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(RESEARCH ARTICLE)

Ethanol leaf extract of *Dialium guineense* produces anti-diarrhoeal and gastrointestinal motility slowing activities in Wistar rats

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Abstract

Dialium guineense leaves have been traditionally used for the treatment of diarrhoeal diseases in Nigeria. The study was conducted to evaluate the anti-diarrhoeal and gastrointestinal motility slowing effects of the ethanol leaf extract of *D. guineense* in Wistar rats. The anti-diarrhoeal and gastrointestinal motility slowing effects of the ethanol leaf extract of *D. guineense* were evaluated using castor oil-induced diarrhoeal model, charcoal meal, and anti-enteropooling tests in Wistar rats. The test groups received various doses (100, 200, and 400 mg/kg) of the extract whereas positive controls received Loperamide (4 mg/kg) or Atropine (5 mg/kg) and negative controls received Normal Saline (10 ml/kg). The phytochemical screening as well as the acute toxicity test of the extract was also performed. The extract produced a dose-dependent significant reduction in the watery nature and frequency of fecal droppings in the castor oil-induced diarrhea. On gastrointestinal transit time and enteropooling, the extract also dose-dependently reduced the small intestinal transit of charcoal meal and intestinal fluid volume in a manner comparable to 5 mg/kg of atropine sulphate and 4 mg/kg of loperamide. The acute toxicity study on the extract revealed an oral LD50 value greater than 5000 mg/kg in mice. The phytochemical constituents detected in the extract were tannins, phenols, resin, alkaloids, saponins, terpenoids, and glycosides. The findings from this study showed that the ethanol leaf extract of *D. guineense* possesses anti-diarrhoeal properties and thus supports the traditional application of the leaf extract in the treatment of diarrhea in Nigeria.

Keywords: Anti-diarrhoeal; Dialium *guineense*; Leaf extract; Phytochemical; Rats

1. Introduction

Globally, there are nearly 1.7 billion cases of childhood diarrheal disease every year. It is the second leading cause of death in children under five years old, accounting for the death of 525 000 children every year. Children under three years old experience on average three episodes of diarrhea every year in low-income countries [1]. The prevalence of childhood diarrhea in Nigeria is 10% [2].

Diarrhoeal illness in early childhood may be associated with long-term adverse cognitive effects and decreased work productivity later in life [3]. Key measures to treat diarrhea in children include rehydration with intravenous fluids in case of severe dehydration, oral rehydration salt (ORS) solution for moderate or no dehydration, and zinc supplements

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to reduce the duration of a diarrhea episode and stool volume [2]. Other measures are traditional remedies, behavioral change communication evaluation as well as prevention approaches by World Health Organization [4]. Despite these measures, diarrhoeal disease remains a big public health challenge in developing countries like Nigeria [5]. Therefore, it becomes very important to identify and evaluate commonly available natural drugs as alternatives to currently used anti-diarrhoeal drugs which are not completely free from adverse effects.

Dialium guineense, also known as Velvet tamarind or black velvet is a genus of a legume belonging to the family of fabaceae and sub-family of caesalpinioideae. The genus *Dialium* comprises five species in West Africa but *Dialium guineense, Dialium dinklagel, Dialium packyphylum* are represented in Nigeria [6]. The plant is widespread in Nigeria. It is commonly known as "icheku" among the Igbo in the Eastern part of Nigeria, as "awin" among the Yoruba in

the western part of Nigeria and as "tsamiyarkurm" among the Hausa in the northern part of Nigeria [7]. The leaves and stem bark are used as folklore remedies for the treatment of infections and other ailments such as diarrhoea, severe cough, bronchitis, wound stomach ache, malaria fever, jaundice, peptic ulcer disease, haemorrhoids and prevention of cancer [8]. Ogu and Amiebenomo, 2012 [9] demonstrated that *Dialium guineense* stem bark extract possessed anti-diarrhoeal activity *in vitro*.

The present study was undertaken to investigate the anti-diarrhoeal effect of the ethanol leaf extract of *Dialium guineense* in Wistar rats.

2. Material and methods

2.1. Collection and Identification of Plant Material

Fresh leaves of *Dialium guineense* were harvested from a farm in Ezza North LGA of Ebonyi State, Nigeria. The plant material was identified and authenticated by Mr. Chijioke Onyeukwu of the department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria where a voucher specimen (No UNH.111) was deposited in the herbarium for reference.

