Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2012, 4(10):4604-4612

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Phytochemical and anti-diarrhoeal properties of ethyl acetate root extract of *Guiera Senegalensis* J. F. Gmelin via oral route

[1]Shettima AY, [1]Karumi Y, [2]Tijjani MA,[1] Sodipo OA, [3]Idris M.

Shettima Abba Yagana, Department of Biochemistry, Faculty of Science, University of Maiduguri, Nigeria
[1]Department of Biochemistry, Faculty of Science, University of Maiduguri – Nigeria,
[2]Department of Chemistry, Faculty of Science, University of Maiduguri – Nigeria,
[3]College of Agriculture, Gujuba, Yobe State-Nigeria.

ABSTRACT

The study investigated the anti-diarrhoeal properties and phytochemical constituents of the ethyl acetate root extract of Guiera senegalensis. The phytochemical components of the extract were evaluated by qualitative test. White Wister strain albino rats weighing between 150-200 g were used to investigate the acute toxicity and the anti-diarrhoeal activity of the ethyl acetate root extract by using the castor oil-induced diarrhoea method. The effect of ethyl acetate extract on contraction of isolated rabbit jejunum and responses of the tissue to acetylcholine were also investigated in vitro. The extract was found to be safe at the doses tested. 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 ethyl acetate extract at a dose of 800 mg/kg inhibited significantly ($p<0.05$) the castor oil-induced diarrhoea by 75.55%. The extract also attenuated the spontaneous contraction of the isolated rabbit jejunum concentration-dependently. It can be concluded that, ethyl acetate extract possess anti-diarrhoeal properties. The possible mechanism of action could be by attenuating the spontaneous contractions of the intestine, thus the study provided a scientific basis for the use of G. senegalensis root extract in the treatment of diarrhoea.

Key words: *G. senegalensis,* ethyl acetate, root extract, anti-diarrhoeal effect, acute toxicity and phytochemical constituents.

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 (WHO) 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 ethyl acetate was tested for its anti-diarrhoeal potentials.

EXPERIMENTAL SECTION

The plant material (root) of *G. senegalensis* was collected in Jere Local Government Area, 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 using a blender and kept in cellophane bag at 4^{o}C before extraction.

Plant Extraction

A 2000 g portion of the weighed, powdered dried root sample was partitioned using ethyl acetate. 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^{0}c[12]. The extract was then stored below ambient temperature.

Phytochemical Screening

Phytochemical analyses of the extract were carried out according to the methods [13] and [14].

Experimental Animals

White Wistar strain albino rats of both sexes weighing between 150–200 g 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 ethnical procedures with investigated animals [15].

Acute Toxicity Test

The method described by [16] with a slight modification in the number of animals was used to determine the safety of the root extract of *G. senegalensis* obtained. Apparently healthy adult albino rats of Wistar strains weighing between (150–200 g) were divided into groups of three into six cages. The extract was dissolved in normal saline and administered via the oral route after the rats were fasted. 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 (1600, 2900 and 5000 mg/kg). The general behaviour of rats was observed continuously for 1h after the treatment and then intermittently for 4h and thereafter over a period of 24h. The rats were further observed for up to 14 days following treatment for any sign of toxicity and mortality.

Evaluation of Anti-diarrhoeal Activities of Ethyl acetate Root Extract of *G. senegalensis in Rats*

The anti-diarrhoeal effect of the extract of root of *G. senegalensis* i.e. the ethyl acetate extract was tested for its antidiarrhoeal effects or activities in normal healthy rats in three different set of experiments.

Effect of Ethyl acetate Root Extract of *G. senegalensis* on Castor Oil-induced Diarrhoea

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 [17]. 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 6 h and the paper was changed every hour after castor oil administration; absence of which was regarded as protection [18].

Effect of Ethyl acetate 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 [19] and [17]. 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 1 ml 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.

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

[17]

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

This was determined according to the method of [20] and [21]. 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.

