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In-Vivo Antidiarrhoea Evaluations of Methanol Leaf Extract and Fractions of *Chrysophyllum albidum*

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Abstract **Original Research Article**

Background and Objectives: In Nigeria, traditional use of medicinal plants, like *Chrysophyllum albidum*, for diarrhoea persists. This study assessed its effectiveness in treating diarrhoea in rats. ***Methods***: Extract and Fractions from the plant were tested for antidiarrhoea effect. The process involved methanol extraction and fractionation using hexane, ethyl acetate, and butanol. Rat models were used to evaluate the effects on castor oil-induced diarrhoea, enteropooling, and gastrointestinal motility. ***Results***: Defecation caused by castor oil-induced diarrhoea was inhibited significantly by 53.55, 78.48 and 74.69 % at 500 mg/kg of methanol extract, ethyl acetate, and n-hexane fraction respectively, and by 50.99 and 63.69 % at 250 and 500 mg/kg of butanol fraction respectively. The volume of intestinal content and weight of intestinal content were reduced by 76.74 and 80 % at 500 mg/kg of ethyl acetate fraction. Also, the volume of intestinal content and weight of intestinal content were reduced by 70.93 and 70.83 % at 500 mg/kg of n-hexane fraction. At 250 and 500 mg/kg, the ethyl acetate fraction inhibited intestinal motility by 58.65% and 76.50% respectively. The n-hexane fraction at 500 mg/kg showed a 66.35% inhibition. ***Conclusion***: These fractions from Chrysophyllum albidum leaves exhibit potential antidiarrhoea compounds, as evidenced across all three study models. Ongoing research aims to isolate these compounds for further investigation.

Keywords: Antidiarrhoea, *Chrysophyllum albidum*, Castor oil, Enteropooling, Intestinal motility.

INTRODUCTION

Diarrhoea, defined by three or more loose stools daily, is a common gastrointestinal ailment, especially impactful in developing nations due to infections and malnutrition. In such settings, malnourished children, particularly those with weakened immunity or HIV, face heightened risks [1]. Annually, millions in third-world countries suffer from and succumb to diarrhoea, making it a critical global issue. For children under five, it stands as the second leading cause of death [2].

Diarrhoea presents in three types: acute watery (like cholera), lasting hours; acute bloody (dysentery); and persistent, over 14 days. Symptoms encompass nausea, cramps, fever, and bloody stools, posing dehydration risks [3]. Rising diarrhoea-related deaths link to bacterial, viral, and parasitic causes, often transmitted through contaminated water. Dehydration remains the gravest concern in diarrhoea cases, amplifying its severity [1].

Diarrhoea types involve varied physiological changes affecting water transport, electrolytes, and motility, altering osmolality, secretion, absorption, and transit time [4]. Medicinal plants offer potential for new antidiarrhoea drugs, bridging non-pharmacological and pharmacological interventions [2]. In rural areas of the developing world, these plant remedies serve as affordable, accessible therapies [5]. Tannins, alkaloids, saponins, flavonoids, steroids, or terpenoids present in many plants contribute to their antidiarrhoea properties,

expanding treatment options beyond conventional medicines [6, 7].

Chrysophyllum albidum holds a prominent place in Nigerian traditional medicine, renowned for its diverse therapeutic applications. Previous studies in West Africa have highlighted its significance in local communities, exploring its potential in both health and food industries. Extensive analysis of the fruit's physical, chemical, and nutritional attributes reveals promising industrial prospects [8-10]. Ethnobotanical investigations underscore the plant's utilization by communities for medicinal and culinary uses [11, 12]. Studies show the leaf extract harbors compounds with antiplatelet and hypoglycemic properties, while the bark's methanol extract contains antiplasmodial substances [13-15]. Despite its widespread use, scientific data on its role in alleviating diarrhoea remains limited. This preliminary study aims to explore potential antidiarrhoea properties in the methanol leaf extract and fractions of *C. albidum*, employing rat models for investigation.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The fresh leaves of *C. albidium* were collected from its natural habitat at Adaegbe village, Enugwu-ukwu, Njikoka Local Government area of Anambra State, Nigeria, in March 2022. The plant was identified and authenticated by Mr Ozioko, a taxonomist in the Department of Botany, Faculty of Natural Sciences, University of Nigeria, Nsukka. A voucher specimen (PCG/474/A/060) was deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka.

