

## Evaluation of the Antidiarrhoea Activity of the Methanolic Extract of *Canna indica* Leaf (Cannaceae)

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### ABSTRACT

*Canna indica*, (Cannaceae) is widely cultivated as a garden plant. It's claimed to be used for cancer, malaria infection and diarrhoea amongst others. The study was designed to ascertain the anti-diarrhoea activity. The leaves were extracted with Soxhlet apparatus using methanol and screened for anti-diarrhoea activity. The screening was done using castor oil-induced diarrhoea, measure of charcoal meal transit and acetylcholine-induced contractions of the isolated rat ileum.

In the castor oil induced diarrhoea, loperamide (5 mg/kg) 50, 100 and 200 mg/kg of the extract were used and compared with a control (tween 80), while in the gastrointestinal transit, atropine (2.5 mg/kg), 100 and 200 mg/kg were used and also compared with a control (tween 80). In both 5 mice were used per group. A dose of 10 mg/ml of the extract was used against acetylcholine induced contractions in the isolated ileum experiments. The extract of *Canna indica* significantly ( $p < 0.050$ ) reduced both the castor oil induced diarrhoea and the charcoal plug transit time in a dose dependent manner. In the castor oil induced diarrhoea, the extract decreased the intraluminal fluid content in mice, with the highest reduction recorded at 200 mg/kg dose of the extract, though this was slightly better than that of loperamide. In the charcoal plug transit, both doses of the extract and atropine significantly ( $p < 0.05$ ) decreased the distance travelled by the charcoal plug in the intestine of the mice, with the 200 mg/kg producing an inhibitory effect higher than that of atropine. The effect of *C. indica* on the isolated rat ileum showed that the extract produced significant ( $p < 0.0001$ ) inhibitory effect on acetylcholine induced contraction. The methanolic extract of *C. indica* possesses anti diarrhoeal properties comparable to atropine and loperamide via reduction of fluid secretion, gastrointestinal motility and acetylcholine-induced contractions.

**Keywords:** *Canna indica*, transit time, diarrhoea, leaf extract.

### INTRODUCTION

Traditional medicine (TM) refers to the sum total of knowledge, skills and practices based on the theories of beliefs and experiences indigenous to different cultures (igbos, Hausa, Yoruba, etc.) used in the maintenance of health and the prevention, diagnosis, improvement or treatment of physical and mental illness<sup>1</sup>. Traditional medicine covers a wide variety of therapies and practices which vary from country to country and region to region. In some countries, it is referred to as alternative or complementary medicine (CAM). Medicinal plants play a key role in human health care and about 80 % of the world population relies on the use of traditional medicine which is predominantly based on plant materials<sup>1</sup>.

From time immemorial, plants have been serving as a source of drug to man and

animals. More than 80 % of modern drugs are obtained from plants. Herbal medicine has different constituents which are effective against diarrhoea, hypertension, diabetes etc. Of particular interest is diarrhoea. The use of herbal drugs in the treatment of diarrhoea is a common practice in Africa<sup>2</sup>. Diarrhoea has been recognised as one of the most important health problem in developing countries<sup>3</sup>. In Nigeria, diarrhoea remains the number one killer among children aged 1 -5 years. The disease accounts for 4-5 million deaths among humans annually<sup>4</sup>.

The plant, *Canna indica* is a native of the Caribbean and Tropical American. It belongs to the family: Cannaceae consisting of 19 species of flowering plants. It is locally called ebesalebo in edo, nkwa ebetri amongst the efik, bakalekale Hausa, aberekamwo in igbo land and iroro amongst the Yorubas. Its

ethnomedicinal uses includes; treatment of malaria in western Nigeria<sup>5</sup>, as a cure for diarrhoea and dysentery and in the treatment of fever, bruises and cut<sup>5</sup>.

The aim of this study is to ascertain its use in the treatment of diarrhoea and its possible mechanism of action.