Effect of Ethyl acetate Root Extract of *G. senegalensis* on Isolated Rabbit Jejunum

A rabbit was fasted for 12 h before the experiment and thereafter it was sacrificed by a blow on the head and exsanguinated. Segments of the jejunum, about 2 cm long were cut. The remaining intestinal contents were removed by flushing using Tyrode's solution of the following composition (mM): [NaCl (136.8), KCl (2.7), $CaCl_2$, (1.3), $NaHCO_3$ (11.9), $MgCl_2$ (0.5), Na_2PO_4 (0.45) and glucose (5.5) at a temperature of 37^oC ($\pm 1^oC$) and aerated with air (95 % O2 and $5CO_2$). The jejunum was suspended in a 25 ml organ bath containing Tyrode's solution. A load of 0.5 g was applied, 1h equilibration time was allowed during which the physiological solution was changed every 15 min. Changes in the tension were recorded with an Ugo Basile microdynometer with isotonic transducer [22].

The responses of the tissue to serial concentrations of acetylcholine 0.2, 0.4, 0.6, 0.8 and 1 mg/ml and extract 0.2, 0.4, 0.6, 0.8 and 1 mg/ml were recorded. Furthermore a fixed concentration of acetylcholine 0.2 mg/ml was interacted with varying concentrations of the extract (0.2, 0.4, 0.6, 0.8 and 1 mg/ml). Each concentration tested was allowed a contact time of 1min followed by washing three times. A resting period of 15 min was allowed before the next addition.

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 and alkaloids.

Acute Toxicity Test

Results indicated that the ethyl acetate 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 Ethyl acetate Root Extract of *G. senegalensis* on Castor Oil-induced Diarrhoea in Rats

The ethyl acetate extract was found to be effective against castor oil-induced diarrhoea on experimental rats at various doses of 200, 400 and 800 mg/kg body weight shown in Table 2. All the doses significantly decreased ($P<0.05$) the total number of faeces produced by administration of castor oil (5.40 ±0.89 at the dose of 200 mg/kg, 3.20 ±0.44 at the dose of 400 mg/kg and 2.20 ±0.44 at the dose of 800 mg/kg) as compared to the castor oil-treated control group (9.0±1.22). The percentage inhibition of castor oil-induced diarrhoea in the extract treated rats was 40.00 ±0.33, 64.44±0.78 and 75.55 ±0.98 % respectively, at 200, 400 and 800 mg/kg dose of extract. The decrease in the number of faeces and the percentage inhibition was dose dependent. The extract at the doses of 400 and 800 mg/kg produced a marked anti-diarrhoeal effect in the rats. The rats that did not receive the extract, showed typical diarrhoea signs such as watery and frequent defecation. The rats that were given the standard anti-diarrhoeal drug loperamide (5 mg/kg) produced an inhibition of 100 % without any sign of diarrhoea and even the normal faeces.

Effect of Ethyl acetate Root Extract of *G. senegalensis* on Intestinal Propulsion [small intestinal transit of charcoal meal (CM)] in Rats

The administration of the ethyl acetate extract also slowed down the propulsion of charcoal meal through the gastro-intestinal tract when compared to the castor oil-treated rats (Table 3). The mean distance travelled by charcoal meal in the extract treated rats (200, 400 and 800 mg/kg) and castor oil-treated rats was 59.76±4.22, 55.84±4.62, 71.28±3.82 and 64.1±1.82 cm, respectively. Atropine on its part produced a marked decrease in the propulsive movement and the intestinal length travelled by charcoal meal was 36.88±6.53 cm. The percentage inhibition of the extract treated rats was 42.98±0.69, 39.89±0.28 and 19.24±0.57 and that of atropine treated rats was 63.76±0.04 as compared to the control group rats which was 29.91±0.83. The effect of the extract at 800 mg/kg (19.24±0.57 %) was lower than that at 200 and 400 mg/kg respectively. The percentage inhibitions were significant ($P<0.05$) and dose-dependent.

Effect of Ethyl acetate Root Extract of *G. senegalensis* on Castor Oil-induced Intestinal Fluid Accumulation in Rats

G. senegalensis ethyl acetate extract (200, 400 and 800 mg/kg) reduced the intestinal fluid accumulation (Table 4) 80.23, 88.70 and 76.84 % with a mean volume of intestinal fluid of 0.70, 0.40 and 0.8 ml respectively relative to the control the percentage inhibition of the extract at 800 mg/kg is lower than that of 200 and 400 mg/kg dose.

Atropine sulphate produced 100±0.00 % inhibition of the intestinal fluid with 0.00 mean volume of intestinal fluid. The inhibition produced by the extract treated rats are significantly different ($P<0.05$) relative to the control rats and the atropine-treated rats.