Reagents and Chemicals

N-hexane (JHD), ethyl acetate (JHD), methanol and butanol (JHD, Hyoscine butyl bromide (Buscopan), Activate charcoal (KUNIMED), Loperamide tablet, Imodium® (Eurolife health care, India), castor oil (Bells).

Equipment

Animal weighing balance, animal cages, forceps, hand gloves, aerator, medicut intravenous oral cannula, dissecting kit, beaker, measuring cylinder, borosil tray, hot air oven, mortar and pestle, mechanical grinder, Sterile polypropylene tubes, centrifuge, rotary evaporator (Model RE 300, by Barloworld Scientific Ltd, UK), electronic weighing balance (Ohaus Corp., USA)

Extraction and Fractionation of the Plant Material

Plant leaves were cleaned, dried, and pulverized after separation from the stalk. Weighing 3.5 kg, the powdered leaves underwent soaking in absolute methanol for 72 hours, with intermittent shaking. Filtration was done through gauze and Whatman grade No 1 paper, the extract was concentrated via evaporation in a rotary evaporator. This resulting extract (5g) underwent liquid-liquid fractionation successively with n-hexane, ethyl acetate, and butanol, as per Onyegbule *et al*., (2014) [16]. After filtration and concentration using a rotary evaporator, the fractions were stored in a sealed container in a refrigerator.

Animals for the Study

Healthy adult male and female Albino Wistar rats weighing 150-200 g were used for the experiments. The animals were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra state, Nigeria. They were maintained under standard environmental conditions with free access to a regular diet and clean water. Ethical approval for the study was obtained from the Enugu State University of Science and Technology Animal Ethics Committee, with the approval number ESUT/AEC/0152/AP091.

Acute Toxicity

The acute toxicity test for the extract and fractions was conducted following Lorke's method [17]. A total of 52 female rats were utilized for this study. In the initial phase of the test, there were 9 rats for the extract and 9 rats each for the three fractions. These 9 rats were divided into 3 groups of 3 rats each. One group received a dosage of 10 mg/kg of the body weight of the sample tested, another group received 100 mg/kg, and the remaining group received 1000 mg/kg. The rats were observed for 24 h.

In the second phase, four doses (2000, 3000, 4000, and 5000 mg/kg body weight) of the test samples were administered. For the extract, 4 rats were used, each receiving one of the specified test doses. Similarly, for each of the 3 fractions, 4 rats were used, with each rat receiving one of the test doses, as was done with the extract. The rats were observed for 24 h

Castor Oil-Induced Diarrhoea Evaluation

The method described by Ezekwesili *et al*., (2010) [18], was followed for this study. The Wistar rats were randomized into 10 groups of 5 per group and fasted for 18 hours with free access to water. The extract and the fractions were administered to the animals as follows.

Group 1 received loperamide (3 mg/kg) orally and served as a positive control.
Group 2 received CMC (10 ml/kg) and served as a negative control
Group 3 received (250 mg/kg) methanol crude extract
Group 4 received (500 mg/kg) methanol crude extract
Group 5 received (250 mg/kg) butanol fraction
Group 6 received (500 mg/kg) butanol fraction
Group 7 received (250 mg/kg) ethylacetate fraction
Group 8 received (500 mg/kg) ethylacetate fraction

Group 9 received (250 mg/kg) n-hexane fraction
Group 10 received (500 mg/kg) n-hexane fraction

One hour after dosing, each rat was given 0.5 ml of castor oil orally for induction of diarrhoea and placed individually in cages in which the floor was lined with white paper. The paper was changed every hour for a total of four hours. During the observation period, the onset of diarrhoea (the time interval in minutes between the administration of castor oil and the appearance of the first diarrhoea stool) and the total number and total weight of fecal output were recorded. Finally, the diarrhoea inhibition percentage was calculated using the formulas described below [19].