## EXPERIMENTAL

### Drugs and Chemicals

Castor oil (Bells, England), Activated charcoal, Loperamide (Bells, England), Chloroform (BDH Chemicals), Methanol (BDH Chemicals).

### Collection and preparation of Plant material

The leaves were collected from the premises of Federal Government Girls' College, Benin City, Nigeria in May 2011. Botanical authentication and Identification was done by Mr Sunny Nweke of the department of Pharmacognosy, Faculty of Pharmacy, University of Benin.

The leaves of *Canna indica* were initially dried under mild shade and thereafter transferred to an oven for further drying at a temperature at 60°C. It was then powdered using an impact laboratory attrition mill (Christy and Morris Ltd. Process Engineers, Cheimsford England Model 475/54). The powdered sample was weighed and stored in a glass jar prior to extraction. The weight of the powdered sample was 490 g.

### Extraction

The powdered sample was subjected to successive soxhlet extraction with methanol. The 490 g dried powdered sample was successively extracted by rebatching the thimble of the soxhlet with 100 g, 100 g, 100 g, 100 g and 90 g respectively with a total of 1200mls of methanol. The extract obtained was concentrated with the aid of a water bath after which the percentage yield was calculated (w/w) using the formula below:

$$\frac{\text{Weight of material extracted}}{\text{Weight of plant material}} \times 100$$

### Animals

Wistar albino rats (105-166 g) and albino mice (25-40 g) of both sexes were used for the study. They were procured from the animal house, College of Medical Sciences, Ambrose Alli University, Ekpoma, Edo State. The animals were kept in cages with temperature varied from 30-40 °c and lighting hours varied between 12-14 hours. The animals were maintained under standard laboratory conditions, had free access to standard feed (Vital feeds R, Nigeria) and water. Animals

were acclimatized for at least 2 weeks before use and fasted over night with free access to water prior to experiments. Ethical conditions governing the conducts of experiments with life animals were strictly observed as stipulated by Zimmermann<sup>6</sup>.

### Ethical Approval

Ethical approval for the study was obtained from the Ethical Committee on the Use of Animals for Experiments, Faculty of Pharmacy, University of Benin in July, 2011.

### Phytochemical Tests

Phytochemical test were carried out on powdered leaves using standard phytochemical procedure<sup>7,8</sup>.

### Pharmacological Tests

#### Small intestinal charcoal plug transit

The effect of *C indica* on charcoal transit time was evaluated using the method of Mascolo<sup>9</sup> modified by Chidume<sup>10</sup>. 25 fasted mice were randomly selected, though they had access to water and placed in 5 groups of 5 each, labelled groups I-IV. Groups I through IV received tween solution (10 ml/kg), atropine (2.5 mg/kg), 100 and 200 mg/kg of *C indica* respectively via an oro-gastric tube. Twenty minutes after drug/extract administration, 0.5 mls of charcoal suspension was administered to each mouse orally via an oro-gastric tube. Thirty minutes later all mice were sacrificed by cervical dislocation, the abdomen opened and the total length of the small intestine removed and measured with a metre rule. The distance travelled by the charcoal plug from the pylorus to the caecum was also measured and expressed as a percentage of the total length of the small intestine. The percentage inhibition of movement was also calculated.

#### Castor oil induced diarrhoea

Intraluminal fluid accumulation was determined by the method of Parimata<sup>11</sup>. Five groups of overnight fasted 5 mice each were administered orally with the extract (50, 100, and 200mg/kg), loperamide (5mg/kg), positive control and tween solution (10 ml/kg) was given to the control group. Sixty minutes later, castor oil (0.3ml) was administered to each mouse orally with an oro-gastric tube. Each mouse was separately placed in a well ventilated cage, with the floor lined with white paper and observed for 4 hours. The parameters observed were the number and weight of wet stools.

