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ANTI-DIARRHEAL AND ANTI-MICROBIAL EFFECTS OF *XYLOPIA AETHIOPICA* SEEDS

Victoria Nonyelum Umeh[1]*, Ugochinyere Amarachukwu Olli[2] and Jude Nnaemeka Okoyeh[3]

[1]Department of Pharmacology and Toxicology, Chukwuemeka Odumegwu Ojukwu University Igbariam campus Nigeria.
[2]Nnamdi Azikiwe University Teaching Hospital, Nnewi Nigeria.
[3]Biology and Clinical Laboratory Science, Department of Mathematics and Sciences, School of Arts and Sciences, One Neumann Drive, Neumann University, Aston, PA, 19014. USA.

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***Corresponding Author**
Victoria Nonyelum Umeh
Department of Pharmacology and Toxicology, Chukwuemeka Odumegwu Ojukwu University Igbariam campus Nigeria.

ABSTRACT

Background: Traditionally, the seed extract of *X. aethiopica* is used as a spice for the prevention of food-induced diarrhea but there is no scientific data to support this claim. **Method:** The powdered seeds of *X. aethiopica* were cold macerated with ethanol and the filtrate dried. Phytochemical analysis and acute toxicity studies were carried out using the extract. The charcoal meal method and the castor oil (1 ml/kg body weight) induced diarrhea models were employed for the gastro intestinal track (GIT) inhibitory effects and anti-diarrheal studies respectively. Twenty five albino rats divided into five groups (n=5) were employed for each arm of the study. In each study, groups 1 and 2 served as negative and positive control having received 10 ml/kgbw normal saline and 2 mg/kgbw loperamide or 1mg/kgbw Atropine (for GIT inhibitory effect) per oral respectively. Groups 3 received 250 mg/kgbw and 4 was administered with 500 mg/kgbw of the extract. Agar well diffusion method was used for the anti-microbial study. The data were analyzed by Statistical Package for Social Sciences using one way ANOVA and the difference between the mean values were considered statistically significant at $p<0.05$. **Result:** The extract exhibited LD50 that is greater than 5000 mg/kg. At 250 and 500 mg/kg doses, the seed extract caused a significant ($p<0.05$) increase in time of diarrhea onset, and significant ($p<0.05$) reduction in GIT motility when compared to control. The extract also displayed anti-microbial effect against the tested micro-organisms. Conclusion: The folkloric use of *X. aethiopica* seeds as anti-diarrhea may therefore be justified.

KEYWORDS: *Xylopia aethiopica*, anti-diarrhea, GIT motility, anti-bacteria, loperamide.

1. INTRODUCTION

1.1 Background: For centuries, herbal medicines have played a vital role in the field of medicine.[1] Herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products that contain as active ingredients parts of plants, or other plant materials, or combinations.[2] In addition, traditional medicine products can be liquid (decoctions, infusions, oily mixtures, and gargles), solids (powders for internal administration with hot maize pap or other drinks), semi-solids (crude balsams, resins, latex) or gaseous (steam inhalation preparations, fumigations like incense).[3]

Xylopia aethiopica is an aromatic tree which grows up to 15–30 m high and about 60–70 cm in diameter.[4] It belongs to the family of *Annonaceae* and it is largely found in West, Central and Southern Africa in the humid forest zones especially along rivers.[4] Xylopia is the Greek word (‘xylon pikron’) for ‘bitter wood’, while aethiopica refers to its Ethiopian origin. Its common names include; African pepper, Guinea pepper, spice tree, Negro pepper, West African pepper and Senegal pepper.[5] It is called Udah by the Igbo speaking tribe of Eastern Nigeria. The leaves are simple, alternate, oblong and elliptic to ovate while the flowers are bisexual, solitary or in 3-5 flowered fascicles or in strange, sinuous, branched spikes, or cymes, up to 5.5 by 0.4 cm and creamy-green. Fruits of *Xylopia aethiopica* look like small, twisted bean-pods which are dark brown, cylindrical, 2.5 to 5 cm long and 4 to 6 mm thick. Each pod contains about 5 to 8 kidney-shaped seeds grains of approximately 5 mm length.[4]

Figure 1: Dried fruits of *Xylopia aethiopica(*Annonaceae).

1.2 Ethno medical uses of *Xylopia aethiopica*

The odiferous roots of the plant are employed in tinctures, administered orally to expel worms and other parasitic organisms from the intestines. The extract is also used in rinsing the teeth and as mouth wash against toothaches.[4] In Eastern part of Nigeria the grounded seeds are added while preparing Okpa (a delicacy made from Bambara nuts) in order to prevent diarrhea experienced by some people after eating Okpa.