Effect of Ethyl acetate Root Extract of *G. senegalensis* on Isolated Rabbit Jejunum

The ethyl acetate root extract of *G. senegalensis*, at increasing concentrations inhibited spontaneous contractions of the isolated rabbit jejunum in a concentration-dependent fashion (Table 5). 0.2, 0.4, 0.6, 0.8 and 1 mg/ml reduced the amplitude in this way 0.5, 0.4, 0.4, 0.3, and 0.3, respectively (Table 1). Application of acetylcholine to the bathing medium of the isolated rabbit jejunum at increasing concentrations greatly increased the contractions of the tissue (Fig.2) .The extract did not affect the contractile action of acetylcholine

.

Acetylcholine acts through muscuranic subtype 2 (M_2) cholinergic receptors. It therefore suggests that the extract does not act on muscuranic cholinergic receptors of the rabbit jejunum. It probably works on the adrenergic receptors of the rabbit jejunum.

DISCUSSION

The root ethyl acetate root extract of *G. senegalensis* did not show any toxic effect because doses 5000 mg/kg did not cause any death or alter the behaviour of normal animals. According to [16], 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 ≤5000mg/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. [23] 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 [24] 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 [25]; [26], respectively and were shown to exhibit antidiarrhoeal properties. Flavonoids have been shown to attenuate contraction of guinea pig ileum induced by some spasmogens [27] and inhibit small intestinal transit [28].
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[29]. 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 [30].

In this study, the investigation of the anti-diarrhoeal effects of the ethyl acetate extract of the root 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 ethyl acetate root extract of *G. senegalensis* exhibited a significant inhibition of castor oil-induced diarrhoea in a dose-dependent manner. The highest dose of 800 mg/kg body weight inhibited the castor oil-induced diarrhoea by 75.55±0.98 % with a mean total number of faeces of 2.20±0.44, when compared with the loperamide (5 mg/kg) treated group that gave 100±0.00 % inhibition. The extract slowed down the propulsion of charcoal meal (CM) through the gastrointestinal tract though not dose-dependently. The highest dose of 800 mg/kg produced an inhibition of 19.24±0.57 % when compared to the other two doses of 200 and 400 mg/kg body weight which produced 42.98±0.69 % and 39.89±0.28 %. The control group treated with atropine-sulphate (3 mg/kg) inhibited the transit of charcoal meal by 63.76±0.04 %. There was a marked reduction in the weight and volume of intestinal contents though not in a dose-dependent manner. The highest dose of 800 mg/kg produced an inhibition of 76.84 % which was lower than that of 200 and 400 mg/kg body weight which gave 80.23 and 88.70 % inhibition respectively.

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

The ethyl acetate root extract of *G. senegalensis* concentration–dependently inhibited the spontaneous contractions of the isolated rabbit jejunum. The results demonstrated that the ethyl acetate extract of root of *G. senegalensis* induced a graded relaxation of the smooth muscle of the gastrointestinal tract, the severity of which depended on the concentration of the extract. According to [31], the property of reducing intestinal contractions is demonstrated by most anti-diarrhoeal agents and this helps in preventing excessive loss of fluid and ingesta. Acetylcholine acts through muscuranic subtype 2 (M_2) cholinergic receptors. It therefore suggests that the extract does not act on the muscuranic cholinergic receptor of the rabbit jejunum since it did not affect the contractile effect of acetylcholine on the rabbit jejunum. It probably works on the adrenergic receptors of the rabbit jejunum. 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 [32]; [33].

The liberation of ricinoleic acid results in irritation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion [34] thereby preventing the re-absorption of NaCl and Water [35].

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 activity 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 [36]. 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 [37].

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 [38];[39].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 *G. senegalensis* ethyl acetate root extract's 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 medicated through inhibition of prostaglandin stimulation of gut motility and / or through calcium antagonist actions [40]; [41]. 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 [42].

Atropine produced a significant reduction in both the intestinal fluid accumulation and transit time, possibly due to its anti-cholinergic effect [43].

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 [33].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.

