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Research Article

# Anti-diarrhoeal Activities of the Methanolic Root Bark Extract of *Cochlospermum planchonii* (Hook f).

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**ABSTRACT**
Antidiarrhoeic properties of the methanolic root bark extract of *Cochlospermum planchonii* was studied using gastric emptying time (GET) in rats, castor oil induced enteropooling and charcoal plug transit time in mice. Diphenoxylate (5 mg/kg) and atropine (3 mg/kg) were used as the reference drugs.The extract of *C. planchonii* significantly ($P < 0.05$) reduced the rate of gastric emptying into the duodenum in a dose dependent manner. The extract (1000 mg/kg) was more effective than diphenoxylate (5 mg/ kg) used in the study.The extract also markedly inhibited the movement of charcoal plug in all doses used dose dependently. Oral administration of the extract (500 and 1000 mg/kg) and the reference drug diphenoxylate (5 mg/kg) significantly ($P < 0.05$) decreased the distance covered by the charcoal plug in the intestine of mice with the highest dose of the extract (1000 mg/kg) producing similar percent inhibition of the charcoal plug with the reference drug by 44% and 44% respectively. In castor oil induced enteropooling, the extract significantly ($P < 0.05$) decreased the intra luminal fluid content in mice. The highest reduction was recorded at 1000 mg/kg of the extract which was similar to that of the reference drug atropine (3 mg/kg). In conclusion, the methanolic root bark extract of *C. planchonii* possessed antidiarrhoeal property comparable to diphenoxylate and atropine by reduction of gastrointestinal motility and fluid secretion.

**Keywords**: anti-diarrhoeal, *Cochlospermum planchonii.*

## INTRODUCTION

Diarrhoea is defined as the frequent passage of liquid faeces and involves both an increase in the motility of the gastrointestinal tract along with increased secretions and a decrease in the absorption of fluid and thus a loss of electrolytes (particularly $Na^+$) and water (Rang *et al*, 2003). Causes of diarrhea include infectious agents, plant toxins, gastrointestinal disorders such as inflammatory and dysmotility problems of gastrointestinal tract and substances that increase gastrointestinal tract secretions (Alquist *et al*, 2001).

Diarrhoea has been recognized as one of the most important health problems in developing countries (Snyder and Merson, 1982). In Nigeria, diarrhea remains the number one killer among children aged 1 –5 years and world wide, the disease accounts for 4-5 million deaths among humans annually (Audu *et al*, 2000). Also in Veterinary medicine, diarrhoea occurs as symptoms or complications of most viral, bacteria, parasitic and nutritional diseases (Susan and Mays, 2005).

To overcome the menace of diarrhoeal diseases in developing countries, the world health organization (WHO) has included a programme for control of diarrhoea which involves the use of traditional herbal medicine (Snyder and Merson, 1982) and medicinal plants have been documented to have advantage in toxicity considerations based on their long term use and one might expect bioactive compounds obtained from such plants to have low animal and human toxicity (Fabricant and Farnsworth, 2001). Based on this, many people have embarked on the use of indigenous plants as remedy against diarrhoeal diseases(Etuk, et al, 2009) and several plants have been reported to be used in treating and managing diarrhoea (Agunu *et al*, 2005).

*Cochlospermum planchonii* belongs to the Family *Bixaceae*, made up of shrubs or small trees of 2- 2.5 m high with young branches and leaves, and the Genus is made up of approximately 40 species. The leaves are alternate, stipules small with sheathing bud. The flowers are bisexual, bright yellow and borne basally, while the fruits are in form of capsules, two veined and usually spiny and the seeds usually many.(Zhang and Zhang, 1990).