Diarrhea is defined as stool emission with an excess weight of 200 grams per day.[6] Diarrhea is the second most frequent fatal childhood disease after pneumonia, estimated to be the cause of 1.34 million deaths in children worldwide.[7]

1.3 Types of diarrhea

Secretary diarrhea

This occurs when absorption of sodium in the villi is impaired while secretion of chloride in the crypt cells continues to increase. In this case the toxins from the microorganisms stimulate secretion of fluid. This occurs during cholera virus infection or food poisoning.

Invasive diarrhea

This results due to the disruption of the intestinal mucosal cells as a result of invasion by bacteria, protozoa, or parasites.

Motility diarrhea

This manifest as a result of increased motility of the gastrointestinal tract (GIT) resulting in a decrease in the transit time of food or drink across the GIT. This gives less chance for the contents to be absorbed, it can occur due to some disease condition such as thyrotoxicosis, hyperkalemia or due to the use of purgatives.

Osmotic diarrhea

Osmotic diarrhea occurs due to decreased absorption of osmotic active substance which draws fluid from the GIT by osmosis hence the stool is left loose or watery. This could manifest due to lactose intolerance and laxatives.

Presently diarrhea is usually managed by replacing fluid and electrolytes, with addition of antibiotic where necessary.[8]

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Collection and authentication of plant materials

Some dried fruits of *Xylopia aethiopica* were bought from Awka main market (Eke-Awka) located at Awka, Anambra State in Nigeria and authenticated by a taxonomist at the department of botany Nnamdi Azikiwe University Awka Anambra State in Nigeria where a herbarium specimen: NAUH-08^{B} (Seed) was kept.

2.1.2. Chemicals, reagents and drugs

Castor oil (NK Industries Ltd), Activated charcoal (Shinkwang Chem. India, Co., Ltd,), Loperamide (Jassen-Cilag AG, Gubelstrasse Switzerland), Tragacanth powder (PJ Enterprises India), Carboxy-methyl-cellulose (Union Chem China), 10% Tween 80 (Allan Chemical Corp India), Ciprofloxacin tablets (Fidson Health care Nigeria), Atropine injection (Wellona Pharma India).

2.1.3. The animals

Adult albino rats (150-200g) of both sexes were obtained from the animal house, Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University Igbariam campus, Anambra State, Nigeria. The animals were handled in compliance with the National Institute of Health guidelines for the use of laboratory animals and approved by the Faculty of Pharmaceutical Sciences Animal Research Ethics Committee with approval number: PHACOOU/AREC/2021/010. After a 24-hour fasting with access to drinking water, the weights of the rats were taken.

2.2. Methods

2.2.1. Preparation of extract

The dry seeds of *Xylopia aethiopica* were washed and further dried at ambient temperature and then grounded coarsely to enable separation of the seeds from the outer

portion. The seeds were further grounded to fine powder. The powdered seed (97g) was cold macerated with 80% ethanol for 48 hours. This was first filtered by passing it through a cotton plug and further filtered with filter paper (Whatman filter paper, No 1). The extract of *Xylopia aethiopica* was freeze-dried to a constant weight and stored at 4°C in an amber-colored bottle until required for experiments.

2.2.2 Qualitative phytochemical analysis

Screening for the presence of secondary metabolites was carried out following the standard phytochemical tests as demonstrated by.[9] The following secondary metabolites were tested for: alkaloids, glycosides, phenol, tannins, flavonoids, terpenoids, saponins, steroids, Coumarins and anthraquinones.

2.2.3 Acute toxicity study

The toxicity test was conducted using the up and down procedure (UDP) adopted by.[10] and revised by.[11] Using this method, the animals were dosed one at a time and the doses were dependent on the response of the first animal to the initial dose. The second animal receives a lower dose if the first animal dies (the initial dose is decreased by a factor of 3.2) or the second animal receives a higher dose if the first animal survives (the initial dose is increased by a factor of 3.2). Three rats weighing 150-200 g were used as starting point. Two rats served as negative control having received 10 ml/kg of distilled water orally while the test animal received a default oral dose of 5000 mg/kg of the extract. The animals were then observed continuously for 4 hours for changes in behavior and any other obvious signs of toxicity and subsequently daily for a total of 14 days for delayed toxicity.

2.2.4. Castor oil induced diarrhea study

The method described by.[12] was employed for this study. Twenty five albino rats divided into five groups (n=5) were used. Groups 1 and 2,which represents the negative and positive control were treated with distilled water (10 ml/kgbw) and loperamide (2 mg/kgbw) respectively. Diarrhea was induced by administering castor oil (1 ml/kgbw) orally to each rat. After one hour of castor oil treatment, two different doses of the extract (250 and 500 mg/kg) were administered orally to groups 3 and 4 respectively. The animals were then housed in individual plastic cages lined with white nonabsorbent paper. Fecal output was assessed by collecting the fecal material for 8 hours after drug administration. The percentage of fecal output (% FOP) was calculated as follows:

$$\% \text{ FOP} = \frac{Ft}{Fc} \times 100$$

Where Ft = fecal weight of each group, Fc = fecal weight of control group.

$$\% \text{ Inhibition of defecation} = \frac{Mo-M}{Mo} \times 100$$

Where, Mo= Defecation of control, M: Defecation of test sample/standard drug.