% Inhibition = (number of wfc – number of wft) / (number of wfc) x 100
Where WFC == wet faeces in the control group and WFT= wet feces in the test group

Castor oil-Induced Enteropooling

The effects of the extract and fractions on intraluminal fluid accumulation were determined using the method described by Robert *et al*., (1976) [20]. Fifty Wistar rats were fasted for 18 hours and divided into 10 groups of 5 rats each.

Group 1 received loperamide (3 mg/kg) and served as a positive control.
Group 2 received CMC (10 ml/kg) and served as a negative control
Group 3 received (250 mg/kg) methanol crude extract
Group 4 received (500 mg/kg) methanol crude extract
Group 5 received (250 mg/kg) butanol fraction
Group 6 received (500 mg/kg) butanol fraction
Group 7 received (250 mg/kg) ethylacetate fraction
Group 8 received (500 mg/kg) ethylacetate fraction
Group 9 received (250 mg/kg) n-hexane fraction
Group 10 received (500 mg/kg) n-hexane fraction

Each rat was given 0.5 ml of castor oil one hour after dosing. One hour later, the rats were sacrificed, and the small intestine was removed after tying the ends with threads and weighed. The intestinal content was collected into a graduated cylinder, and their volume was measured. The intestine was reweighed, and the difference between the full and empty intestines was calculated [19].

% Inhibition = (Vfc –Vft) / (number of Vfc) x 100
Where Vfc = volume of fluid content in the control group, Vft = volume of fluid content in the test group

Gastrointestinal Motility Test

The intestinal motility test was carried out according to the methods of Qnais *et al*., (2005) [21], and Meite *et al*., (2009) [22]. Fifty Wistar rats were fasted for 18 hours and divided into 10 groups of 5 animals per group.
Group 1 received Hyoscine butyl bromide (5 mg/kg) and served as a positive control.
Group 2 received CMC (10 ml/kg) and served as a negative control
Group 3 received (250 mg/kg) methanol crude extract
Group 4 received (500 mg/kg) methanol crude extract
Group 5 received (250 mg/kg) butanol fraction
Group 6 received (500 mg/kg) butanol fraction
Group 7 received (250 mg/kg) ethylacetate fraction
Group 8 received (500 mg/kg) ethylacetate fraction
Group 9 received (250 mg/kg) n-hexane fraction
Group 10 received (500 mg/kg) n-hexane fraction

Thirty minutes later, each animal was orally given a charcoal meal (10 % charcoal in 5 % gum acacia; 0.2 ml/ mouse). The animals were sacrificed 30 minutes later, and the small intestine was isolated immediately. The distance traveled by the charcoal meal from the pylorus to the caecum was measured, and the percentage of Inhibition of movement was calculated as follows [19].

Peristaltic Index (PI) = (Distance traveled by charcoal) / (Total length of the intestine) × 100
% inhibition of motility PI (Control-test) / (PI control) x 100

Data obtained from the study were analyzed using Statistical Packing for Social Sciences (SPSS-25). Results were expressed as mean ± SEM. Raw data were subjected to one-way analyses of variances (ANOVA) followed by post hoc Tukey's test. $p< 0.05$ was considered statistically significant.