*C. planchonii* have been employed in folk or traditional medicine in Nigeria and other West African countries for treatment of different kinds of diseases and other social and religious uses which include treatment of infertility, diabetis mellitus, malaria, premenstrual pain, hepatobilliary affections, gonorrhea and management of jaundice (Burkill,1985 and Igoli *et al*, 2005). Literature search shows that the methanolic extract has been shown to be as effective as chloroquine for the treatment of uncomplicated malaria caused by *Plasmodium falciparium* (Benoit –Vical, 2003) and the chloroform extract of the stem bark have trypanocidal activity

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(Atawodi, 2005). Also *C. planchonii* has been used traditionally for treatment of diarrhea.

This study was carried out to establish the pharmacological basis for the use of *C. planchonii* for diarrhoea management in Nigerian traditional medicine with focus on the possible mechanisms of action using gastrointestinal propulsion and secretion models.

## MATERIALS AND METHODS

### Collection and extraction of plant material.

The plant materials were collected from the premises of University of Agriculture, Markudi, Benue state, Nigeria and was identified as *Cochlospermum planchonii* (Hook f.) by Mr. A. Ozioko of Bioresources Development and Conservation programme (B.D.C.P.) Aku Road, Nsukka, Enugu State. A voucher specimen was deposited in the herbarium of the Department of Botany, University of Nigeria, Nsukka.

The root barks were pilled off and cut into small pieces using a knife and dried under mild sunlight. They were pulverized into a coarse powder of about l mm in diameter using a hammer mill. The coarse powder of the plant material was extracted using cold maceration method. The plant material (400 g) was extracted in 80% methanol for 48 hours with intermittent shaking at 2 hours interval. The extract was filtered using Whatman No. 1 filter papers and later concentrated in vacou using rotary evaporator connected to cold water circulator and a pressure pump at $40^0$c and 210 milibar. The percentage yield( w/w) of the extract was determined using the formula below:

$$\frac{\text{Weight of the material extracted}}{\text{Weight of plant material}} \times \frac{100}{1}$$

### Animals:

### Rats and Mice.

Mature albino Wistar rats (105- 166g) and mice (25-35 g) of both sexes bred in the laboratory Animal units of the Faculty of Veterinary Medicine, University of Nigeria Nsukka were used for the experiment. The temperature varied between 25-$30^0$c and lighting hour varied between 12-14 hours with relative humidity of about 40-60%. The animals were kept in stainless steel cages and clean drinking water provided *ad libitum* to them while they were fed with standard commercial pelleted feed (Vital feed®, Nigeria). Ethical conditions governing the conducts of experiments with life animals were strictly observed as stipulated by Zimmerman (1983) and Ward and Elsea (1997).

### Phytochemical spot tests.

Phytochemical spot tests were carried on the crude extract using the methods of Harbourne (1991) and Trease and Evans (1996).

### Effect of *C. planchonii* extract on gastric emptying time

The method of Droppleman *et al* (1980) was used in evaluating the effect of *C.*

*Planchonii* extract on gastric emptying time in rats.

### Preparation of the test meal.

Ten grammes of methylcellulose (methocil® ) was slowly added to 200 ml of ice cold water while being agitated in a blender at 20,000 r. p. m for five minutes. Disodium monoglucoate (10 g) was dissolved in 100 ml warm water and was blended into the methocil solution. Casein (16 g), 8 g of granulated confectioners' sugar and 8 g of corn starch were added. The ingredients were added slowly and thoroughly mixed for 2 minutes resulting in formation of semi- solid homogenous paste of about 325 ml. The test meal was refrigerated for 48 hours to allow the trapped air in the meal to escape before use. The test meal was used for the experiment.

Twenty five mature albino Wistar rats of both sexes (104 - 166 g) were fasted for 12 hours and water made available *ad libitum*. The animals were randomly grouped into five groups of five rats each. Each rat was given 3.0 ml of the test meal with each meal having an average weight of 3.28 g by stomach intubation.