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

S/No	Constituents / Test	Extract
1	Carbohydrates	**EA**
	i. General test (Mollisch's test)	+
	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	
	1. Dragendorff's	+
	ii. Mayer's reagent	+

Key: *(+) present (-) absent,* *EA – Ethyl acetate*

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

Treatment Control (1)	Dose (mg/kg)	Total number of faeces	Percentage inhibition (%)
(Normal saline)	-	9.0±1.22	0.00±0.00
Extract 2	200	5.40±0.89[a]	40.00±0.33[a]
Extract 3	400	3.20±0.44[b]	64.44±0.78[b]
Extract 4	800	2.20±0.44[c]	75.55±0.98[c]
Control drug (Loperamide)	5	0.00	100.00±0.00

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

Table 3: Effect of Ethyl acetate Root Extract of *G. senegalensis* on Intestinal Propulsion (transit time) of Charcoal Meal (CM) in Rats

Treatment	Dose (mg/kg)	Mean intestinal length (cm)	Mean distance traveled by (CM)	Percentage inhibition travelled by (CM) (%)
Control (normal saline)1	-	91.46±10.82	64.1±1.82	29.91±0.83
Extract 2	200	104.8±13.62	59.76±4.22[a]	42.98±0.69
Extract 3	400	92.9±3.59	55.84±4.62[b]	39.89±0.28
Extract 4	800	88.26±9.08	71.28±3.82	19.24±0.57
Control drug (atropine sulphate)	3	101.76±6.50	36.88±6.53	63.76±0.04

Values are mean ± SD of five replicates. Values with different alphabets superscript are statistically significantly different (P<0.05) relative to normal control.

Table 4: Effect of Ethyl acetate 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 value of Intestinal fluid (ml)	Percentage inhibition of intestinal fluid (%)
Control Normal(1) saline	-	4.04±0.07	3.54±0.24	0.00±0.00
Extract 2	200	0.73±0.07[a]	0.70±0.33[a]	80.23
Extract 3	400	0.64±0.01[b]	0.40±0.24[b]	88.70
Extract 4	800	0.94±0.09[c]	0.82±0.20[c]	76.84
Control drug (atropine sulphate)	3	0.13±0.04[d]	0.00[d]	100±0.00

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

Table 5: Mean Contractile Response of Isolated Rabbit Jejunum Exposed to Graded Concentrations of Ethyl acetate Root Extract of *G. senegalensis* and Acetylcholine

Dose mg/ml	Amplitude of contraction (cm)		
	Extract	Acetylcholine	Extract + 0.2mg/ml acetylcholine
0.2	0.5	0.9	1.2
0.4	0.4	1.1	1.35
0.6	0.4	1.0	1.5
0.8	0.3	1.0	1.6
1	0.3	1.0	1.6

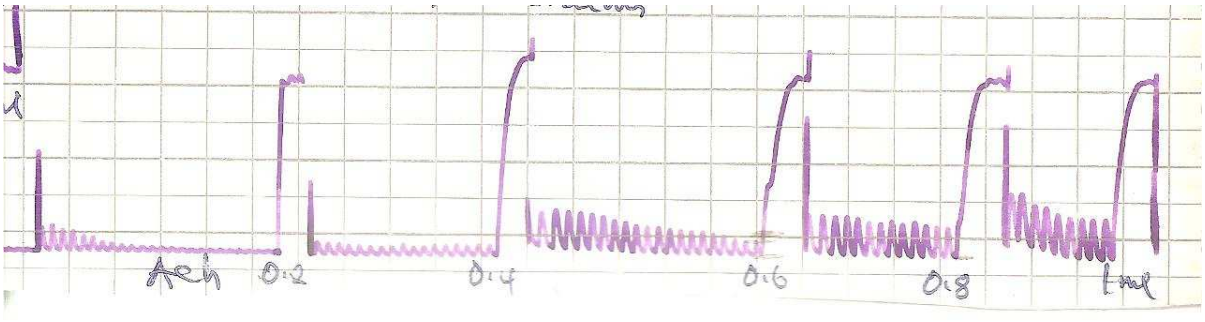

Fig. 1: Effect of varying concentrations (mg/ml) of acetylcholine (Ach) on contraction of isolated rabbit jejunum.

