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Phytochemistry and Effects of Ethanol Leaf Extract of *Meytenus senengalensis* (Lam) on Castor-oil Induced Diarrhoea in Albino Rats

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Abstract
Meytenus senengalensis (Lam). Ethanol leaf extract was investigated for its phytochemical contents as well as anti-diarrhoea effects. The ethanol leaf extract subjected to qualitative phytochemical screening. Graded doses of the extract (200, 400 and 800 mg/kg) were administered orally to the three groups of rats (n = 5) before induction of diarrhoea with castor oil. Another two groups of animals were treated with normal saline (control) and loperamide, a conventional anti-diarrhoea drug respectively. Gastro-intestinal transit of charcoal meal and gastro-intestinal enteropooling with the same graded doses of the ethanol leaf extract were used. The extract produced a significant inhibition of the castor-oil induced diarrhoea. The gastro-intestinal transit of charcoal meal was also reduced by the various graded doses of the extract used in this study. The phytochemical analysis of the ethanol leaf extract revealed the presence of secondary metabolites such as cardenolides, cardiac glycosides, flavonoids, saponins, tannins and terpenoids. The findings suggest that, the ethanol leaf extract of *M. senengalensis* possesses antidiarrhoeal effect, which could be related to inhibition of gastro-intestinal motility and secretion.

Key words: Phytochemical screening; *Meytenus senengalensis*; Anti-diarrhoea; Albino Rats.

Introduction

Diarrhoea is ranked high among some of the serious health problems claiming lives of children and immune compromised patients in the developing countries accounting for more than 5 million deaths worldwide each year in infants and children of less than 5 years. (Shoba and Thomas 2001). In order to reduce the impact of diarrhoea in developing countries, international organizations such as WHO have encouraged the research and use of traditional remedies of proven efficacy in remote communities as a tentative approach before accessing standardized medical treatment (Atta and Mouneir 2004).

Since time immemorial, humans have been using such plants for the management of various conditions. Many of such plants have well been scientifically proven to be effective through experimental bioassay and the active principle have been isolated and developed into drugs used in conventional orthodox medicine. (Shoba and Thomas 2001).

Maytenus senegalensis Lam. Exell.; locally called *'Bokaroro', 'Kunkushewa' or Namijin tsada',* in Hausa; *Tultulde or 'Yare-lesdi'* in Fulani; *'Afor-juru'* in Igbo and *'Sepolohun'* in Yoruba languages belongs to the family Celestraceae. It is a tall shrub with young branches often spiny, bearing leaves and flowers. The leaves appear grey in colouration. The plant flowers in October and found widely distributed in Tropical and Subtropical Africa (Burkill, 1995). The decoction of the root bark of *Maytenus senegalensis* is widely used in Sudan and other African countries in the traditional medicine to treat malaria (Khalid *et al.*, 2007).

Therefore, the paucity of scientific basis for the use of ethanol extract *M. senengalensis* as anti-diarrheal remedy in traditional medicine is yet to be investigated. In furtherance of the search for potent medicinal agents from plant sources, the anti-diarrheal properties of the ethanol extract of *M. senengalensis* in rats was evaluated.

The aim of this work therefore to investigate the anti-diarrheal properties of the ethanol extract of *M. senengalensis*

Materials and Methods

Sample Collection and extraction

Fresh samples of the leaves of *Meytenus senengalensis* were collected from Bauchi town in Nigeria. The plant was identified and authenticated at the Department of Biological sciences, University of Maiduguri, Nigeria where a voucher specimen No Chem/17/003 was deposited at the Herbarium of the Department of Chemistry University of Maiduguri. The leaves were cleaned, air dried under shade to a constant weight then pulverized to fine powder 500 g of the powder was exhaustively extracted using soxhlet extractor with 85% ethanol as solvent. The crude ethanol extract was concentrated to dryness at 50 ^{0}C using an oven (DHG-9030A) Gallenkamp England. The extract was secured in air-tight container until required.

Experimental Animals

Seventy-five (76) adult albino rats of both sexes weighing 100 and 185 g were obtained from the Department of Veterinary Anatomy, University of Maiduguri for this study. They were kept in a well-ventilated plastic cages in the Department of Veterinary Physiology Laboratory, Faculty of Veterinary Medicine University of Maiduguri for 10 days to acclimatize to the Laboratory condition before the commencement of the experiment. The rats were feed with Vital Feed Grower's mash (Grand Cereal Nig. Ltd. Jos, Plateau State Nigeria) and tap water provided *ad libitum.* All animals were handled according to the international guiding principle for Biomedical Research Involving Animals (CIOMS, 1985) as adopted by the ethical committee of the Faculty of Veterinary Medicine, University of Maiduguri, Nigeria.

Phytochemical Screening

Phytochemical screening of the extract was carried out according to the method of Trease and Evans (2002).