2.2. Extraction of the Plant Material

The leaves were washed, air-dried, and pulverized to a coarse powder. The coarse powder (600g) of *D. guineense* was soaked in 1.5L of ethanol for 48 h at room temperature. The extract was obtained by filtration followed by evaporation of the solvent on the organ bath at a reduced temperature (45˚C). The recovered extract was stored in an airtight container and used for the study.

2.3. Phytochemical Analysis

The phytochemical screening of ethanol extract of *D. guineense* leaf was carried out to determine the presence of the following compounds; alkaloids, glycosides, phenols, resins, saponins, flavonoids, steroids, tannins, terpenoids, carbohydartes, proteins, fats and oil using standard procedures by Mukherjee (2006) as well as Oloyede (2005) [10], [11].

2.4. Experimental Animals

Wistar albino rats (180-200 g) of both sexes were obtained from the Animal Unit, Faculty of Medicine, Ebonyi State University, Abakaliki, Nigeria. They were kept in the animal house of the College of Health Sciences, Ebonyi State University, Abakaliki. The rats were housed in clean plastic cages and cared for under a controlled temperature of 25 $\pm$1°C, 12 h light and 12 h dark cycle, and 60% humidity. They were fed on a standard diet and had free access to water. The animals were handled according to the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animal [12].

2.5. Acute Toxicity Study of the Extract

The LD_{50} of the leaf extract was tested to determine its safety following the method as described by Lorke (1983) method [13]. The study was done in two phases. In the first phase, nine mice were randomized and divided into three groups of three mice per cage. The mice in each group were orally administered with 10 mg/kg, 100 mg/kg, and 1000 mg/kg of the leave extract respectively. The animals were observed in the first four hours and 24 hours for signs of toxicity such as muscle paralysis, respiratory distress, hyperactivity, writhing, and mortality. This was followed by the second phase in which 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of the extract were administered to the other three groups with three mice per cage. The signs of toxicity and mortality were observed for 24 h, 48 h, and 72 h.

2.6. Induction of Diarrhoea with Castor Oil

The method described by Akuodor *et al.* [14] was employed to demonstrate the effect of the extract on castor oil-induced diarrhea models in rats. Twenty rats that fasted for 24 h with access to water were randomized into four groups of five rats each. Group 1 which served as the negative control received 10 ml/kg of normal saline. Group 2 was administered 4 mg/kg of loperamide (standard drug) as positive control while 3, 4, and 5 were administered 100, 200, and 400 mg/kg of the extract orally. At 30 minutes after the treatment, rat in all the groups was administered 1 ml castor oil orally to induce diarrhea. The rat in each group was placed in a separate cage with adsorbent paper lined on the floor. The diarrhea episode was observed for 4 h and the total number of diarrhea (drops) was recorded and compared with the controls. The percentage (%) inhibition of diarrhea was calculated following the formula [15].

$$\% \text{ inhibition of defecation} = (A\text{-}B) \times 100$$

Where A is the mean number of defecation caused by castor oil and B is the mean number of defecation caused by drug or extract.

2.7. Intestinal Transit Test

The effect of the extract on gastrointestinal transit or motility was evaluated according to the method described by Akuodor *et al*. [16]. Twenty rats were randomly divided into four groups of five rats each. They were fasted for 24 h before the test with free access to water. Group 1 which served as a negative control was treated with 10 ml/kg normal saline. Group 2 which served as a positive control was given 5 mg/kg of atropine sulphate (standard drug) while 3, 4, and 5 received 100, 200 and 400 mg/kg of ethanol leaf extract of *D. guineense* orally respectively. Thirty minutes after this drug administration, 1 ml of marker-charcoal meal (5% deactivated charcoal suspension in 10% tragacanth) was administered orally to all rats. At 30 minutes after charcoal meal administration, all the animals were sacrificed. The small intestine of each animal was excised and the distance travelled by the charcoal meal was expressed as a percentage of the length of the small intestine according to the expression.

$$\% \text{ inhibition } = \frac{\text{Mean length of intestine } - \text{ mean distance travelled by meal}}{\text{Mean length of intestine}} \times 100$$

2.8. Castor Oil-Induced Enteropooling

The effect of ethanol leaf extract of *D. guineense* on intestinal fluid accumulation or castor oil-induced enteropooling in rats was done according to the method of Akuodor *et al.* [14]. Twenty rats were randomly divided into four groups of five rats each. Group 1 which served as negative control received 10 ml/kg normal saline, while Groups 2 serving as a positive control was administered 4mg/kg of loperamide (standard drug), 3, 4, and 5 received 100, 200 and 400mg/kg of the extract, respectively. All were administered orally. Immediately after this administration, 1 ml of castor oil was administered orally to all the rats. After 30 minutes, all the rats were sacrificed. The small intestine was excised and the intestinal contents were milked quantitatively into a measuring cylinder to obtain the volume and mass of the intestinal content was determined by using the formula [15].

$$\% \text{ inhibition of intestinal fluid } = \frac{\text{Control test extract}}{\text{Control}} \times 100$$

2.9. Statistical Analysis

Data were expressed as the mean ± standard error of the mean (SEM) of five determinations. The significance of the difference between means was determined using a one-way analysis of variance (ANOVA) followed by Dunnetts's *post hoc* test [17]. Statistical significance was established at $p < 0.05$.