2.2.5. Effect of ethanol seed extract of *X. aethiopica* on gastrointestinal transit

The effect of the extract on normal gastrointestinal transit was assessed using charcoal meal method as described by.[12] Four groups of albino rats of both sexes (n=5) were used for the experiment. The rats were starved for 24 hours with unrestricted access to drinking water before the treatment. Groups 1 and 2 served as negative and positive control, having received distilled water (10 ml/kgbw) and Atropine (1mg/kgbw) respectively while groups 3 and 4 were treated (p.o) with 250 and 500mg/kgbw of the extract. After 5 minutes, 1ml of charcoal meal (5 % charcoal suspension in 2% aqueous tragacanth) was administered orally to each animal. The rats were allowed 15 minutes before being sacrificed by cervical dislocation. The intestine was tied at the position of charcoal plug (in order to secure the position), and then the intestine from pylorus to ileocaecal junction was removed carefully and stretched out. The intestinal distance moved by the charcoal plug from the pylorus was measured and expressed as percentage of the total distance from the pylorus to the ileocaecal junction. The peristaltic index and percentage of inhibition was calculated by using the following formula.[13]

$$\text{Peristalsis index} = \frac{\text{Distance travelled by charcoal mean}}{\text{Length of small intestine}} \times 100$$

$$\% \text{ inhibition} = \frac{Dc-Dt}{Dc} \times 100$$

Where, Dc: Distance travelled by the control, Dt: Distance travelled by the test group.

2.2.6 Anti-microbial study on ethanol seed extract of *Xylopia aethiopica*

The antibacterial assay of the extract was carried out using the agar well diffusion assay as described by [14]. The antimicrobial activity of the extract was tested against four standard clinical bacterial isolates namely: *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli* and two fungal isolates; *A. niger* and *C albicans*. The stock solutions of 0.5ml McFarland standard bacterial and fungal suspensions of each of the test isolates were prepared to be used for the antimicrobial assay. Mueller-Hinton agar and Sabouraud Dextrose agar were prepared according to the manufacturer's specification and were aseptically dispensed into 90 mm sterile agar plates and allowed to set. The sterile agar plates were inoculated with the test culture by surface swab method using a sterile swab stick. Then a sterile cork borer was used to make five wells (8 mm in diameter) on each of the MHA and SDA plates. Aliquots of 80 µl of each extract dilutions, reconstituted in DMSO at concentrations of 50, 25, 12.5, 6.25 and 3.13 mg/mL were applied in each of the wells in the culture plates previously inoculated with

the test organisms. Ciprofloxacin (5 µg) and miconazole (50 µg) served as the positive control in the antibacterial and antifungal assays respectively; while DMSO served as the negative control.

The cultures were incubated at 37^{o}C for 18-24 h for the antibacterial assay and at 25-27^{o}C for 48h for antifungal assay. The antimicrobial property of the extract was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). The size of the zone of inhibition indicates the activity of the extract against the test microorganisms.

2.2.7 Statistical Analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS version 20) using one way ANOVA, followed by post-hoc turkey's test for multiple comparisons. The data were expressed as mean ± standard error of mean (SEM). Graphical representation was done using Microsoft Excel 2010. The difference between the mean were considered significant at $p<0.05$.

3. RESULTS

3.1. Extraction and total yield

The weight of the crude extract obtained was 50g representing a percentage yield of 47.7%.

3.2. Phytochemical analysis

The phytochemical analysis revealed the abundance of terpenoid, alkaloid, phenol, tannins, saponin and cardiac glycoside while steroid and anthraquinone were in moderate amount (Table 1).

3.3 Acute toxicity study (LD50)

Oral administration of the extract up to 5000 mg/kgbw dose produced no change in behavior, neither was there any mortality in any of the groups. Therefore, the LD_{50} of ethanol seed extract of *X. aethiopeca* is above 5000 mg/kgbw.

3.4. Castor oil induced diarrhea

Treatment with 250 and 500 mg/kg of ethanol seed extract of *X. aethiopeca* caused a significant ($p<0.05$) increase in time of onset of diarrhea, significant ($p<0.05$) reduction of wet feces (WF), total feces (TF) and a significant ($p<0.05$) inhibition of diarrhea when compared to the control. Although the seed extract exhibited a dose–dependent anti-diarrhea effect, this inhibition was lower than that of the reference anti-diarrhea drug, loperamide (Table 2).

