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METHANOL EXTRACT AND SOLVENT FRACTIONS OF *PAULLINIA PINNATA* LINN. (SAPINDACEAE) EXHIBIT ANTI-DIARRHOEAL POTENTIAL USING *IN VIVO* MODEL

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ABSTRACT

Background and aim: To experimentally justify the ethnomedicinal claim of the usage of *Paullinia pinnata* in the management of diarrhoea.

Methods: Antidiarrhoeal activity of crude extract and solvent fractions at 100 - 400 mg/kg was done using castor oil-induced diarrhea and gastrointestinal motility test using rats.

Results: LD_{50} was noted to be greater than 5 g/kg. *Paullinia pinnata* methanol extract (PPME) gave a decrease in faecal output in a non-dose dependent manner at tested doses, with the highest and lowest inhibitions found to be 71.53% and 57.53% at 200 and 400 mg/kg, respectively. The PPME (200 mg/kg) reduced stooling frequency in a manner comparable to that of the standard drug, Loperamide® (75.92% at 5 mg/kg). No activity was observed for the aqueous fraction at 50 and 100 mg/kg, unlike the DCM fraction which produced 44.50% and 63.50% inhibitions at 50 and 100 mg/kg, respectively. Also, a dose-dependent increase in inhibition of gastrointestinal (GI) motility (56.45% and 64.49%) was observed with 100 and 200 mg/kg PPME, respectively. However, a sharp reduction in activity (29.4%) was noted at the highest dose (400 mg/kg) compared to 59.16% in animals administered with 5 mg/kg atropine (positive control). DCM fraction also showed a similar pattern of activity as observed in castor oil-induced diarrhoea, with dose-dependent increase in antidiarrhoeal activity. The 50 and 100 mg/kg doses inhibited GI motility by 48.68% and 58.05 %, respectively.

Conclusion: This study suggests that *P. pinnata* methanol leaf extract possesses antidiarrhoeal potential with bioactive compounds residing in the non-polar fraction.

Keywords: Antidiarrhoeal, atropine, castor oil-induced diarrhoea, gastrointestinal motility, loperamide, *Paullina pinnata.*

INTRODUCTION

Diarrhoea is increased fluidity, frequency, or volume of bowel movements. It may be acute or chronic and can be very serious in infants and elderly people because of the risk of severe, potentially fatal dehydration [1]. Diarrhoea occurs worldwide and causes 4% of all deaths and 5% of health loss to disability [2–4]. In Africa, it is four times more common among children with HIV and seven times more common among adults with HIV than their HIV-negative household members [4]. Since ancient times, diarrhea has been recognized as one of the most important health problems afflicting mankind, particularly those populations in socio-economically backward, and developing, third-world countries. Globally, diarrhea been estimated to kill about 2.2 million people annually, the majority of whom are infants and children below the age of 5 years [5–7].

Diarrhea is a disorder characterized by the discharge of semisolid or watery fecal matter from the bowel three or more times in a day [7,8]. There are numerous forms of medications used to manage diarrhea, which include from antibiotics to non-antibiotics depending on the cause of the diarrhea [6,10–12]. Different limitations have been mentioned regarding these therapeutics, which include secondary effect of the drugs, unavailability and high cost of the therapeutics to locals. These necessitated quests for alternative forms of medicine [such as medicinal plants] to complement available therapeutics used in the management of diarrhoea.

Numerous studies have validated the traditional use of anti-diarrheal medicinal plants by investigating the biological activity of extracts of such plants, which have antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water absorption or reduce electrolyte secretion [13].

Paullinia pinnata Linn. (Sapindaceae) is commonly called “bread and cheese plant” and “sweet gum”, and the major Nigerian local names include: Ogbe-okuje (Yoruba) and Omekpa (Hausa). It is an African woody vine whose fruits are widely eaten and its leaves are used in traditional medicine for the treatment of malaria [14]. The plant is a sub-woody or woody climber that originates from tropical America but now domesticated to Savanna region of Tropical Africa and Madagascar. Traditionally, the leaves of the plant are used in various forms for the treatment of colic, dysentery and diarrhea, to prevent miscarriage, as uterotonics to ease child birth, lactogene and also to help sterility, to ease menstrual discomfort, treatments of rheumatism, internal and external swellings, dermatological aberrations and treatment of ulcers [14]. The aim of the present research was to evaluate the anti-diarrhoeal activity of crude methanol extract and solvent fractions of *P. pinnata leaves* using *in vivo* experimental assay.