Thirty minutes prior to administration of test meal, the rats were treated as follows: Group 1 served as the untreated control and received 10% Tween 20 solution (10 ml/kg b.w) while rats in group 2 received diphenoxylate (5 mg/kg b.w.) which served as the treated control The test groups 3, 4, and 5 were treated with 250, 500 and 1000 mg /kg b.w of CPE respectively. This was followed by the administration of the test meal. The rats were sacrificed by cervical dislocation 30 minutes after administration of the test meal. Laparatomy was done on the rats and the stomach removed after tying both the pyloric and the cardiac ends with a silk tread. The full stomach of each rat was weighed with an analytical balance (Mettler H30). The rats´ stomach were cut open through the greater curvature, rinsed with water, excess moisture removed by gently sponging with tissue paper and immediately re-weighed. The difference between the weight of the full stomach and the empty stomach was indicative of the amount of the feed remaining in the stomach. The values were subtracted from the weight of the 3.0 ml of the test meal (3.28 g) to obtain the quantity of meal emptied from the stomach during the test period and the percent meal emptied was calculated.

### Effect of *C. planchonii* extract on charcoal meal transit time

The effect of *C. planchonii* on charcoal meal transit time was evaluated using the method of Mascola *et al* (1994), modified by Chidume *et al* (2001). Twenty five female mice weighing between 27-35 g were fasted for 12 hours but allowed free access to drinking water. They were randomly divided into five groups (1-5) of five mice each. They were treated as follows: The mice in group 1 received 10% Tween solution (10 ml/ kg b.w). Group 2 received dihenoxylate (5 mg /kg b.w) and those in groups 3, 4, and 5 received *C. planchonii* extract (250, 500 and 1000 mg/ kg b.w) respectively, all by stomach intubations. Five minutes after drug administration, 0.5 ml of 10% charcoal suspension in 5% acacia gum was administered to each mouse by stomach intubations. Thirty minutes later, all the mice were sacrificed by cervical dislocation, the abdomen opened and the total

length of the small intestine measured with a calibrated ruler. The distance traveled by the charcoal plug from the pylorus to caecum was determined and expressed as a percentage of the total length of the small intestine. Also the percent inhibition of movement was calculated by subtracting the percentage traveled from 100%.

**Effect of *C. planchonii* extract on Castor oil - induced enteropooling**.

Intraluminal fluid accumulation was determined by the method of Robert *et al* (1976). Twenty five mature male mice were fasted for 12 h with free access to clean water. They were randomly divided into five groups (1 – 5) with 5 mice each.

Group 1 received 10% Tween solution (10 ml/ kg b.w) and served as untreated control while, group 2 received atropine sulphate (3 mg/kg b.w) (positive control) and groups 3, 4 and 5 received 250, 500 and 1000 mg/kg b.w of the extract respectively. They were treated by stomach intubations. One hour post treatment, each mouse was given 0.5 ml castor oil by oral gavage. Two hours after administration of castor oil, the mice were sacrificed by cervical dislocation, laparatomized, the small intestine located and tied at the pyloric and ceacal junction and cut out. The small intestine was weighed with analytical weighing balance (Mettler $H_3O$). The content of each intestine was milked into a graduated test tube and the volume recorded. The empty small intestine was reweighed and the difference in weight between the full and empty small intestine was recorded as the weight of the intestinal content.

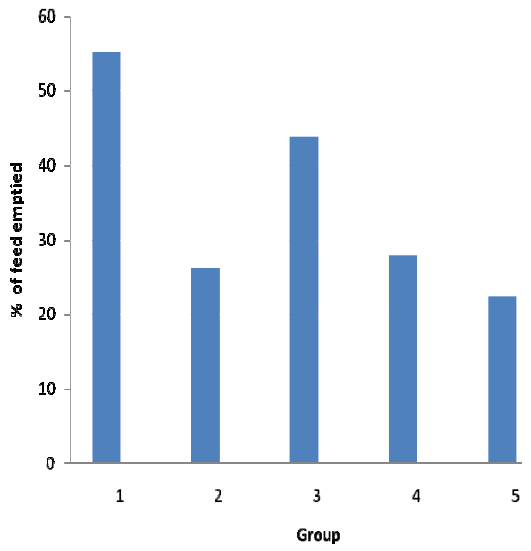


**Fig. I** : Effect of CPE on percent test meal emptied from test rats' stomach.

## RESULTS

### Extraction

The percentage yield was 8.55% w/w dry matter. The extract was ox-blood in colour and had oily pungent odour

### Phytochemical spot tests.

A preliminary phytochemical spot test of the crude extract of *C. Planchonii* root bark showed the presence of carbohydrates, tannins, glycosides and flavonoids.

