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RESEARCH ARTICLE

ANTI-DIARRHEAL ACTIVITY OF ETHANOL AND CHLOROFORM SEED EXTRACT OF *COLA NITIDA* IN EXPERIMENTALLY INDUCED DIARRHEA

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Abstract

Objective: *Cola nitida* has been used in traditional medicine to treat diverse ailments including diarrhea. This study is carried out to investigate the antidiarrheal activity of the ethanol and chloroform seed extract of *Cola nitida* in wistar albino rats.
Methods: The ethanol and chloroform extracts of *Cola nitida* were evaluated with different doses (100 mg/kg, 300 mg/kg and 650 mg/kg of animal weight) orally for antidiarrheal activity using castor oil induced-diarrhea, gastrointestinal motility test and castor oil-induced gastroenteropooling in Wistar albino rats. The observed activity was compared to standard antidiarrheal drug Lopermaide hydrochloride (2 mg/kg) and to distilled water (10 ml/kg) which served as the negative control.
Results: *Cola nitida* ethanol extract at 150, 300 and 650 mg/kg showed 55.64%, 59.73%, and 71.34% inhibition in gastrointestinal motility respectively. A significant reduction in diarrheal episodes ($p<0.0001$) was also observed with 650 mg/kg of both extracts showing 100% inhibition. A reduction in the volume of fluid in the small intestine was also seen, this was however not significant. The chloroform extract of *Cola nitida* on the other hand produced a significant reduction in volume and weight of small intestinal content ($p<0.05$) with 650 mg/kg showing a 92.73% inhibition of intestinal fluid accumulation.
Conclusion: The ethanol and chloroform extract of *Cola nitida* showed anti-diarrheal activity in animal model by decreasing the frequency of defecation and by reducing gastrointestinal motility and intraluminal fluid accumulation in the intestine.
Keywords: Anti-diarrheal, *Cola nitida*, gastro-intestinal motility, seeds.

INTRODUCTION

Diarrheal disease is the second main cause of mortality in children below five years old, and is accountable for the mortality of approximately 525 000 children each year[1]. As a result of this, The World Health Organization set in motion a control program in 1988 to investigate traditional medical practices and other associated areas[2]. Diarrhea can continue for many days, and can cause the body to lose water and salts needed to survive. Formerly, the principal causes of deaths from diarrhea were severe dehydration and fluid loss. Certain factors including bacterial infections are now likely to account for an increasing number of all diarrhea-related deaths. Diarrhea occurring in undernourished children and people living with HIV could be potentially fatal[3]. In Ghana, it is the third main cause of mortality in children under the age of five[4]. Diarrhea has undesirable effects on the growth and development of cognitive ability in children[5]. An approximated 94% of the burden of diarrheal disease is attributed to the environment, and is associated with risk factors such as contaminated drinking water, low socio economic condition, lack of adequate sanitation and poor hygiene[6].

Cola nitida (*Vent.*) Schott and Endl. fruits have been employed traditionally as an aphrodisiac, appetite suppressant, to alleviate morning sickness, migraine, and indigestion[7]. It has also been used to relieve inflamed or wounded skin[8]. The bitter twigs of *C. nitida* have also been used for teeth and gum cleaning[9]. *C. nitida* is indigenous to West Africa and the nuts are obtained from cola trees. Cola has a broad number of species that have been widely cultivated some of which are *C. anomala*, *C. verticillata* (Thonn.) Stapf, *C. acuminata* (Pal. de Beauv.) Schott and Endl. and *C. nitida* (Vent.) Schott and Endl. are the most prevalent of the edible species[10]. The fruits are commonly used

by students, drivers, and other menial workers to prevent hunger and thirst and as stimulant to keep awake and combat exhaustion[11]. Cola trees are best known for their seeds or nuts which are rich in caffeine and other secondary metabolites such tannins, phenols and xanthine[12]. It was also reported that the plant had antidepressant and antidiarrheal activity[11]. It is known that castor oil induces diarrhea through its active metabolite, ricinoleic acid which causes small intestine peristalsis which in turn leads to changes in the permeability of electrolytes in the intestinal mucosa[13]. Ibeh *et al*., evaluated the antispasmolytic and anti-diarrheal activity of the methanol extract of *C. nitida*[14] and since cola nut is believed to possess antidiarrheal activity by traditionalists, this research is carried out to investigate the effect of the ethanol and chloroform crude extract of *C. nitida* on diarrhea. When confirmed pharmacologically, *C. nitida* stands the chance of further studies to isolate the active constituent responsible for activity.