Acute Toxicity Study

The up and down procedure (Dixon, 1991) was used to evaluate the oral acute toxicity of the ethanol leave extract of *M. senengalensis.* Five rats weighing 100–185 g were randomly selected and used for the experiment. They were housed individually in cage for 10 days prior to treatment to allow for acclimatization to the laboratory condition. The rats were fasted overnight but allowed free access to water. Freshly prepared ethanol extract was orally administered at a limit dose of 5,000 mg/kg. One rat was dosed and observed for 48 hours for sign of toxicity or death. Since the rats survived, the same procedure was adopted until all the rats were treated at the same limit dose of 5,000 mg/kg and observed for 48 hours.

Effect of Ethanolic Leaf Extract on Castor-Oil Induced Diarrhoea

The method of Williamson *et al*. (1996) was used to evaluate the effect of ethanol leaf extract on castor-oil induced diarrhoea. Twenty-five (25) Wistar strain albino rats of both sexes weighing between 160 – 185 g were used for this experiment. The rats were denied feed for 12 hours but were provided with water. They were divided at random into five groups of 5 rats each. Group A was given 2 ml distilled water orally. Groups B, C and D were dosed orally with 200, 400 and 800 mg/kg of the extract respectively. Group E was given 5 mg/kg Loperamide (RPG Life Science Ltd, Anleshwar) interperitoneally as the standard drug. The rats were separated and caged singly with white blotting paper. After one hour, each rat was given 1ml of castor oil (®Bellson and Co. Ltd, England), orally and observed for 6 hours for wet or dry faeces. The wet faeces of each rat were counted and recorded at the end of the experiment. The percentage protection was calculated using the formula: (Nwafor and Okwuasaba, 2001).

Effect of Ethanol Leaf Extract on Castor-Oil Induced Enteropooling

The method of Williamson *et al*. (1996) was used to evaluate the effect of ethanol extract on intraluminal fluid accumulation in rats. Twenty-five (25) albino rats weighing between 150 and 170 g were used in this experiment. The rats were fasted overnight and then divided into five groups of five rats each. Group A serves as control and was given 2 ml normal saline orally. Group B, C and D were given 200, 400 and 800 mg/kg of the extract orally respectively. Group E was treated with 3 mg/kg atropine sulphate interperitoneally to serve as control. After one hour, each rat was administered 1 ml of castor oil. One hour after the castor oil treatment, the rats were humanely sacrificed and the intestine removed and weighed. The content of the intestine was collected by milking and the weight of the empty intestine and the content were also measured and calculated.

$$\% \text{ protection} = \frac{\text{Mean defecation of control group} - \text{mean defecation of treated group}}{\text{Mean defecation of control group}}$$

Effect of the extract on Gastrointestinal Transit of Charcoal Meal in Rats

The method of Williamson *et al*. (1996) and Chitme et al, (2003) were used to evaluate the effect of ethanol leave extract on gastrointestinal transit of charcoal meal in rats. Twenty-five (25) albino rats weighing between 140 and 190 g were used in the experiment. The rats were denied feed for eighteen hours but allowed access to water. They were divided at random into five groups of five rats each. Group A serves as control and was given 2 ml of distilled water orally. Groups B, C and D were given 200, 400 and 800

mg/kg of the extract respectively. Group E was treated with 3 mg/kg atropine sulphate interperitoneally as the standard drug. After 10 minutes, I ml of charcoal meal (5% activated charcoal) [Kochlight Laboratories Ltd England] suspension in 10% aqueous solution of acacia powder was given orally to each rat. The rats were sacrificed after 30 minutes and the abdomen opened. The distance traveled by the charcoal meal was measured and expressed as the percentage of the total length of the intestine. The percentage intestinal transit of the charcoal meal was calculated using the formula:

$$\% \text{ intestinal transit} = \frac{\text{Movement of charcoal (cm)}}{\text{Total length of intestine (cm)}} \times 100$$

A reduction in the gastrointestinal propulsion of the charcoal meal as an antidiarrhoeal effect (Nwafor, 1998).

Ethical Statement

All animals were handled according to the international guiding principle for Biomedical Research Involving Animals (CIOMS, 1985).

Statistical Analyses

Results were presented as Mean ± SD and the differences among means were analysed using one-way analysis of variance (ANOVA) using computer software GraphPad Prism Version 5 (GraphPad Software, San Diego, California, USA). $P \leq 0.05$ was considered significant. Some results were also expressed in percent (%).

Results

Phytochemical screening

The qualitative phytochemical screening of the ethanol leave extract of *M. senengalensis* is shown in Table 1. The result revealed the presence of carbohydrates, cardenolides, cardiac glycosides, flavonoids, saponins, tannins and terpenoids.