### Effect of extract on gastric emptying time in rats

The effect of CPE on percent meal emptied from rats stomach is presented in Fig. 1. The result showed that diphenoxylate (5 mg/kg b.w) and the extract tremendously delayed gastric emptying with CPE (1000 mg/kg b.w) decreasing the percent meal emptied by more than half of the negative control group (10% Tween 20 solution). Also the quantity of the test meal (g) emptied in group 1 (negative control) was 1.808 ± 0.11 and was significantly ($P < 0.05$) reduced by diphenoxylate and the extract in a dose dependent

### Effect of the extract on charcoal transit time.

The result of *C. planchonii* extract on charcoal meal transit time in mice is presented in Table 1. The result showed that the all the doses of the extract (250, 500 and 1000 mg/kg) reduced the distance travelled by the charcoal plug in a dose dependent manner. The reference drug, diphenoxylate (5 mg/kg) and the extract at 500 and 1000 mg/kg significantly ($P < 0.05$) inhibited the charcoal movement in a dose dependent manner when compared with the negative control group with the extract at the dose of 1000 mg/kg having a little higher percent inhibition than the reference drug.

**Table I**. Effect of *C. planchonii* extract on charcoal transit time in mice

| Group | Treatment | Length of stomach (cm) Mean ± S.E.M | Distance covered (cm) mean ±S.E.M | % Inhibition |
|---|---|---|---|---|
| 1 | Tween 20 solution (10ml/kg) | 42.4± 1.50 | 42.4± 1.50 | 0 |
| 2 | Dihenoxylate (5mg/ kg) | 39.6± 0.51 | 22.2± 0.86 | 43.9 |
| 3 | CP (250 mg/kg) | 41. ± 1.65 | 33. 4 ± 1.38 | 18.7 |
| 4 | CP (500 mg/kg) | 42.5± 1.22 | 29.5± 1.09 | 30.6 |
| 5 | C.P (1000 mg/kg) | 43.4± 1.43 | 23.2± 1.39 | 45.9 |

### Effect of the extract on castor oil-induced enteroproolmg in mice.

The result of the effect of *C. planchonii* extract on castor oil enteropooling is presented in table III. Studies on the castor oil induced enteropooling showed that both the extract (500 and 1000 mg/kg) and the reference drug atropine (3 mg/kg) significantly ($p < 0.05$) decreased both the intra luminal volume of fluid accumulation and

the weight of intestinal content in mice when compared to the control group that received10% Tween 20 solution (10 ml/kg.).

**Table II.**Effect of *C. planchonii* extract of on castor oil-induced enteroproolmg in mice.

| Group | Treatment | Volume of intestinal content (ml) Mean ± SEM | Weight of intestinal content (g) Mean ± S.E.M |
|---|---|---|---|
| 1 | Tween 20 solution (10ml/kg) | 0.42 ± 0.04 | 0.43 ± 0.03 |
| 2 | Atropine (3mg/kg) | 0.30 ± 0.03 | 0.21 ± 0.04 |
| 3 | CP (250 mg/kg) | 0.40 ± 0.05 | 0.41 ± 0.06 |
| 4 | CP (500 mg/kg) | 0. 38 ± 03 | 0.35 ± 0.02 |
| 5 | CP (1000mg/kg) | 0.28 ± 0.04 | 0.23 ± 0.08 |

**DISCUSSION AND CONCLUSSION**

The antidiarrhoeal properties of *C. planchonii* was studied using gastric emptying time (GET) in rats, charcoal meal transit time and castor oil induced enteropooling in mice. These models are justified because according to Havagiray, *et al*, (2004), in some diarrhoeas, the secretory component predominates while other diarrhoeas are characterized by hypermotility of the gastrointestinal tract.