3. Results

3.1. Phytochemical Screening

Phytochemical screening of the extract revealed the presence of alkaloids, glycosides, phenols, resins, saponins, tannins, terpenoids, carbohydrates and proteins while flavonoids, steroids, fats, and oils were not detected (Table 1).

3.2. Acute Toxicity Test

There was no mortality observed upon oral administration of the ethanol leaf extract of *D. guineense* in mice, even at doses as high as 5000 mg/kg. The ethanol leaf extract did not produce any clinical signs of toxicity in mice during 72h observation period. Hence the LD_{50} in mice was considered to be greater than 5000 mg/kg.

3.3. Effect of the ethanol extract on Castor Oil-Induced Diarrhoea

The ethanol leaf extract at 100 mg/kg, 200 mg/kg and 400 mg/kg produced a dose-dependent and significant ($p<0.05$) protection of rats against castor oil-induced diarrhea which led to the decreased number of feces. Loperamide a standard anti-diarrhea drug produced a higher inhibitory effect than the highest dose of *D. guineense* (400 mg/kg) used (Table 2).

3.4. Effect of the ethanol extract on intestinal transit

Administration of the extract produced dose-dependent and significantly ($p<0.05$) slowed the propulsive movement and transit of charcoal meal. The atropine sulphate at 5 mg/kg showed a more anti-motility effect than 400 mg/kg of the extract used.

3.5. Anti-enteropooling effect

The ethanol leaf extract dose-dependently and significantly *($p<0.05$)* decreased the masses and volumes of intestinal fluid with corresponding increases in inhibition of the intestinal fluid content **-** induced enteropooling model. The intestinal fluids of the animals pre-treated with the ethanol leaf extract of *D. guineense* and loperamide were found to be more viscous than those of rats treated with distilled water.

Table 1 The phytochemical constituents of ethanol leaf extract of *Dialium guineense*

Metabolites	Relative Presence
Alkaloids	+
Glycosides	++
Phenols	+++
Resins	++
Saponins	++
Tannins	++
Terpenoids	++
Carbohydrates	+++
Proteins	+++
Flavonoids	-
Steroids	-
Fats and Oil	-

Key: + =Present in low quantity; ++ = Present in moderate quantity; +++ = Present in large quantity; - = absent

Table 2 The effect of ethanol leaf extract of *D. Guineese* on Castor oil-Induced diarrhea in rats

Drug	Dosage (mg/kg)	Diarrhea episode in 4 h	% Protection
Distilled water	10ml/kg	10.00±1.08	-
Loperamide	6	0.25±0.25	98[b]
D. guineense	100	5.75±1.55	43[a]
D. guineense	200	5.00±0.71	50[a]
D. guineense	400	4.50±1.50	55[a]

Values are mean ± SEM (n=4) [a]Significantly different from control at $p < 0.05$; bSignificantly different from control at $p < 0.01$

Table 3 The effect of ethanol leaf extract of *D. guineese* on intestinal transit time in rats

Drugs	Dosage (mg/kg)	Intestinal length (cm)	Distance travelled by charcoal meal (cm)	% Inhibition
Distilled water	10ml/kg	78.50±3.66	65.00±4.62	-
Atropine	5	82.00±0.0	11.00±2.25	83[b]
D. guineense	100	92.25±2.95	31.50±5.90	52[a]
D. guineense	200	94.00±5.02	29.50±3.56	55[a]
D. guineense	400	84.00±5.34	24.63±7.42	63[a]

Values are mean ± SEM (n=4) [a]Significantly different from control at $p < 0.05$; bSignificantly different from control at $p < 0.01$

Table 4 The effect of ethanol leaf extract of *D. Guineese* on castor oil induced intestinal fluid accumulation in rats

Drugs	Dosage (mg/kg)	Volume of intestinal contents (ml)	% inhibition of secretion
Distilled water	10mg/kg	1.70±0.44	_
Loperamide	6	0.15±0.17	91[b]
D. guineense	100	0.63±0.1	63[a]
D. guineense	200	0.38±0.06	78[b]
D. guineense	400	0.25±0.03	85[b]

Values are mean ± SEM (n=4) [a]Significantly different from control at $p < 0.05$; [b]Significantly different from control at $p < 0.01$.