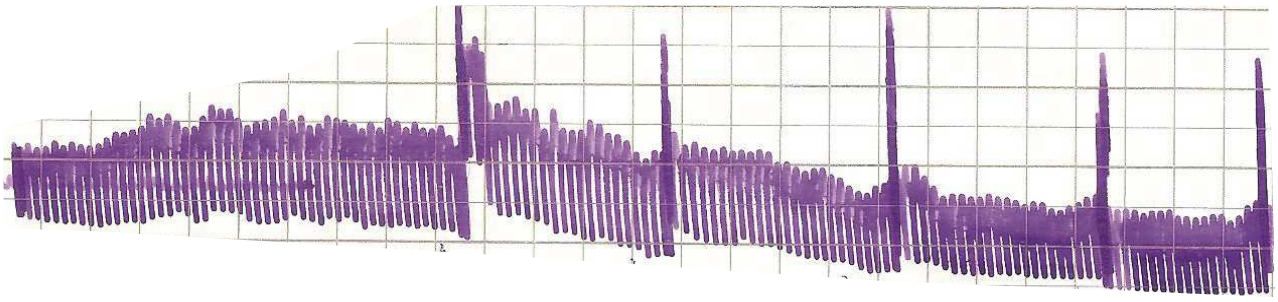

Fig. 2: Effect of varying concentrations (mg/ml) of extract (E) on spontaneous contraction of isolated rabbit jejunum.

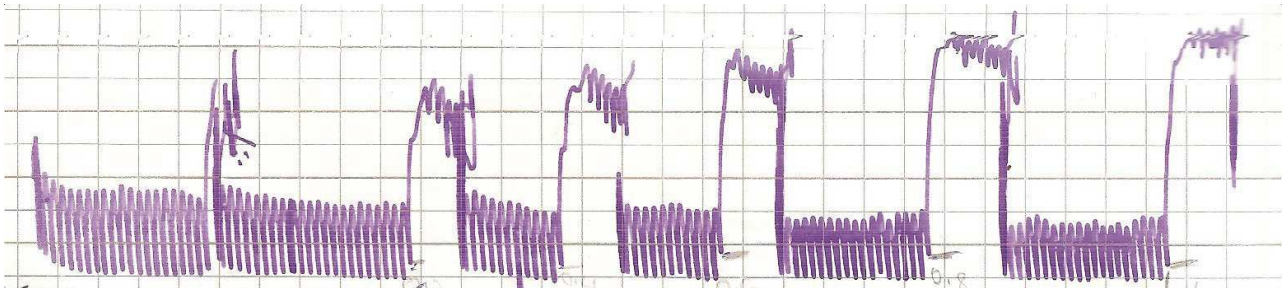

Fig. 3: Combined effects of varying concentrations of extract and acetylcholine (Ach) mg/ml on isolated rabbit jejunum.

CONCLUSION

The results of this study revealed that the ethyl acetate root 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 the root of *G. senegalensis* as a traditional folk anti-diarrhoeal medicine.

REFERENCES

[1] R Audu; SA Umilabu; RK Renner, JA Awodiji, *J. Nig. Infec. Control Association*., **2000**,(3)15,10-14.
[2] A Agunu; S Yusuf; GO Andrew; AU Zezi, EM Abdulrahman, *J. Ethnopharmacol*., **2005,** 101:27-30.
[3] AH Atta, SM Mouneir, *J. Ethnopharmacol*., **2004**, 92:303-309
[4] RA Dahanukar; RA Kulkarni, NN Rage, *Indian J. Pharmacol*, **2000**, 32: 581-583.
[5] OA Salawu; AY Tijjani; IC Obidike, BA Chindo, *J. Phytomed. Therap*, **2007,** 12, 44-50
[6] J Hutchinson, JM Dalziel, *Flora of West Tropical Africa* vol. 1, part1. Crown agents for oversea governments and administration, London, **1954**, 275.
[7] L Touzeau, Lesarbes foragers cledtazore Sahaliere le l, Afruque. (Dissetatim) Talouse: Eole, Nat. Vetenin, **1973,**125.
[8] G E B El-Gazali; M S El-Tohami, AAB El- Egami, *Medicinal Plants of the Sudan*, **1994,** 64-65.
[9] O Silva; S Barbosa, E Gomez, *Int. J. Pharmacol*, **1997,**12, 190-191.
[10] AB Sallau; GC Njoki; AB Olokisi; AU Wurochekke; AA Abdulkadir; S Isah; MS Abubakar, S Ibrahim,. *J. Med. Sci*, **2005** 5, 2880-2883.
[11] MW Iwu; AR Duncan, CO Ukunji, New antimicrobials of plant origin in: *Perspective on New Crops and New Uses*. (Janick. J. Editor) ASHS press, Alexandria, V.A. **1999**, 457-462.
[12] JB Harborne, Phytochemical Methods, A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, **1998**,279.
[13] GE Trease, WC Evans, *A Textbook of Pharmacognosy*, Bailliere Tindall, London, **2005,** 76-87.
[14] A Sofowora, *Medicinal Plants and Traditional Medicine in Africa*, John Wiley and Sons. Ltd. New York, **2008**, 78-80.
[15] National Institute of Health, Guidelines for handling laboratory animals, **1985,** 106-108.
[16] D Lorke, *Archives of Toxicology***, 1983,** 54: 275-287
[17] PA Nwafor, H Hamza, *J. Natural Remedies***, 2007,** 7: 71-79.
[18] MU Diumo; AA Izzo; B Mazzoni; A Bologynes, F Capasso, *J. Pharm. Pharmacol*. **1996,** 48: 760-762,
[19] N Mascolo; AA Izzo; G Autore; F Barboto, F Capasso, J .*Pharmacol. Exp. Therap*, **1994,** 268: 288-295.
[20] A Robert; JF Nezamis; C Lancaste; AJ Hanchar, MS Klepper, *Prostaglandins*, **1976,** 11:809-828.