Table1: Effects of crude extract and solvent fractions of the leaf of *C. albidum* on castor oil-induced diarrhoea

Treatment/Dose (mg/kg)	Onset of diarrhoea (Min)	No of wet faeces	Total No of faeces	Average weight of Wet faeces (gm)	Average weight of total feces (gm)	% inhibition of defecation	%WWFO	%WTFO
Control	79.77±0.33	10.55±1.36	11.09±0.67	0.43±0.09	0.46±.0.03			
Loperamide (3mg/kg)	175±1.54	1.08±0.60	2.90± 0.50	0.08±0.03	0.12 ± 0.02	89.76*	18.60*	26.09*

Methanol extract (250 mg/kg)	109.67±12.15	6.83± 0.70	8.50± 0.72	0.20±0.02	0.24 ± 0.08	35.26	46.51	52.17
Methanol extract (500 mg/kg)	145.0± 21.77	4.90±0.50	6.40± 0.68	0.14±0.03	0.17 ± 0.03	53.55 *	32.56*	36.96*
Butanol fraction (250 mg/kg)	104.33 ± 6.14	5.17± 0.40	5.67± 0.49	0.15±0.07	0.19 ± 0.04	50.99*	34.88*	44.19*
Butanol fraction (500 mg/kg)	136.00 ± 29.02	3.83± 0.87	4.33± 1.05	0.12±0.06	0.14 ± 0.06	63.69*	27.91*	32.56*
Ethylacetate fraction (250mg/kg)	123.33 ± 23.81	5.40 ± 1.00	7.33± 1.08	0.19±0.05	0.21 ± 0.06	48.82	44.19	36.96*
Ethylacetate fraction (500mg/kg)	173.8± 1.03*	1.20±0.49	2.27±0.72	0.09±0.08	0.14 ± 0.03	78.48*	20.93*	30.43*
n-hexane fraction (250 mg/kg)	140.5± 19.99	7.17± 0.65	9.67± 0.76	0.22±0.09	0.26 ± 0.04	32.04	51.16	56.52
n-hexane fraction (500 mg/kg)	152.50±26.89*	2.67±0.76	3.17±0.83	0.10±0.04	0.15±0.04	74.69 *	23.26*	34.88 *

Values are expressed as mean of 5 replicates ±SEM. Values with (*) are significantly comparable to the control values (p<0.05). WWFO = Weight of wet fecal output, WTFO = weight of total fecal output

Table 2: Effects of crude extract and solvent fractions of the leaf of *C. albidum* on enteropooling

Treatment/Dose (mg/kg)	Volume of intestinal contents (ml)	% inhibition	Weight of intestinal contents (gm)	% inhibition
Control	0.86 ± 0.07		1.20 ± 0.03	
Loperamide (3 mg/kg)	0.19 ± 0.08	77.91*	0.26 ± 0.03	94.00*
Methanol (250 mg/kg)	0.64 ± 0.04	25.58	0.77 ± 0.05	35.83
Methanol (500 mg/kg)	0.50 ± 0.03	41.86	0.52 ± 0.02	56.67
Butanol fraction (250 mg/kg)	0.58 ± 0.03	32.56	0.81 ± 0.04	33.33
Butanol fraction (500 mg/kg)	0.49±0.08	43.02	0.73±0.08	39.17
Ethyl Acetate fraction(250 mg/kg)	0.22 ± 0.02	74.42*	0.47 ± 0.02	60.83*
Ethyl Acetate fraction(500 mg/kg)	0.20 ± 0.05	76.74*	0.24 ± 0.02	80.00*
n-hexane fraction (250 mg/kg)	0.38±0.08	55.81*	0.40 ± 0.08	66.67*
n-hexane fraction (500 mg/kg)	0.25±0.04	70.93*	0.35±0.07	70.83*

Values are expressed as mean of 5 replicates ±SEM. Values with (*) are significantly comparable to the control values (p<0.05)

Table 3: Effects of crude extract and solvent fractions of the leaf of *C. albidum* on intestinal motility