4. Discussion

The phytochemical analysis of the ethanol leaf extract of *D. guineense* revealed the presence of alkaloids, proteins, carbohydrates, glycosides, phenols, resins, saponins, tannins and terpenoids which shows that the plant is of high pharmacological importance. Earlier studies showed that anti-diarrhoeal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, steroids, and terpenoids [18], [19]. These constituents have been reported to possess anti-diarrhoeal activity through the inhibition of intestinal motility, anti-secretory effect, and anti-microbial action [20].

The ethanol leaf extract of *D. guineense* did not cause any mortality or showed any visible signs of toxicity in the animals even up to 5000 mg/kg. This shows that *D. guineense* is a relatively non-toxic substance and may be responsible for its widespread use in different ethno-therapeutic interventions. Thus it can safely be used as a pharmacological agent especially in the treatment of diarrhoeal diseases in children.

The leaf extract showed significant activity in reducing the frequency of castor oil-induced diarrhea which is comparable to that of the standard anti-diarrhoeal drug, loperamide. Castor oil causes diarrhea through inhibition of intestinal Na^+K^+ATPase activity which reduces normal fluid absorption, activation of adenylate cyclase mediated active secretion, stimulation of prostaglandin secretion, and nitric oxide [21], [22]. Inhibitors of prostaglandin biosythensis delay castor oil-induced diarrhea [23], [24]. Loperamide can antagonize diarrhea caused by castor oil, through its anti-motility, anti-secretory activities, and inhibitory effect on prostaglandin. It is most likely that the extract may share a similar mechanism of action with loperamide. This finding agrees with observations made by other researchers [9], [25].

Also, the oral administration of leaf extract of *D. guineense* significantly reduced gastrointestinal propulsion as observed by the decrease in transit motility of charcoal meal. This activity is comparable to that of atropine which was used as a reference drug and which is known to reduce intestinal motility through its anticholinergic effect [26]. This observation suggests the ability of the plant to alter the normal peristaltic movement in the intestine leading to a decrease in the movement of intestinal contents thus allowing greater for absorption. It is also possible that flavonoids present in the extract may have contributed to the antidiarrheal effect through its ability to inhibit intestinal motility and hydro-electrolytic secretion [24]. This finding is in line with the reports of other researchers [9], [25].

In the fluid accumulation study, the leaf extract dose-dependently and significantly reduced the volume of the rat intestinal contents. This activity may promote reabsorption of material in the intestines thus leading to the prevention or cure of diarrhea when present. This observation is comparable to a similar report which has established the anti-enteropooling effect of the plant [9], [25]

5. Conclusion

The result of this investigation indicated that the ethanol leaf extract of *D. guineense* has significant anti-diarrhoeal, anti-motility, and anti-enteropooling effects. This validates its folk use in the treatment of diarrhea. Further studies are required to evaluate the exact mechanism of action of this plant and possibly to determine the active compound responsible for the observed anti-diarrhoeal activity.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

The experiments were carried out following the institutional guidelines of the Ethics Committee of Ebonyi State University (EBSU/DRIC/UREC/ 04/052). The animals were handled according to the guidelines of the National Institute of Health Guide for the Care and Use of Laboratory Animal.

References

[1] World Health Organization. Diarrhoeal disease: Health topics [Internet]. 2017.

[2] World Health Organization. Diarrhoea: why children are still dying and what can be done Geneva: UNICEF/WHO. [Internet]. 2009.

[3] Guerrant RL, Kosek M, Moore S, Lorntz B, Brantley R, Lamb AAM. Magnitude and Impact of Diarrheal Diseases. Arch Med Res. 2002; 33(4): 351–5.

[4] Ezeigbo II, Ejike CECC, Ezeja MI, Eneh O. Antioxidant and anti-diarrhoea activity of Manniophyton africanum leaf extract in mice. Cont J Anim Vet Res. 2010; 2: 41–7.

[5] Oloruntoba EO, Folarin TB, Ayede AI. Hygiene and sanitation risk factors of diarrhoeal disease among under-five children in Ibadan, Nigeria. Afr Health Sci. 2014; 14: 1001–1011.

[6] Omotayo FO. Plants of south-Western Nigeria. University of Ibadan, Nigeria. 1999; 139.