### Isolated Tissue Experiment

The effect of *C indica* was also evaluated on acetylcholine-induced contractions. The rat ileum was used for this. The rats were sacrificed by cervical dislocation, the abdomen was quickly opened up and the ileum located. 1.5-2.0 cm of the ileum was removed about 15 cm proximal to the ileocolic valve. The contents were expelled by flushing with tyrode's solution. The ileum was suspended in an organ bath in tyrode solution with composition: sodium chloride 40g, sodium hydrogen carbonate 5g, D-glucose 5g, potassium chloride 1g, sodium hydrogen phosphate 0.25g, magnesium chloride 0.5g and calcium chloride 1.32g all in 5 litres. Baseline was set with 7N tension (0.7g weight) as the 0 tension i.e. calibration of the equipment. The speed was maintained at 5.0mm/min while the sensitivity was 6.0. The preparation was maintained at 37 °C and aerated with 95 % oxygen and 5 % carbon dioxide. The response of the isolated ileum was recorded on an Ugo Basile recorder via an isometric force transducer.

The tissue was allowed to equilibrate for 30 mins during which the spontaneous contraction of the tissue was observed. Thereafter a dose response curve for acetylcholine was constructed using 1 to 1000 µg/ml of acetylcholine with a contact time of 30 s and resting time of 1 min 30 s (2min. time cycle). The dose response curve for acetylcholine was then repeated in the presence of 10 mg/ml of the extract. The response by each drug administered was expressed as the percentage of the highest contraction produced by the highest concentration as shown below:

$$\text{Percentage response (\%)} = \frac{\text{Response}}{\text{Highest response}} \times 100$$

### Statistical analysis

Results are expressed as mean  $\pm$  standard error of mean (S.E.M). The results were analysed with Graph pad instat, version 2.05a, student t- test and considered significant when  $P < 0.05$ .

## RESULTS AND DISCUSSION

### Phytochemistry

Phytochemical screening revealed the presence of reducing sugars, phenolic compounds, tannin, saponins, flavanoids and anthracene derivatives. In this study phytochemical screening revealed the presence of tannins, saponins and flavonoids. Flavonoids are known for their anti-inflammatory, antidiarrhoeal and

antithrombotic effect. Previous works have also shown that the inhibitory effect on diarrhoea may be attributed to the presence of saponins and flavonoids<sup>12,13</sup>.

### Effect on small intestinal charcoal meal transit

In the control group, the charcoal meal transverse the longest distance of  $0.37 \pm 0.2\%$  of the total length of the intestine. Treatment with the doses of the C.I extract produced a decrease in the propulsive movement of the charcoal meal which was significant ( $p < 0.05$ ) at the doses of 100mg/kg and 200mg/kg as compared to the control. The decrease in the distance traversed by the charcoal marker was dose dependent. The effect of doses of C.I, 100 and 200 mg/kg were better than that of atropine a known muscarinic inhibitor (fig 1). The highest dose of the extract produced the greatest inhibitory effect of 87.65 % as opposed to 60 % produced by atropine. The antidiarrhoeal properties of *C indica* were studied using charcoal meal transit time and castor oil induced diarrhoeal in mice. These models are justified because according to Havagiray<sup>14</sup>, in some diarrhoea's the secretory component predominate while other diarrhoea's are characterized by hyper motility of gastrointestinal tract. Gastric emptying is the act of evacuation of the content in the stomach by normal peristaltic movement and a parameter strictly connected with the activity of smooth muscle of the stomach<sup>15</sup>.

From the result, *Canna indica* delayed the rate of gastric emptying into the duodenum in a dose dependent manner. The exact mechanism by which the extract delayed gastric emptying is not clear, but may probably be due to an inhibitory effect on muscarinic receptor. Studies have shown that activated charcoal readily adsorbs drugs and chemicals on the surface of the intestine thereby preventing absorption<sup>16</sup>, hence charcoal meal model was used to study the effect of *Canna indica* on peristaltic movement. The extract decreased the distance travelled by the charcoal plug. According to Bruton<sup>17</sup> the property of reducing intestinal contractions and consequently intestinal transit time was demonstrated by *C indica*. The reduction in transit time may be due to anticholinergic effect<sup>18</sup>.