Treatment Dose (mg/kg)	Length of small intestine (cm)	Distance moved by the charcoal meal (cm)	Peristaltic index (%)	% inhibition
Control	56.17 ± 1.42	51.32± 1.94	91.37 ± 2.25	
Hyoscine butyl bromide (5 mg/kg)	56.83 ± 1.11	14.00 ± 1.41	24.63 ± 0.32	73.04
Methanol (250 mg/kg)	58.33 ± 0.80	46.12 ± 2.43	79.07 ± 1.37	13.46
Methanol (500 mg/kg)	55.67 ± 1.12	40.05 ± 1.09	71.94 ± 0.27	21.27
Butanol fraction (250 mg/kg)	59.17±1.38	42.15±1.96	49.47±3.47	45.86
Butanol fraction (500 mg/kg)	59.50±1.41	30.00±1.33	50.42±0.48	44.82
Ethylacetate fraction (250 mg/kg)	51.37 ± 2.01	21.22 ± 1.65	41.31 ± 1.53*	54.79*
Ethylacetate fraction (500 mg/kg)	56.13±1.08	12.06±0.46	21.46±1.39*	76.51*
n-hexane fraction (250 mg/kg)	55.50±1.09	41.00±1.39	73.87±2.60	19.15
n-hexane fraction (500 mg/kg)	50.17 ± 1.89	17.27 ± 2.43	34.42 ± 1.91*	62.33*

Values are expressed as mean of 5 replicates ±SEM. Values with (*) are significantly comparable to the control values (p<0.05)

RESULTS AND DISCUSSION

Acute Toxicity

No death was recorded after 24 hours of administration of the various doses of the extract and fractions in both phase 1 and phase 2 of the study. Two doses of the extract and the fraction 250 mg/kg and 500 mg/kg were used for the study, since up to 5000 mg/kg there was no sign of toxicity and there was no death.

Castor Oil-Induced Diarrhoea Evaluation

The results of the effects of the extract and fractions on castor oil-induced diarrhoea are presented in table 1. The standard control used in the study which was loperamide delayed onset of diarrhoea and inhibited defecation by 89.76 %. Methanol plant extracts at a dose of 500 mg/kg delayed the onset of diarrhoea and significantly (p<0.05) inhibited diarrhoea occurrence by 53.55 %. Butanol fraction at 250 and 500 mg/kg significantly (p<0.05) inhibited the frequency of stooling by 53.55 and 63.69 % respectively. The ethyl acetate fraction at 500 mg/kg significantly (p<0.05) delayed the onset of stooling and inhibited diarrhoea by 78.48 %. Also, n-hexane fraction at 500 mg/kg significantly (p<0.05) delayed the onset of stooling and inhibited diarrhoea by 74.69 %. These significant inhibitions of defecation also had positive significant effect on the weight of wet faecal output, and weight of total fecal output, as both the weight of wet faecal output and total faecal output reduced significantly.

Castor Oil-Induced Enteropooling

The results of the effects of the extract and fractions on castor oil-induced enteropooling are presented in Table 2. The ethyl acetate fraction at 250 mg/kg and 500 mg/kg significantly (p<0.05) compared to the control decreased both the volume of intestinal content and weight of the intestinal contents by 74.42 and 60.83 %, and by 76.74 and 80.00 %. The n-hexane fraction at 250 mg/kg and 500 mg/kg significantly (p<0.05) decreased both the volume of intestinal content and weight of the intestinal contents by 55.81 and 66.67 %, and by 70.93 and 70.83

Intestinal Motility Test

The result of the effects of the extract and fractions is presented in table 3. The ethyl acetate fraction at 250 mg/kg and 500 mg/kg inhibited the movement of a charcoal meal within the small intestine and decreased the PI significantly ($p < 0.05$) by 54.79 and 76.51 % compared to the negative control. The n-hexane fraction at 500 mg/kg also inhibited the movement of a charcoal meal within the small intestine and decreased the PI significantly ($p < 0.05$) by 62.33 % compared to the negative control.

