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EFFECT OF EXTRACT AND FRACTIONS OF STEM BARK OF *MORINDA LUCIDA* BENTH (RUBICACEAE) ON CASTOR-OIL INDUCED DIARRHEA IN MICE

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ABSTRACT

Morinda lucida Benth is a common Nigerian medicinal plant generally used to cure malaria parasite infection, though there is a lack of scientific reports on its capacity to treat diarrhea. The current study evaluated the effect of extract and fractions of stem bark of *Morinda lucida* Benth (Rubicaceae) on castor-oil-induced diarrhea in mice. The ground (500 g) was macerated in 2500 mL of methanol for 72 h, thereafter filtered and concentrated to obtain methanol extract. The extract (10 g) was subjected to gradient elution to afford different fractions. The phytochemical screening method was used to investigate the constituents of extract and fractions. The method of Lorke's was employed in the acute toxicity study. The evaluation of anti-diarrhea activity was carried out by the castor-Oil-induced gastro-intestinal motility model. The secondary metabolites present in the extract and fractions were alkaloids, flavonoids, saponins, tannins, terpenoids, cardiac glycoside, anthraquinone glycoside, and steroids. The Acute toxicity indicated no mortality or any adverse behavioral change even at 5000 mg/kg body weight. The extract at 200 mg/kg dose gave better antidiarrheal activity than the 400 mg/kg dose indicating that the effect is not dose dependent. Among the fractions ethyl acetate at 400 mg/kg showed better activity and indicated no significant difference when compared to loperamide at 2 mg/kg dose. When compared, the antidiarrheal activity of the extract and fractions differed significantly at ($p<0.05$) with negative control. *Morinda lucida*'s stem bark has an outstanding antidiarrheal effect as a result of its copious bioactive constituents.

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INTRODUCTION

The passage of liquid or irregular stools along with an increase in the frequency of bowel movements and pain in the abdomen

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Is commonly referred to as diarrhea [1]. In underdeveloped nations, it is one of the main causes of death. The World Health Organization [2] reports that diarrhea sickness kills 525,000 children annually, the highest cause of death in children below age five, only surpassed by pneumonia. In Nigeria, diarrhea causes mortality in younger children, accounting for over 16% of all pediatric fatalities annually. The prevalence of diarrhea and its consequences has instigated the search for alternative medicine to ameliorate the effects especially in the rural areas. *M. lucida* belonging to RUBICACEAE family is a medium-sized tree growing up to 18-25 meter tall with green-shiny leaves [3]. It is a common medicinal plant used in African traditional medicine, growing around the tropical regions of Central and West Africa [4,5]. Different plant parts of *M. lucida* alone and in combination with other plant parts are extensively used to treat of various diseases: malaria, sickle cell, hypertension, as a tonic and diabetes [6-10]. However, using a castor oil-induced diarrhea model in mice, the study attempted to evaluate the antidiarrheal property extract and fractions of *M. lucida* stem bark.

MATERIALS AND METHODS

Collection and Preparation of the Plant Material

Morinda lucida stem bark was harvested from *Morinda lucida* tree located beside the Faculty of Pharmaceutical Sciences, Enugu State University of Science and Technology (ESUT), Agbani, Nkanu Local Government Area of Enugu State, Nigeria. It was authenticated in the Department of Pharmacognosy, ESUT where a Herbarium specimen with Herbarium number with FPS/Cog/13001 was deposited. The stem bark was air-dried under shade for 14 days, thereafter it was ground to a coarse particle.

Experimental Animals

The mice were sourced from the animal house of the Department of Pharmacology, Enugu State University of Science and Technology Agbani, Enugu. They were kept in aluminum cages then made available free access to drinking water and feed. Prior to the investigation, the mice were acclimatized within laboratory environment for 24 h. The use of laboratory animals was approved ethically, and reference number ESUT/FPS/PHA/2023/028 was received.

Extraction and Fractionation

The pulverized sample (778.50 g) was macerated in 2500 mL of methanol and kept for 3 days with intermittent agitation for extraction. The filtrate obtained after filtration was concentrated using a rotary evaporator at reduced pressure to get methanol extract. The extract (56.6 g) was dissolved in methanol and mixed with silica gel (70-230 mesh), dried and triturated to obtain fine particle which was loaded into the vacuum liquid chromatography column. The elution was carried out using (500 mL, n-hexane), (1200 mL, ethyl acetate), n- (400 mL, butanol), and (500 mL, methanol) to obtained the various fractions respectively.