MATERIALS AND METHODS

Plant collection and authentication

Leaves of *P. pinnata* were collected from Okada town in Ovia North-East Local Government Area of Edo State, Nigeria in 2019. The plant was collected and identified by Dr. A. S. Odewo, a taxonomist from Forest Research Institute of Nigeria (FRIN) and authenticated with voucher number IUO/19/278 at the herbarium section of Department of Pharmacognosy, College of Pharmacy, Igbinedion University, Okada where voucher specimen was deposited.

Preparation of plant material

The leaves of the plant were detached from the whole plant, rinsed in water and spread on a clean sack devoid of sand. This was followed by air drying of the plant material in the laboratory for 2 weeks. The dried leaves were reduced to coarse powder with the aid of a mechanical grinder.

Extraction and solvent partitioning

Powdered plant (1.2 kg) was extracted with absolute methanol using Soxhlet apparatus. Resultant extract was concentrated to dryness *in vacuo* using a water bath [HH-S Water bath; Searchtech Instruments] at temperature of 60 °C, and dried residue refrigerated at 4ºC until required.

Crude methanol extract obtained (50 g) was partitioned into dichloromethane (DCM) and water using separatory funnel and yield of fractions determined.

Preliminary phytochemical screening

Phytochemical screening was carried out using standard methods described by [14–17]

Laboratory animals and ethical approval

The experiment was performed with the approved permission of the Igbinedion University Animal Ethical Committee (IUO/Ethics/22/PHA/005) and in accordance with approved national and international guidelines for the care and use of laboratory animals.

Mice of both sexes (20 - 27 g) and male and female Wistar rats of 200 – 270 g and 145–180 g, respectively were sourced from Central Animal House of Igbinedion University, Okada, Nigeria. They were fed with standard rat pelleted diet (Bendel Feeds and Flower mill, Edo State, Nigeria), and water *ad-libitum* and maintained under hygienic standard laboratory conditions (temperature, 25 °C; photoperiod, 12 h of light and 12 h darkness).

Acute toxicological study

Lethal dose (LD_{50}) of the methanol extract was examined using single and daily oral doses of 10, 100, 1000 mg/kg (Phase 1); 1600, 2900 and 5000 mg/kg (Phase 2). The mice were observed continuously for 1 hour for any gross behavioral changes, deaths and intermittently for the next 6 hours and then again at 48 hours after dosing. Animals that survived were kept under observation for 7 days [17].

Antidiarrhoeal activity

***Castor oil-induced diarrhoea in rats*:** The rats were fasted for 24 h prior to the experiment, but had free access to water. Six rats were randomly assigned to each of the following groups, Group 1 served as negative control (distilled water; 10 mL/kg):- group 2 represent the positive control (5 mg/kg; loperamide®), groups 3 to 5 received 100 – 400 mg/kg of the extract: groups 6 to 7 also received 50 and 100 mg/kg of aqueous fraction, while groups 8 to 9 were given 50 and 100 mg/kg of DCM fraction. All drugs were administered by gavage as a single bolus, and 1 hour thereafter animals were orally administered 10 ml/kg of castor oil. Treated animals were kept in separate metabolic cages with transparent plastic container beneath the cage and lined with Whitman filter paper (pre-weighed) to collect faeces. Following castor oil administration, weight of the faeces (wet and dry) obtained were determined upon 8 hours of observation [18,19].