[7] Nwosu MO. Plant resources used by traditional women as herbal medicines and cosmetics in Southeast-Nigeria. Arztezeitschrift fur NaturheilverFahren. 2000; 41(111).

[8] Bero J, Ganfon H, Jonville M, Frédérich M, Gbaguidi F, DeMol P, et al. In vitro antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. J Ethnopharmacol. 2009; 122(3): 439–44.

[9] Ogu GI, Amiebenomo R. Phytochemical analysis and in vivo anti-diarrhoeal Potentials of Dialium guineense stem bark-extract. J Intercult Ethnopharmacol. 2012; 1(2): 105–110.

[10] Mukherjee PR. Quality control of herbal drugs, an approach to evaluation of botanicals. 13th ed. New Delhi: Business Horizones Publishers. 2006.

[11] Oloyede OI. Chemical Profile of Unripe Pulp of Carica papaya. Pakistan J Nutr. 2005; 4(6): 379–81.

[12] National Research Council of the National academies. Guide for the care and use of laboratory animals. 8th ed. Washington DC: National Academies Press. 2011.

[13] Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983; 4(4): 275–87.

[14] Akuodor GC, Ibrahim JA, Akpan JL, Okorie AU, Ezeokpo BC. Phytochemical and anti-diarrhoeal properties of methanolic leaf extract of Maerua crassifolia Forssk. European J Med Plants. 2014; 4(10): 1223–31.

[15] Qnais EY, Elokda AS, Ghalyun YYA, Abdulla FA. Antidiarrheal Activity of the Aqueous Extract of Punica granatum. (Pomegranate) Peels. Pharm Biol. 2007; 45(9): 715–20.

[16] Akuodor GC, Muazzam I, Usman-Idris M, Megwas U A, Akpan JL, Chilaka KC, et al. Evaluation of the Antidiarrheal Activity of Methanol Leaf Extract of Bombax Buonopozense in Rats. Ibnosina J Med Biomed Sci. 2011; 3(1): 15–20.

[17] Dunnett C, Goldsmith C, Buncher CR, T. JY. Statistics in the pharmaceutical industry. 2nd ed. New York NY: Marcel Dekker. 1993.

[18] Akuodor GC, Mbah CC, Essien AD, Akpan JL, Ezeokpo BC, Iwuanyanwu TC, et al. Ulcer-protective and anti-diarrhoeal effects of the aqueous stem bark extract of Bridelia ferruginea in rodents. Pharmacologia. 2012; 3(11): 591–597.

[19] Al-Rehaily AJ, El-Tahir KEH, Mossa JS, Rafatullah S. Pharmacological Studies of Various Extracts and the Major Constituent, Lupeol, obtained from Hexane Extract of Teclea nobilis in Rodents. Nat Prod Sci. 2001; 7(3): 76–82.

[20] Daswani PG, Brijesh S, Tetali P, Antia NH, Birdi TJ. Antidiarrhoeal activity of Zingiber officinale (Rosc.). Curr Sci. 2010; 98(2): 222–229.

[21] Mascolo N, Izzo AA, Gaginella TS, Capasso F. Relationship between nitric oxide and platelet-activating factor in castor-oil induced mucosal injury in the rat duodenum. Naunyn Schmiedebergs Arch Pharmacol. 1996; 353: 680–684.

[22] Capasso F, Mascolo N, Izzo AA, Gaginella TS. Dissociation of castor oil-induced diarrhoea and intestinal mucosal injury in rat: effect of NG-nitro-L-arginine methyl ester. Br J Pharmacol. 1994; 113(4): 1127–1130.

[23] Awouters F, Niemegeers CJ, Lenaerts FM, Janssen PA. Delay of castor oil diarrhoea in rats: a new way to evaluate inhibitors of prostaglandin biosynthesis. J Pharm Pharmacol. 1978; 30(1): 41–5.

[24] Di Carlo G, Autore G, Izzo AA, Maiolino P, Mascolo N, Viola P, et al. Inhibition of intestinal motility and secretion by flavonoids in mice and rats: structure-activity relationships. J Pharm Pharmacol. 1993; 45(12): 1054–9.

[25] Nworgu ZAM, Ifebuna A. Evaluation of the antidiarrhoeal activity of the aqueous leaf extract of Dialium guineense in mice. J Pharm Bioresour. 2015; 12(1).

[26] Longanga-Otshudi A Vercruysse A. Contribution to the ethnobotanical, phytochemical and pharmacology studies of traditionally used medicinal plants in the treatment of dysentery and diarrhoea in Lomela area, Democratic Republic of Congo (DRC). J Ethnopharmacol. 2007; 71(3): 411–423.