Phytochemical Screening of Extract and Fractions

Qualitative phytochemical analysis of extract and fractions of *M. lucida* stembark was carried out according to the standard method [11-12].

Acute Toxicity Study

The Lorke's method described preciously was employed [13]. For the first phase, 10, 100 and 1000 (mg/kg) of extract was administered to Group A - C. For second phase 1600, 2900 and 5000 (mg/kg) of extract was administered to Groups D - F. Observable sign for any behavioral changes and mortality in the mice was monitored for 24 h after treatment.

Evaluation of Anti-Diarrhea Activity

Castor Oil-Induced diarrhea Model as previously described was used [14]. Forty (40) mice of both sexes weighing 17-29 g were fasted for 18 hours without access to drinking water and food. The animals were grouped into seven, having five mice per group. The Negative control group 1 was given normal saline, positive control group 2 treated with 2 mg/kg of loperamide, methanol extract group 3 and 4 received (200 mg & 400 mg/kg) doses, ethyl acetate fraction group 5, n-butanol fraction group 6, methanol fraction group 7 received 400 mg/kg dose respectively. After an hour, each mouse was given 5% activated charcoal suspension 1 mL, and 0.5 mL of castor oil orally. The mice were slaughtered after 30 minutes to remove the small intestine from the pylorus to the cecum. Then, measurements were made of the full length of the intestine and the length of the intestine covered by the charcoal meal. The peristaltic index and percentage of inhibition were expressed using equations 1 and 2.

Peristalsis index = $\frac{DT}{L1} \times 100$ .....Equation 1

Where: DT = distance charcoal meal traveled

LI = length of the small intestine

Percentage of inhibition = $\frac{DC-Dt}{Dc} \times 100$ .....Equation 2

Where: Dc = the mean distance control group traveled

Dt = the mean distance test group traveled

Statistical Analysis

The mean ± standard error of means, One-way ANOVA and post-hoc Turkey's multiple comparison test were used to evaluate statistical differences between groups. When the p-value was less than 0.05, the findings were deemed significant.

Table 1: Percentage yield of extract and fractions of stem bark of *M. lucida*

Extract/Fractions	Yield (g)	Percentage Yield (%)
Methanol extract	72.07	9.3
n-hexane fraction	2.6	4.6
Ethyl acetate fraction	13.70	24.2
n-butanol fraction	8.10	14.3
Methanol fraction	10.20	18.02

Table 2: Phytochemical constituents of extract and fractions of *M. lucida* stem bark.

Phytoconstituent	Methanol extract	*n*-hexane fraction	Ethyl acetate fraction	n-butanol fraction	Methanol fraction
Alkaloid	+	+	+	-	-
Flavonoid	+	-	+	+	+
Saponins	+	-	+	+	+
Tannins	+	-	-	-	+
Terpenoids	+	+	+	-	-
Cardiac glycosides	+	-	+	+	+
Anthraquinones glycosides	+	-	+	+	+
Steroids	+	+	+	-	-

Key: + present and - absent

Table 3: Phase 1 and 2 acute toxicities

	Dose (mg/kg)	Dose (mg/kg)	Dose (mg/kg)
Phase 1			
Methanol extract	10	100	1000
Mortality	0/3	0/3	0/3
Phase 2			
Methanol extract	1600	2900	5000
Mortality	0/3	0/3	0/3

Table 4: Effect of extract and fractions on castor oil-induced diarrhoea in mice.

Group	Samples	Dose	Length of intestine (cm)	Distance charcoal travelled (cm)	Peristalsis Index
A	Negative control	5ml N/saline	40.30±2.18	37.10±2.26	92.06%
B	Loperamide	2mg/kg	40.70±1.34	8.20±0.93*	20.15%
C	Extract	200mg/kg	40.90±1.57	11.18±1.75*	27.33%
D	Extract	400mg/kg	36.50±1.74	24.60±1.64*	67.40%
E	EAF	400mg/kg	40.06±1.90	10.70±1.90*	26.71%
F	*n*-BF	400mg/kg	34.50±1.04	28.67±0.67	83.09%
G	MF	400mg/kg	35.20±1.68	14.00±1.52*	39.77%

Measurements were statistically analyzed as mean ± SEM. The significant difference was set at *p<0.05. * Represent a group where there is a significant difference in distance travelled by charcoal when compared with the negative control. EAF = ethyl acetate fraction, *n*-BF = *n*- butanol fraction and MF = methanol fraction.