Table 1: Phytochemical Constituents of Ethanol Leaf Extract of *Meytenus senengalensis*

S/No.	Phytochemicals Constituents	Test	Result
1.	**Alkaloids**	Dragendorff's	-
		Mayer's	-
2.	**Anthraquinones**		
	Combined Anthraquinones	Borntrager's	-
3.	**Carbohydrates**		
	General test	Molisch's	+
	Monosaccharide	Barfoed's	+
	Free reducing sugar	Fehling's	+
	Combined reducing sugar	Fehling's	+
	Ketoses	Salivanoff's	+
4.	**Cardenolides**	Keller-Kiliani's	+
		Legas	-
5.	**Cardiac glycosides**		
	Salkowski's	L-Buchard's	+
	Lieberman-Buchard's	L-Buchard's	+
6.	**Flavonoids**	Shinoda's	+
		Ferric chloride	+
		Lead acetate	+
		NaOH	-
7.	**Phlobatannins**		-
8.	**Saponins**	Frothing's	+
9.	**Soluble starch**		-
10.	**Tannins**	Ferric chloride	+
		Lead acetate	+
11.	**Terpenoids**		+

Key (+) = Present (-) = Absent

Acute toxicity study

The administration of ethanol leaf extract at a dose of 5,000 mg/kg orally to a group of rats did not cause any death. However, toxicity signs like depression and anorexia were noticed in the treated rats.

Effect of Ethanolic Leaf Extract on Castor-Oil Induced Diarrhoea in Rats

The effect of ethanolic leaf extract of *M. senengalensis* on castor oil induced diarrhoea in rats is presented in Table 2. The result showed percentage protection after oral administration of different doses of the extract was dose dependent and statistically significant (P<0.05) when compared with the control. Rats in group A (negative control group) were given normal saline orally and compared with the treated groups, which were given different doses (200 – 800 mg/kg) of the extract intraperitoneally i.p. The percentage protection conferred on the rats by the ethanolic extract was doses-dependent with 800 mg/kg producing 53.4 % protection. However, the standard drug (loperamide 5 mg/kg) gave the highest protection of 97.6 %, while this group treated with 200 mg/kg and 400 mg/kg concentration of the extract had percentage protection of 13.9 % and 32.4 %, respectively.

Table 2: The Effect of Ethanolic Leaf Extract of *Meytenus senengalensis* on Castor-Oil Induced Diarrhoea Rats

Group	Extract/Dose (mg/kg)	Mean total No. of faeces	Mean No. of Wet faeces in 6 hours	Percentage (%) Protection
A	Control (saline)	10.8 ± 0.84^{a}	2.6 ± 2.51^{a}	00
B	Extract (200 mg/kg)	8.0 ± 2.12^{b}	2.4 ± 0.55^{b}	13.9
C	Extract (400 mg/kg)	7.2 ± 1.79^{b}	1.8 ± 0.45^{b}	32.5
D	Extract (800 mg/kg)	5.4 ± 2.30^{b}	1.4 ± 0.55^{b}	53.4
E	Loperamide 5 mg/kg	0.2 ± 0.44^{b}	0.4 ± 0.55^{b}	97.6

Within column, mean with the sane superscript are statistically significant P<0.05 when compared with negative control group.

Effect of the Ethanol Leaf Extract on Castor-Oil Induced Enteropooling in Rats

The result shown Table 3. Percentage intestinal fluid accumulation after oral administration of castor oil at different doses of the extract. The percentage fluid accumulation in the group treated with 200, 400 and 800 mg/kg of the extract were 41.1 %, 36.2 % and 26.3 % respectively. The percentage intestinal fluid accumulation in the control group was 42.6%, while those treated with the standard drug was 20.9 % and were statistically not significant (P>0.05).

Table 3: The Effect of Ethanolic Leaf Extract of *Meytenus senengalensis* on castor oil induced enteropooling rats

Group	Extract/Dose (mg/kg)	Weight of intestine + content (g)	Weight of empty intestine (g)	Weight of content (g)	Percentage (%) of fluid accumulation
A	Control (saline)	4.32 ± 0.60^{a}	3.4 ± 0.46^{a}	1.45 ± 0.23^{a}	42.6
B	Extract (200 mg/kg)	4.93 ± 0.69^{a}	3.4 ± 0.40^{a}	1.40 ± 0.70^{a}	41.1
C	Extract (400 mg/kg)	4.52 ± 0.27^{a}	3.4 ± 0.55^{a}	1.25 ± 0.37^{b}	36.2
D	Extract (800 mg/kg)	4.94 ± 0.36^{a}	3.8 ± 0.53^{b}	1.00 ± 0.14^{b}	26.3
E	Atropine (3 mg/kg)	4.32 ± 0.62^{a}	4.3 ± 0.17^{b}	0.90 ± 0.15^{b}	20.9

Within column, mean with the same superscript are statistically significant P>0.05 when compared with control group.