### Effect on castor oil- induced diarrhoea

There was a significant ( $P = 0.05$ ,  $P < 0.0001$ ,  $P < 0.0001$ ) reduction in both weight and number of stool produced on treatment with 50, 100 and 200 mg/kg of the C. I extract as compared to the control (tween 80) respectively. This

inhibitory effect increased as dose was increased pointing to a dose dependent effect. The highest reduction in both the number and weight of stool was by the 200 mg/kg dose, where a reduction of the weight of stools from  $0.37 \pm 0.2$  to  $0.21 \pm 0.05$  g and no of stools from  $12.4 \pm 1.76$  to  $6.0 \pm 0.87$  was observed. The standard drug (loperamide) also significantly reduced ( $P < .0001$ ) both the number and weight of stool. The effect of 200 mg/kg of C.I is similar to that of loperamide (Table 1).

The effect of *C.indica* on castor oil induced diarrhoea showed that both the extract and loperamide reduced intraluminal fluid accumulation. Castor oil increases volume of intestinal content by preventing the reabsorption of water and the liberation of ricinoleic acid from castor oil which results in irritation and inflammation of the intestinal mucosa leading to the release of prostaglandins which results in stimulation of motility and secretion and prevention of reabsorption of sodium chloride and water [19]. The prevention of intraluminal fluid secretion by *C indica* in these studies may be due to inhibition of prostaglandins biosynthesis with resultant decrease in the secretion of fluid into the lumen.

#### Effect on isolated tissue experiment

The isolated ileum was stimulated using acetylcholine and the contractions were noted (fig 2). The contractions were observed to increase with increase in the concentrations of acetylcholine administered, such that the minimal dose of 0.2  $\mu$ g/ml gave a percentage response of 8.8, while the maximum dose of 800  $\mu$ g/ml gave a percentage response of 100. The acetylcholine produced the lowest contractions at 0.2 $\mu$ g/ml while the highest contraction *E*<sub>max</sub> was obtained at 800  $\mu$ g/ml. The effect of the extract was monitored by the extent to which each concentration attenuated the contractions induced by acetylcholine. At a concentration of 10mg/ml, the extract of *C. indica* inhibited the contractions produced by 0.2-800  $\mu$ g/ml of acetylcholine.

The contraction of 8.8 % with 0.2  $\mu$ g/ml acetylcholine was reduced to 4.18% by the extract. Similarly, the highest contraction (100%) produced by the Acetylcholine was reduced to 44.69%.

The inhibitory effects of the extract on acetylcholine induced contraction of the rat ileum were observed to be significant ( $p < 0.0001$ ).

In the rat isolated ileum, the extract inhibited acetylcholine induced contraction in a dose dependent manner, such that the maximum dose of acetylcholine used (0.8 mg/ml) was inhibited. Smooth muscle contraction depends not only on extracellular calcium but also on intracellular calcium<sup>19</sup>. The inhibitory effect of the extract on acetylcholine induced contractions may be due to blockade of muscarinic receptors and interference with calcium movement. The blockade appears non-competitive, as the dose response curve was shifted to the right, *E*<sub>50</sub> was increased and *E*<sub>max</sub> unattainable.

#### CONCLUSION

It can be concluded that the methanolic extract of the leaves of *Canna indica* is effective against diarrhoea as claimed by herb medicine, and thus a useful alternate to orthodox drugs. Its anti diarrhoeal effect may be mediated via muscarinic receptor blockade and inhibitory effect on prostaglandins biosynthesis.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### AUTHORS' CONTRIBUTIONS

Author Ofeimu designed the study, performed the statistical analysis, wrote the protocol, Author Owolabi wrote the first draft of the manuscript, while Author Oluyole managed the literature searches and the analyses of the study. All authors read and approved the final manuscript.