[21] OG Dicarlo; N Mascolo; AA Izzo, F Capasso, *Phytother, Res***, 1994,** 8: 42-45
[22] FK Amos; P Gamaniel; P Akah, C Wambebe, *J. Ethanopharmacol*, **1998,** 60**:** 209 – 212.
[23] AS Aime; P Kirti; D Drissa; S Lassine; CC Jean; F Gilles; D Sylvie; T Yves, C Pierre, *J. Medicinal Plants Research*, **2011,** 5: 1639-165
[24] ET Williams; JT Barminas; J Akinniyi, AWilliam, *Afri. J. Pure Appl. Chem***, 2009,** 3(8): 152-157.
[25] MA Rahman, CC Wilcock, *Bangladesh J.Botany*, **1991,** 20: 175- 178.
[26] CA Palombo, *Phytother. Res*, **2005,** 20: 717-724.
[27] PJ Macander, *Progress in Clinic. Biol. Res*, **1986,**213: 489- 492.
[28] S Viswanathan; SP Thirugnana, JS Bapha, *Archives Internationals Pharmacodynamie et de Therapic***, 1984,** 270: 151- 157.
[29] HP Rang; MM Dale; JM Ritter, K Moore, *The Gastro-intestinal Tract Pharmacology*, Churhill, Livingstones. Edinburgh, **2003,** 367-379.
[30] AJ Akindele, OO Adeyemi, *J. Ethnopharmacol*, **2006,** 108: 20-25.
[31] HV Ammon; PJ Thomas, S Philips, *Clinic. Investigation*, **1974,** 53:374-379
[32] WC Watson, R Gordon, *Biochem. Pharmacol***, 1962,** 11: 229-236.
[33] NF Pierce; CJ Carperter; HZ Elliot, WB Greenough, *Gastroenterol***, 1971,** 60:23-32.
[34] KD Tripathi, *Essentials of Medicinal Pharmacology*. Jaypee Brothers Medical Publishers New Delhi, **1994,** 775.
[35] HV Ammon, KH Soergel, *Diarrhoea in Berk JE Gastroenterology*, Philadelphia, Saunders, **1985,** 125-14.
[36] A Galvez; B M E Zarzuelo; MD Crespo; M Lorente, J Jimenez, *Planta Medica*, **1993,** 59: 333- 336.
[37] OA Longanga; A Vercruysse, A Foriers, *J. Ethnopharmacol*, **2000** 71: 411- 423.
[38] W.H.O. The Rationale Use of Drugs in the Management of Acute Diarrhoea in Children, **1990,** 17
[39] BG Katzung, *Basic and Clinical Pharmacology*. Boston McGraw Hill, **2004,** 50.
[40] V Theoderau; J Fioramont; T Hachet, I Bueno, *Gut*, **1991,** 32: 1355-1359.
[41] JH Brown, P Taylor, Muscarinic receptor agonists and antagonist, in: Hard man, JG, Limbird LE, Goodman and Gilman. *The pharmacological Basis of therapeutics*. McGraw Hill, New York, **1996,** 20