Gastrointestinal motility test: Animals were fasted for 24 hours and randomly assigned to groups: group 1 received distilled water

(negative control; 10 mL/kg), group 2 atropine (positive control; 5 mg/kg) and groups 3-5 extract received 100, 200 and 400 mg/kg, respectively. Also, groups 6 and 7 received 50 and 100 mg/kg of aqueous (AQ) fraction, respectively while groups 8 and 9 animals were given 50 and 100 mg/kg of DCM fraction, respectively. Immediately after the administration of the above treatments, all animal received 400 mg/kg of charcoal meal. One hour after, rats were sacrificed and the small intestine was removed from the pyloric sphincter to the ileocoecal junction. Thereafter, the distance covered by charcoal was measured and expressed as a percentage of the overall length of the small intestine using the equation below.

Intestinal transit [%] = [D/L] x 100 ……. [1]
where D = distance covered by charcoal [in metres] and L = intestinal length (in metres).

Data analysis

Data were presented as mean ± SEM of six replicates. One Way ANOVA was done to compare means of different groups as well as a Duncan multiple range test to analyze differences among different means and the interaction between the variables using Graph padprism-7 computer software packages. Differences at $p \leq 0.05$ was considered statistically significant.

RESULTS

Yields of plant extracts and solvent fractions

The plant material yielded approximately 50 g extract corresponding to 4.17%. Partitioning of 40 g of methanol extract gave 25.21 g (63.03%) AQ fraction and 10.98 g (27.45%) DCM fraction.

Preliminary phytochemical Screening

Preliminary phytochemical screening reveals the presence of flavonoids, saponins, steroids, reducing sugars, glycosides, terpenoids, phenolics compounds as shown in Table 1 below.

Table 1: Phytochemical screening of *P. pinnata* methanol extract

Phytochemical	Inference
Phenolics	+
Flavonoids	+
Saponins	+
Alkaloids	+
Steroids	+
Terpenoids	+

+ = present

Acute toxicity study

Results from the acute toxicological studies revealed that PPME exhibited $LD_{50} > 5$ g/kg when administered orally and no toxic symptoms or adverse behavioral changes were observed as zero mortality was recorded after 24 hours and 1 week after the single oral administration of the extract at all doses.

Result of *P. pinnata* on castor oil induced diarrhoea in rats

The administration of methanol extract to diarrhoeal animals produced a reduction in the amount of feaces across all doses tested resulting in highest and lowest inhibitions of 71.53% and 57.66% at 200 and 400 mg/kg, respectively (Table 2). It was more active than the negative control which gave no inhibition. There was no activity observed for the AQ fraction while a dose-dependent increase in activity was observed in the DCM fraction at 50 and 100 mg/kg producing 44.50% and 63.50 % inhibitions, respectively.

Table 2: Effect of crude methanol extract and solvent fractions of *P. pinnata* on castor oil induced diarrhoea

Treatment	Dose (mg/kg)	Weight of faeces	% Inhibition of diarrhoea
Negative Control (10 mL; distilled water)	10 mL/kg	1.37±0.46	-
Positive control (Loperamide)	50 mg/kg	0.33±0.03*	75.92*
Methanol extract	100 mg/kg	0.51±0.15*	62.77*
Methanol extract	200 mg/kg	0.39±0.13*	71.53*
Methanol extract	400 mg/kg	0.58±0.03*	57.66*
Aqueous fraction	50 mg/kg	2.12±0.13	0
Aqueous fraction	100 mg/kg	2.38±0.04	0
Dichloromethane fraction	50 mg/kg	0.76±0.14	44.52
Dichloromethane fraction	100 mg/kg	0.50±0.03*	63.50*

The values above are mean of six replicates. n=6. Mean ± SEM. Values with superscript *indicate significant difference at P≤0.05 relative to values obtained from animals administered with distilled water (negative control; 10 mL/kg). Values with no superscript *indicate no significant difference at p≤0.05 relative to values obtained from animals administered with distilled water (negative control; 10 mL/kg) using ANOVA (non-parametric)

Effect of crude methanol extract and solvent fractions of *P. pinnata* on gastrointestinal motility in rats

A dose-dependence increase in inhibition of gastrointestinal motility was observed with methanol extract at 100 and 200 mg/kg (Table 3). Conversely, no activity was observed with the AQ fraction. DCM fraction also showed a dose-dependent increase in activity (48.68 and 58.05 %) at 50 and 100 mg/kg, respectively.