Effect of Ethanol Leaf Extract on Gastrointestinal Transit of Charcoal Meal in Rats

The effect of the ethanol extract on intestinal transit of charcoal meal in rats is shown in Table 4. In the control group given saline, the charcoal meal travelled a distance of 65.6 ± 3.87 cm which represent 84.1 % of the total intestinal length. The administration of the extract at 200, 400 and 800 mg/kg body weight significantly reduced (P≤0.05) the distance travelled by the charcoal meal to 29.3 ± 7.46, 26.6 ± 3.83 and 21.3 ± 2.30 respectively when compared to the control. These respectively represent 31.5, 28.7 and 28.5 % of the total transit. The group treated with atropine exhibited the shortest transit of 21.3 ± 2.30 cm when compared to the control.

Discussion

The administration of ethanol leaves extract of *M. senengalensis* orally did not produced any death in the treated animals. The absence of mortality and or any serious toxicity is an indication of relative safety of the extract. According to OECD (2008), the LD_{50} of any extract given at a dose of 2000 or 5000 mg/kg using the up and down method of Dixon (1991) is higher than the limit dose if 3 or more of the rats out of 5 survived (within 14 days); such extract is therefore considered to be safe.

In this study, the effect of ethanol leaf extract of *Meytenus senengalensis* on diarrhea experimentally induced by castor oil in rats showed that it markedly reduced the frequency of defecation, number of diarrhea stools and wetness of the fecal

droppings. The castor oil-induced diarrhea model in rats allows the observation of measurable changes in the number of stools (Chitme et al., 2004). The remarkable reduction in frequency of defecation and number of diarrhoea stool may be as a result of the presence of pharmacologically active substances such as tannins in the extract which also has the ability to reduce water and electrolytes secretion into the small intestine which suggests that the extract may enhance electrolyte absorption from the intestinal lumen (Chitme et al., 2004). This is similar to the action of loperamide, the standard drug used in this study which is known to inhibit gastrointestinal secretion and motility. (Jafri & Pasricha).

The effect of ethanol leaf extract of *M. senengalensis* on enteropooling showed that the extract reduced both the weight and volume of intraluminal contents. These effects, which are direct consequences of reduced water and electrolytes secretion into the small intestine, suggest that the extract may promote inhibition of hyper-secretion. However, since electrolyte absorption determines the efficiency of nutrient absorption (Duggan *et al*., 2002), it is likely that the enhanced electrolyte absorption by the extract may have encouraged the absorption of other intestinal contents.

Table 4: The Effect of Ethanolic Leaf Extract of *Meytenus senengalensis* on Gastrointestinal Transit of Charcoal in Rats

Group	Extract/Dose (mg/kg)	Total length of intestine	Movement of charcoal (cm)	Percentage (%) intestinal transit
A	Control (saline)	78.0 ± 10.72[a]	65.6 ± 3.87 [a]	84.1
B	Extract (200 mg/kg)	93.0 ± 5.95 [a]	29.3 ± 2.46 [b]	31.5
C	Extract (400 mg/kg)	91.6 ± 2.91 [a]	26.6 ± 3.83 [b]	28.7
D	Extract (800 mg/kg)	81.6 ± 7.66 [a]	23.3 ± 1.58 [b]	28.5
E	Atropine (3 mg/kg)	78.6 ± 8.64 [a]	21.3 ± 2.30 [b]	27.0

Within column, mean with the same superscript are not statistically significant P>0.05 when compared with negative control group.

The effect of the extract on gastric motility causes significant ($p<0.05$) decrease in the intestinal transit time. Pre-treatment with the extract suppressed the propulsive movement or transit of charcoal meal through the gastrointestinal tract which clearly indicates that the leaf extract may be capable of reducing the frequency of stooling in diarrheal conditions and the effect of the extract in this respect is similar to that of atropine which was used as a standard drug in this work.

The ethanol extract also exhibited physiological active substances. These substances play active role in pharmacology and healing processes in diseases. Tannins which were detected in the extract are known to have andiarrheal effect that denature proteins in intestinal mucosa by forming tannates which make intestinal mucosa more resistant to chemical alteration and reduce secretion (Havagiray *et al.,* 2004). Saponins on the other hand exhibit their action by interfering with bacterial cell membrane leading to dehydration and cell death (Harborne, 1998; Reynold, 1998). Glycosides are known to have diuretic effect (Havagiray *et al.,* 2004).

In conclusion the results of this study showed that the ethanol leaf extract of *M. senengalensis* possesses antidiarrheal properties since it inhibited castor oil induced diarrhoea, decrease gastrointestinal transit of charcoal and slightly reduced fluid enteropooling in the gut. Therefore, this work has further proven the traditional use of the plant for the treatment of diarrhoea.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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