MATERIALS AND METHODS

Plant Material

C. nitida seeds were obtained from Akwatia, a town in the Eastern Region of Ghana. The samples were identified and authenticated by Madam Anna Naa Quarley Quartey of Department of Pharmacognosy and Medicinal Chemistry School of Pharmacy, Central University, Ghana. A specimen of the sample was submitted to the University's herbarium with number CN003.

Plant Preparation and Extraction

The *C. nitida* seeds were crushed into granules using mortar and pestle. A quantity of the comminuted granules equivalent to 2580.16 g was extracted with ethanol (70%) and 1500 g extracted with 1500 ml of chloroform by cold maceration[15]. The mixture was shaken vigorously to enhance the extraction process and filtered after seven days to obtain the filtrate. The filtrate was evaporated using rotary evaporator (Drawell RE100 pro) to obtain the dry crude extract which was then stored in a refrigerator at 4ºC until ready for animal experimentation.

Phytochemical Screening

The ethanol and chloroform crude extracts of *C. nitida* were investigated for the presence of the following phytochemical constituents: phlobatannins, tannins, flavonoids, saponins, reducing sugars, alkaloids, cardiac glycosides using standard methods[15].

Experimental Animal

Wistar albino rats (95-120 g) of both sexes obtained from The University of Ghana Animal House were employed for this experiment. The rats were kept in standard plastic cages in a room with controlled 12 hrs light and dark cycle. They had unrestricted access to clean water and were fed with standard pelleted commercial feed. The animals were allowed to acclimatize for 14 days before the experiments. The study was carried out according to the National Research Council Guide for the Care and Use of Laboratory Animals[16] and Organization for Economic Cooperation and Development (OECD) guidelines[17]. The experiments were carried out in the Pharmacology Laboratory of Central University Ghana.

Acute Toxicity Study

The acute toxicity of *C. nitida* was determined through the oral route. The rats were fasted for 24 hours and doses up to 2000 mg/kg[17] of the ethanol and chloroform extract of *C. nitida* were administered to rats of weight between the range of ninety to one hundred gram (90-100 g) orally and rats were observed closely for the first six hours and subsequently periodically for seven days for mortality and any delayed toxic manifestations.

Gastro-Intestinal Motility Test

Gastrointestinal (GI) motility test was carried out according to standard methods[18] with slight modifications. Transit time of gastrointestinal content was measured at three doses of the ethanol and chloroform *C. nitida* extract (150 mg/kg, 300 mg/kg and 650 mg/kg) with distilled water (10 ml/kg) as negative control and Loperamide hydrochloride (2 mg/kg) as positive control. All administrations were done orally with an oral gavage. All animals were administered 1 ml of activated charcoal which served as a marker one hour after pretreatment. Animals were then sacrificed by cervical dislocation. The small intestines (from pylorus to caecum) were harvested and distance travelled by activated charcoal was measured and percentage inhibition of gastrointestinal motility was calculated. **Castor Oil-Induced Diarrhea Test**

This test was performed according to standard methods[19,20]. Rats were fasted for 18 hours and were divided into five groups. The nature of fecal matter (put into three categories solid, semi-solid, liquid), and frequency of defecations were measured over a period of 6 hrs. Rats in the first group were administered distilled water (10 ml/kg), group two received standard drug Loperamide hydrochloride (2 mg/kg) while groups three, four and five received 100 mg/kg, 300 mg/kg and 650 mg/kg of ethanol and chloroform extracts of *C. nitida* seeds respectively. Castor oil (1 ml) was used to induce diarrhea in all experimental groups one hour after administration. Rats were placed in individual cages lined with absorbent paper. Percentage inhibition of diarrhea was calculated.

Castor Oil-Induced Gastroenteropooling test

The activity of *C. nitida* on the inhibition of the accumulation of intraluminal fluid was ascertained by measuring the volume and weight of fluid accumulated in the small intestine over a period of time[21]. Rats were placed into five groups of five and pretreated as described above. One hour after pretreatment, rats were administered 1 ml of castor oil and were sacrificed after another hour by cervical dislocation. The small intestine from the pylorus to caecum was harvested and the contents of each small intestine was emptied in a graduated measuring cylinder and weighed. The volume and weight was recorded and percentage inhibition of secretion was calculated.

Statistical Analysis

Statistical analysis was carried out using Graph Pad Prism 8.0. All data were summarized as mean±SEM (n=5). Multiple comparison tests were ascertained by one-way ANOVA along with post-hoc Tukey's honest

significant difference (HSD) test. $p<0.05$ was taken as statistically significant.

RESULTS

Phytochemical Screening

Results of different chemical tests on the ethanol and chloroform extracts of the seeds of *C. nitida* showed the presence of flavonoids, tannins and other constituents.