Table 3: Effect of crude methanol extract and solvent fractions of *P. pinnata* on gastrointestinal motility in rats

Treatment	Dose (mg/kg)	Distance travelled by charcoal (cm)	% Inhibition
Negative Control (10 mL; distilled water)	10 mL/kg	24.98±4.95	-
Positive control (Atropine, 5 mg/kg)	50 mg/kg	10.20±3.18*	59.16*
Methanol extract	100 mg/kg	10.88±1.04*	56.45*
Methanol extract	200 mg/kg	8.87±1.78*	64.49*
Methanol extract	400 mg/kg	17.63±3.42	29.42
Aqueous fraction	50 mg/kg	20.2±3.5	19.14
Aqueous fraction	100 mg/kg	28.99±1.65**	0
Dichloromethane fraction	50 mg/kg	12.82±0.87*	48.68
Dichloromethane fraction	100 mg/kg	10.48±0.78*	58.05*

The values above are mean of six replicates. n=6. Mean ± SEM. Values with superscript *indicate significant difference at P≤0.05 relative to values obtained from animals administered with distilled water (negative control; 10 mL/kg) Values with no superscript *indicate no significant difference at p≤0.05 relative to values obtained from animals administered with distilled water (negative control; 10 mL/kg) using ANOVA (non-parametric).

DISCUSSION

Medicinal plants have been used for treatment of different ailments from time immemorial [14,20]. Previously, *P. pinnata* has been reported for various activities such as analgesic and anti-inflammatory, antimicrobial, radical scavenging anti-

malarial, antioxidant, anxiolytic effect, aphrodisiac [14,21].
Active metabolites such as steroids, saponins, flavonoids, reducing sugars, glycosides, phenolic compounds and terpenoids found *P. pinnata* are responsible for its medicinal properties [15,19,22]. Flavonoids are known to modify the production of cyclooxygenase 1 and 2 and lipo-oxygenase thereby inhibiting prostaglandin production. The anti-diarrhoeal activity of PPME may be through the prevention of prostaglandin biosynthesis.

Acute toxicity study of PPME showed LD_{50} > 5g/kg indicating that the plant is safe orally.

Castor oil induces diarrhoea through its active metabolite, ricinoleic acid which increases peristaltic activity, stimulates gastrointestinal secretions and irritates the intestinal mucosa. Castor oil also promotes the biosynthesis of prostaglandins which causes irritation and inflammation of the intestinal mucosa to stimulate the motility and secretion.

The PPME at 200 mg/kg, has a potential to reduce the frequency of stool at a dose comparable to that of the standard (loperamide; 5 mg/kg; 75.92 %) used as control. It can therefore be said that only a small dose of methanol extract is required for anti-diarrhoeal effect, and thus less amount of drug in the system and less toxic effect. However, fractionation of the methanol extract further showed that the active principles might be non-polar compound(s) due to the loss in activity observed for aqueous fraction at both doses (50 and 100 mg/kg). This is in agreement with the findings of other workers [14,15].

In the presence of castor oil in the gut, prostaglandins are secreted, which in turn induces gastrointestinal motility. Hypermotility is one of the different pathophysiological conditions that characterize diarrhoea. The charcoal meal test was carried out to determine the effect of *P. pinnata* extract on gut motility. A dose-dependent increase in activity was observed with the extract at lower doses of 100 and 200 mg/kg, whereas at a higher dose (400 mg/kg) there was a sharp reduction in activity implying that extract is more effective at the lower doses. These results corroborate the work done by Barnes [15] and also experimentally justify that *P. pinnata* exhibits antidiarrhoeal attribute.

CONCLUSION

The results of the study suggest that the methanol extract of the leaves of *P. pinnata* possesses antidiarrhoeal properties. Moreover, the compound(s) responsible for the activity is/are suggested to be of non-polar nature.

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CONFLICT OF INTEREST

No conflict of interest

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