RESULTS

Percentage Yield

The yield and percentage yield presented in table 1 showed that ethyl acetate faction gave the best yield indicating that the stem bark of *M lucida* has abundance of moderately polar compounds while *n*- hexane fraction gave the poor yield among the fractions.

Phytochemical Constituents of Stem Bark of *M. lucida*

The qualitative phytochemical screening revealed the presence of mostly polar bioactive constituents in the extract and fractions as shown in table 2.

Acute Toxicity

The acute toxicity study result obtained showed no mortality in both phases 1 and 2 experiment as presented in the table 3.

Anti-Diarrheal Activity

The extract, ethyl acetate and methanol fractions were able to significantly reduce the distance travelled by the charcoal meal at doses of 200mg/kg and 400mg/kg whereas n-butanol fraction did not exhibit antidiarrheal activity at dose of 400 mg/kg.

DISCUSSION

This study confirmed the antidiarrheal properties of the stem bark of *Morinda lucida*. The following bioactive constituents: Alkaloids, flavonoids, saponins, tannin, steroids, terpenoids, cardiac glycoside, and anthraquinone glycoside were found in *M. Lucida* stem bark. The present result corroborated previous findings that *Morinda lucida's* therapeutic properties were attributed to its bioactive flavonoids, anthraquinones, terpenoids, saponins, alkaloids, and cardiac glycosides [15]. The acute toxicity study of extract showed no mortality and behavioral changes within 24 h of the study even at dose up to 5000mg/kg. It established the safety of the *Morinda lucida* stem bark in treatment of ailments [16-17].

The castor oil releases autocoids and prostaglandins which are implicated in the pathophysiology of diarrheal because ricinoleic acid induces diarrhea by stimulating secretory processes and release of the endogenous prostaglandins causing diarrhea. It alters the permeability of electrolytes in the intestinal mucosa, so leading to Na^+/K^+ ATPase inhibition in the intestine [18-19]. The antidiarrheal evaluation indicated that the methanol extract and fractions of *Morinda lucida* stem bark demonstrated a very good anti-diarrhea activity comparable to standard drug. The methanol extract had better activity at 200mg/kg (69.87% and 27.33%) indicating that the observed effect was not a dose dependent. The ethyl acetate fraction at 400mg/kg gave best antidiarrheal activity with percentage inhibition of 71.2% among the fractions which is similar to the standard drug whose percentage inhibition was 77.90%. The results showed that phytoconstituents present in *M. lucid* stem bark were responsible for the observed activity. Flavonoids and anthraquinones which are present in the most active fraction have been reported to exhibit antidiarrheal activity by restricting intestinal motility and hydroelectrolytic secretions just like loperamide [20-21].

CONCLUSION

There was a reasonable reduction in the rate of gastrointestinal motility in mice by the extract and fractions of *M. lucida* stem bark. Ethyl acetate fraction demonstrated significant reduction among the fractions. This may be due to the plant's abundance of bioactive metabolites such as terpenoids, flavonoids, and anthraquinones. This study provided insight to the potential use of the *M. lucida* stem bark for management of diarrhea condition.

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AUTHORS' CONTRIBUTION

The conceptualization and proofread of the manuscript were done by Ali Ibeabuchi Jude, the literature collection and first draft of manuscript were done by Ugwu Obiora Celestine, Adonu Cyril Chekwube and Omeh Romanus Chijioke. The experimentation and data analysis were done by Asogwa Felix Kenolisa, Okorie Ndidiamaka Hannah, Okonkwo Raymond Maduabuchi, Obidiegwu Onyeka and Onyegbulam Patrick Chibueze while Okoye Festus Basden Chiedu supervised the work.

CONFLICT OF INTEREST

The authors declared no conflict of interest

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