Acute Toxicity Test

Administration of doses up to 2000 mg/kg of the ethanol extract of *C. nitida* orally did not produce any mortality nor any visible toxic manifestations. The chloroform extract of *C. nitida* produced mortality at 2000 mg/kg (50%), no deaths were observed when the dose was reduced to 1000 mg/kg.

Gastrointestinal motility test

A significant dose-dependent inhibition of intestinal motility was observed by the ethanol ($p<0.01$ to $p<0.001$) and chloroform ($p<0.05$) extract of *C. nitida* compared to the negative control as described in the table below. Loperamide hydrochloride produced the highest inhibition on gastrointestinal motility activity than the highest dose of both the ethanol and chloroform extracts.

Table 1: Results of phytochemical screening of ethanol and chloroform seed extract of *C. nitida*.

Phytochemical Constituent	*C. nitida* (Ethanol extract)	*C. nitida* (Chloroform extract)
Tannins	+	+
Saponins	+	+
Phlobatannins	+	+
Reducing Sugars	+	+
Alkaloids	+	+
Flavonoids	+	+
Cardiac glycosides	+	+
Phenols	+	-
Anthraquinones	+	+

Present (+); Absent (-)

Table 2: Effect of ethanol and chloroform extracts of *C. nitida* on inhibition of gastrointestinal motility.

Treatment group	Dose (mg/kg)	Average length of small intestine/cm	Distance travelled by charcoal meal/cm	% inhibition
Distilled water	10	74.58	62.10±4.46	16.73
Loperamide HCl	2	86.06	18.36±4.60****	78.67
C. nitida (Ethanol Extract)	150	77.46	34.36±5.15**	55.64
	300	75.00	30.20±3.22***	59.73
	650	77.80	22.30±5.54****	71.34
C. nitida (Chloroform Extract)	150	70.20	66.6±0.58*	5.13
	300	70.60	63.2±3.11*	10.48
	650	77.40	68.1±4.42*	12.02

Mean±SEM (n=5). *$p<0.05$ ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$

Table 3: Effect of ethanol and chloroform extract of *C. nitida* on castor oil-induced diarrhea in rats.

Treatment groups	Dose (mg/kg)	Average no. of watery stools±SEM	%inhibition of diarrhea
Distilled water	10	12.00±0.4472	0.00
Loperamide HCl	2	1.000±0.3162****	91.67
C. nitida (Ethanol Extract)	150	5.200±1.393****	56.67
	300	2.000±1.140****	83.33
	650	0.000±0.000****	100
C. nitida (Chloroform Extract)	150	3.2±0.8602****	73.33
	300	2.4±1.122****	80.00
	650	0.000±0.000****	100

Mean±SEM (n=5). **** $p<0.0001$

Castor oil-induced diarrheal test

The ethanol ($p<0.0001$) and chloroform ($p<0.0001$) extracts of *C. nitida* significantly inhibited diarrhea with 650 mg/kg of both extracts producing 100% inhibition of diarrhea. All doses of the ethanol and chloroform *C. nitida* extracts significantly reduced the frequency of watery stools.

Castor oil-induced gastroenteropooling

The chloroform extract of *C. nitida* caused a significant decrease in the volume ($p=0.0039$) and weight of small intestinal content ($p<0.05$) with 650 mg/kg producing 92.73% reduction in volume of small intestinal content. A reduction was also observed with the ethanol extract, this was however not significant.

DISCUSSION

Plant or plant parts are used traditionally for the management of diarrheal episodes without any scientific evidence to validate their use. This study was conducted to assess the antidiarrheal activity of the ethanol and chloroform extracts of *C. nitida*, which are considered to be effective in the management of diarrhea among the Ashantis in Ghana. Castor oil-induced diarrhea test model, gastrointestinal motility test and castor oil-induced enteropooling test were employed to ascertain the anti-diarrheal activity of *C. nitida* extracts in this study. Castor oil-induced diarrhea test is employed to evaluate the anti-diarrheal effect of plants. Ricinoleic acid, the active constituent of castor oil is implicated in its diarrheal effect by stimulating

peristaltic activity in the small intestine which leads to a change in permeability of electrolyte in the intestinal mucosa. It can also stimulate the release of endogenous prostaglandins which in turn result in the stimulation of secretion and motility[22]. Alkaloids, tannins, flavonoids and saponins are some of the phytochemical constituent present in the ethanol and chloroform extracts of *C. nitida* (Table 1). Flavonoids have been reported to inhibit intestinal motility and prostaglandin synthesis by altering the synthesis of the cyclo-oxygenase enzymes[23,24]. Tannins present in the ethanol and chloroform extracts of *C. nitida* will form precipitates with the proteins present in the small intestine to form tannates which will in turn make the mucosa resistance to any chemical change and therefore reduce peristalsis and secretion[25,26].

