stool and the reduction in the weight and volume of the intestinal contents, as well as a marked reduction in intestinal transit. This signifies the usefulness of this model and the clinical effect of the extract.

CONCLUSION

The results of this study revealed that the methanol/ extract of *G. senegalensis* root possess anti-diarrhoeal activity. This is due to its inhibitory effect on castor oil-induced diarrhoea, gastrointestinal propulsion and fluid accumulation. This property establishes the use of *G. senegalensis* as a traditional anti-diarrhoeal medicine.

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