The in vivo antidiarrhoea activity of crude extracts and solvent fractions was evaluated in three models of diarrhoea using castor oil. Castor oil is known to induce diarrhoea by enhancing the volume of intestinal content by preventing water reabsorption. This effect is attributed to ricinoleic acid found in castor oil, which stimulates irritation and inflammation of the intestinal mucosa. Consequently, this leads to the release of prostaglandins, causing alterations in mucosal fluid and electrolyte transport. As a result, the prevention of NaCl and water reabsorption triggers a hypersecretory response, ultimately leading to diarrhoea [23-25]. Enteropooling is the accumulation or pooling of fluid within the intestines, primarily in the lumen of the gastrointestinal tract. In the case of castor oil, the relevance of enteropooling arises from substances such as ricinoleic acid, which is present in castor oil. This acid can induce alterations in fluid and electrolyte transport in the intestines, potentially leading to the build-up of fluid and the onset of diarrhea [20].

The effectiveness of medicinal plants in treating diarrhoea is associated with their phytochemical makeup, encompassing flavonoids, tannins, saponins, sugars, sterols, and/or terpenes [22-26]. Research indicates that the leaves of *C. albidum* harbor most of these phytochemicals [27], contributing significantly to the observed antidiarrhoea effects in this study. The mechanisms underpinning the anti-diarrhoeal properties of extracts from traditional medicinal plants include antisecretory actions, facilitating intestinal absorption, antispasmodic or antimotility effects, and anti-microbial actions [28, 29].

Following the administration of CO at a dosage range of 0.1–0.3 ml, diarrhoea onset typically transpires within 1 to 2 hours [30]. In this research, a 0.5 ml dose of castor oil-induced diarrhoea in negative controls, lasting an average of 79.77 minutes. The study demonstrates a dose-dependent antidiarrhoeal effect of the extract and its fractions, as detailed in table 1. This suggests that the phytochemicals responsible for this action are present in the polar, semi-polar, and nonpolar solvents utilized in this investigation.

The presence of flavonoids is crucial for the antisecretory properties found in medicinal plants [31]. Consequently, the activity observed across all three doses of the ethyl acetate and n-hexane fractions in a castor-induced enteropooling antidiarrhoeal model might be attributed to the dosage-dependent existence of tannins and flavonoids within them. Flavonoids and tannins share certain characteristics, particularly in their classification, wherein condensed tannins derive from the polymerization of flavonoids [32].

The presence of phytochemicals like tannins, flavonoids, terpenoids, and alkaloids in medicinal plant extracts is linked to their anti-motility effects [33]. Hence, the observed reduction in motility at doses of 250 mg/kg and 500 mg/kg using the ethyl acetate fraction, and at 500 mg/kg using the n-hexane fraction, could be ascribed to distinct semi-polar and nonpolar constituents present within these fractions.

CONCLUSION

The study's findings revealed that the ethyl acetate and n-hexane fractions extracted from C. albidum consistently exhibited significant antidiarrhoea effects across the three models employed in this research. Moreover, both the methanol extract and butanol fraction also demonstrated notable antidiarrhoea effects. These results validate the traditional medicinal use of this plant for treating diarrhoea, underscoring the need for additional investigations to isolate the precise

phytochemicals responsible for this activity. Further studies are imperative to elucidate the molecular mechanisms through which these compounds operate in alleviating diarrhoea symptoms.

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Conflict of Interest: There are no competing interests.

Author Contributions

EAP, EII and ECO conceived and designed the study. EAP carried out the study. EII and ECO supervised the study. EAP and EII analyzed the data. EAP, EII, and ADL contributed to the writing of the manuscript. Authors reviewed and approved the final manuscript

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Ethics Approval

Ethical approval for the study was obtained from the Enugu State University of Science and Technology Animal Ethics Committee, with the approval number ESUT/AEC/0152/AP091.

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