Table 4: The effect of the ethanol and chloroform seeds extract of *C. nitida* on castor oil-induced gastroenteropooling.

Treatment groups	Dose (mg/kg)	Average weight of small intestinal content±SEM	Average volume of small intestinal content ±SEM	% Reduction in volume of intestinal content
Distilled water	10	1.354±0.16	1.10±0.02	0
Loperamide HCl	2	1.574±0.13	0.42±0.19	61.82
C. nitida	150	1.148±0.36	0.44±0.19	60.00
(Ethanol	300	1.186±0.13	0.40±0.18	63.64
Extract)	650	1.574±0.35	0.60±0.28	45.45
C. nitida	150	0.632±0.13**	0.46±0.09	58.18
(Chloroform	300	1.920±0.17*	0.58±0.15	47.27
Extract)	650	0.600±0.15**	0.08±0.08**	92.73

Mean±SEM (n=5). *$p<0.05$, **$p<0.01$ compared to the control

This study showed that ethanol and chloroform extracts of *C. nitida* had anti-diarrheal effect in all experimental models employed. In the gastrointestinal motility test, the extracts decreased the transit of charcoal meal dose dependently (Table 2). The ethanol extract at 150 mg/kg, 300 mg/kg and 650 mg/kg showed higher inhibition of gastrointestinal motility (55.64%, 59.73% and 71.34%) compared to the chloroform extract at the same doses (5.13%, 10.48% and 12.02%). The percentage inhibition of gastrointestinal motility was comparable to that shown by the standard drug Loperamide hydrochloride. A reduction in motility in the gastrointestinal tract lengthens the time substances spend in the intestine thereby allowing for more water absorption[27]. It can therefore be postulated that the reduction in gastrointestinal propulsion observed may be as a result of the anti-motility properties of the constituents present in the *C. nitida* extracts. Studies have reported the anti-diarrheal activity of tannins and flavonoids as a result of their ability to reduce motility in the small intestine[28,23]. In the castor oil-induced diarrhea test, the ethanol and chloroform extract of *C. nitida* produced significant decrease in the number of watery stools which may be due to its ability to inhibit the synthesis of prostaglandin stimulated by the action of castor oil. Maximum anti-diarrheal effect was seen with the highest dose (650 mg/kg) of the ethanol extract of *C. nitida* (Table 3) rather than the standard antidiarrheal drug Loperamide hydrochloride. This might also be as a result of the phytochemical constituents like tannins, alkaloids and saponins present in the extracts (Table 1) that may increase the time for water and electrolyte absorption by inhibiting intestinal motility[29]. For the castor oil-induced gastroenteropooling test, the chloroform extract of *C. nitida* showed better activity compared to the ethanol extract (Table 4). The chloroform extract was able to significantly inhibit the accumulation of intraluminal fluid relative to the control and the maximum reduction in volume of small intestinal content was shown by the highest dose of the extract. It can therefore be postulated that the inhibition of intestinal fluid accumulation observed may be as a result of the inhibition of the release of prostaglandin and consequently increasing the reabsorption of water and electrolytes. All doses of the ethanol extract of *C. nitida* showed better anti-diarrheal activity in the gastrointestinal motility test and castor oil-induced diarrhea test than the chloroform extract which showed significant activity in the castor oil-induced gastroenteropooling test. This activity may be as a result of phytochemical constituents in the extracts working singly or together.

CONCLUSIONS

This study showed that the ethanol and chloroform seed extract of *C. nitida* extract possessed significant anti-diarrheal activity which may be as a result of the presence of phytochemical constituents like tannins, flavonoids, saponins and alkaloids. This study therefore provides pharmacological basis for the use of *C. nitida* for the management of diarrhea in some rural communities in Ghana.

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AUTHOR'S CONTRIBUTION

Doe P: writing original draft, methodology, investigation. **Ametepey NK:** formal analysis, data curation, conceptualization. **Mshelia VC:** writing, review and editing. **Otchere DB:** supervision,

methodology, formal analysis. **Fofana O:** data curation, conceptualization. **Amissah AA:** writing, review, and editing. **Yeboah PN:** methodology, data curation. **Lamptey Mills HN:** writing, review, and editing, data curation. **Ansah-Abrokwah E:** writing, review and editing, data curation. Final manuscript was read and approved by all authors.

DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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