Gastric emptying is the act of evacuating the content of the stomach by normal peristaltic movement and a parameter strictly connected with the activity of the smooth muscles of the stomach (Bertaccini *et al*, 1981). It is determined by a balance between the force and frequency of gastric peristalsis and the resistance to flow offered by the pylorus (Mountcastle, 1974). Rapid gastric emptying time has been observed in patients with functional diarrhea (Charles *et al*, 1997). From our results, *C. planchonii* significantly ($p < 0.05$) 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 its relaxant effect on the stomach muscles. One of the causes of diarrhoea among other things is dysmotility problems of the gastrointestinal tract (Alquist *et al*, 2001) and drugs that have effect on the motility or propulsion rate of the gastrointestinal tract may have antidiarhoeal effect (Otimenyin *et al*, 2008). Since the extract at the highest dose (1000 mg/kg) had a better effect than the reference drug, it then follows that *C. planchonii* may be beneficial in diarrhea management.

Studies have shown that activated charcoal readily adsorbs drugs and chemicals on the surface of the intestines, thereby preventing absorption (Levy, 1982), hence charcoal meal study was employed to study the effect of *C. planchonii* on peristaltic movement. The extract and the reference drug diphenoxylate significantly ($P < 0.05$) decreased the distance traveled by the charcoal plug in a dose dependent manner with the percent inhibition of movement being a bit higher in the highest dose of the extract (1000 mg/kg) than the reference drug. According to Bruton (1996), the property of reducing intestinal contractions (and consequently, intestinal transit) is demonstrated by most antidiarrhoeal drugs. This property was shown by *C. planchonii* from the study. Reduction of intestinal transit time may possibly be due to its anticholinergic effect (Brown and Taylor, 2000). Diphenoxylate used in the symptomatic control of diarrhea, works through its antimotiliy effects (Aliu, 2007). *C. planchonii* may also be acting through the same mechanism.

The effect of *C. planchonii* on castor oil induced enteropooling showed that the reference drug atropine (3 mg/kg) and the extract in all the doses used significantly ($p < 0.05$) reduced both the intraluminal fluid accumulation and the weight of intestinal content in a dose dependent manner. Castor oil increases volume of intestinal content by prevention of the re- absorption of water and the liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa leading to release of prostaglandins which results in stimulation of motility and secretion and the prevention of re- absorption of NaCl and water (Pierce *et al*, 1971). The prevention of intraluminal fluid secretion by *C. planchonii* in this study may be due to inhibition of prostaglandin biosynthesis with resultant decrease in secretion of fluid into the lumen or may be due to promotion of absorption of water and electrolytes in the gut. Suppression of intestinal fluid accumulation by the extract might also suggest inhibition of gastrointestinal function (Nwafor *et al*, 2000).

Phytochemical screening of the methanolic root bark extract of *C. planchonii* revealed the presence of carbohydrates, tannins, glycosides and flavonoids. Some of the chemical constituents present in the root extract have been shown to have antidiarrhoeal activity. Antidiarrhoeal and antidysentric properties of medicinal plants have been reported to be due to tannins, flavonoids, reducing sugars/glycosides among others (Longanga *et al*, 2000). Flavonoids and sugars obtained from selected medicinal plants were shown to exhibit antidirrhoeal properties (Palombo, 2005) and the inhibitory activity of flavonoids on intestinal motility in a dose related manner was earlier reported (Dicarlo *et al*, 1994).

We therefore suggest that flavonoids and or other chemical constituents present in the extract might be responsible for the antidirrhoeal activity of *C. planchonii.*

In conclusion, the results of this study indicate that *C. planchonii* possesses anidiarrhoeal properties which may be due to its inhibitory effects on both gastrointestinal propulsion and fluid secretion. However, more work is

required to determine the exact mechanism of action of the extract and to isolate and characterize the active principle.

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