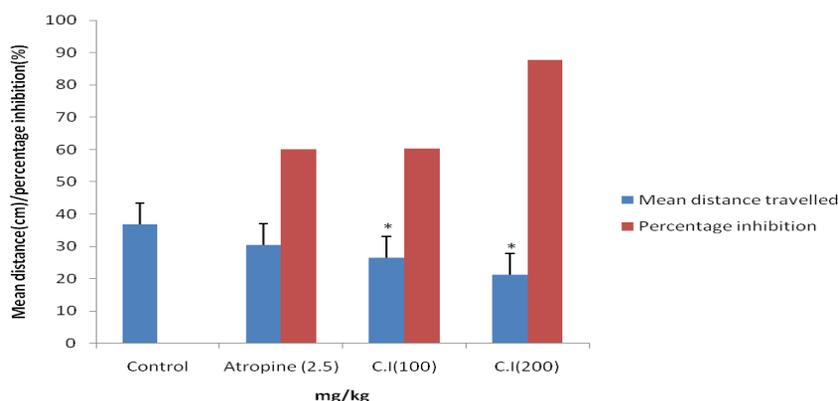
**Table 1: Effect of the methanol extract of the leaves of *Canna indica* on castor oil induced diarrhoea in mice**

Treatment (mg/kg)	Wt of stool (mg)	% Inhibition	No of stool	% Inhibition
Control (2ml/kg)	0.37 $\pm$ 0.20	-	12.40 $\pm$ 1.76	-
C.I (50)	0.30 $\pm$ 0.07 <sup>a</sup>	17.8	9.6 $\pm$ 4.60 <sup>a</sup>	22.6
C.I (100)	0.27 $\pm$ 0.06 <sup>b</sup>	28.05	7.0 $\pm$ 1.30 <sup>b</sup>	43.54
C.I (200)	0.21 $\pm$ 0.05 <sup>b</sup>	42.65	6.0 $\pm$ 0.80 <sup>b</sup>	51.61
Loperamide (5)	0.21 $\pm$ 0.05 <sup>b</sup>	45.4	6.4 $\pm$ 3.90 <sup>b</sup>	48.38

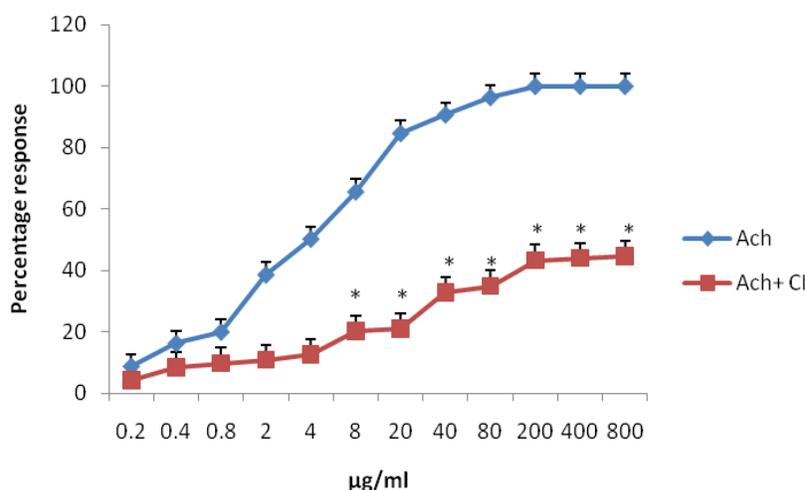
<sup>a</sup>*P* = .05 and <sup>b</sup>*P* < .0001 significantly lower than that of the control.

C.I: Methanol extract of *Canna indica*

Values are mean  $\pm$  SEM.



**Fig. 1: Effect of the ethanol extract of *Canna indica* on small intestinal charcoal meal transit in mice**  
<sup>a</sup> $P = .05$  and <sup>b</sup> $P < .001$  significantly different from the control.



**Fig. 2: Effect of the methanol extract of *Canna indica* on acetylcholine-induced contraction** <sup>\*</sup> $P < 0.0001$  significantly different from the acetylcholine treated group.  
 CI: Methanol extract of *Canna indica*

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