3.5 Effect of ethanol seeds extract of X. aethiopeca on gastric transit

The extracts (250 and 500 mg/kg) significantly ($p < 0.05$) inhibited the intestinal transit of charcoal meal in a dose-dependent manner. The extract was able to cause a percentage GIT inhibition of 7.32% (250mg/kgbw), 34.90% (500 mg/kgbw), while atropine, at 1mg/kgbw, caused 90 %GIT inhibition (Table 3).

3.6 Anti microbial study of the seed extract

At concentration range of 6.25-50 mg/ml the seed extract of *Xylopia aethiopica* showed activities against all the test bacteria and fungi with Inhibition Zone Diameters (IZDs) ranging from 2mm to 11 mm (Table 4). Highest antibacterial and antifungal activities were recorded against *P. aeruginosa* (IZD = 5mm) and *A. niger* (IZD = 11) respectively.

Table 1: Phytochemical screening of ethanol seed extract of *xylopia aethiopica.*

Terp	Gly	Alk	Flav	Phe	Tan	Ster	Sapo	Anthr	C /gly
+++	-	+++	-	+++	+++	++	+++	++	+++

Key: Absent = -, mildly present = +, moderately present =++, Strongly present =+++

Terp= terpenoid, Gly=glycosides, Flav=flavonoids, Phe= phenol, Tan=tannins, Ster=steroids, Sapo=saponins, Anthr=anthraquinone, C /gly=cardiac glycosides

Table 2: Anti-diarrheal effect of ethanol seed extract of *X. aethiopica*.

Dose (mg/kg)	Onset of DH (min)	No WF	Total number F	Average weight		Percentage		
				WF (gm)	TF gm	DH inhibition	Wt of WF	wt of TF
Dist/water 10ml	83.54±2.00	8.34±0.55	10.16±1.03	0.50±0.05	0.55±0.13	0	0	0
Loperamide 2mg	190.23±0.56*	3.17±0.76*	4.30±0.20*	0.10±0.73*	0.15±0.9*	65.42*	22.00*	27.27*
250mg ESEX	111.19±1.65*	6.55±2.00	9.97±0.44	0.31±1.77*	0.35±2.00	31.16*	62.00*	63.60*
500mg ESEX	150.00±0.76*	4.45±0.38*	5.22±0.34*	0.20±3.05*	0.27±1.6*	50.21*	40.00*	49.09*

Values are represented as mean ± standard error of mean (n=5). *p<0.05: Statistically significantly different from the control group.

Key: ESEX= Ethanol seed extract of *Xylopia aethiopica*, DH=diarrhea, F=feces, WF=wet feces, AWF=average weight of feces, WF= wet feces gm, ATF= average weight total feces, wt=weight.

Table 3: Effect of ESEX on gastrointestinal motility.

Dose/ kg	Length of small intestine (cm)	Distance moved by the charcoal meal (cm)	Peristaltic index (%)	% Inhibition
Dist water 10ml	69.5 ± 0.32	41.00 ± 0.54	58.99 ± 0.48	0
Atropine 1mg	77.0 ± 1.87	4.00 ± 0.88	5.19 ± 2.00	90.24
ESEX 250mg	97.00 ± 2.44	38.00 ± 1.56	39.18± 1.00	7.32
ESEX 500mg	112.23 ± 0.21	23.00 ± 1.69	20.49 ± 0.77	34.90

Values are represented as mean ± standard error of mean (n=5). *p<0.05: Statistically significantly different from the control group. Key: ESEX= Ethanol seed extract of *Xylopia aethiopica*.

Table 4: Antimicrobial activity of ethanol seed extract of *Xylopia aethiopica.*

Concentration (mg/mL) / Inhibition zone diameter (mm)							
Test Organisms	**50**	**25**	**12.5**	**6.25**	**3.13**	**Positive control (Ciprofloxacin)**	**Negative control (DMSO)**
S. aureus	2±0	0±0	0±0	0±0	0±0	10	0
B. subtilis	4±0	3±0	3±0	2±0	0±0	12	0
E. coli	0±0	0±0	0±0	0±0	0±0	6	0
P. aeruginosa	5±0	4±0	0±0	0±0	0±0	11	0
						Miconazole	**DMSO**
A. niger	11±0	8±0	6±0.7	5±0	5±0.7	15	0
C. albicans	7±0	6±0	6±0.7	4±0	2±0.7	6	0

4. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion

Diarrhea may occur when there is a change in active ion transport through decreased sodium absorption or increased chloride secretion, change in intestinal motility, increase in luminal osmolarity; and/or increase in tissue hydrostatic pressure.[15] Osmotic diarrhea occurs due to decreased absorption of osmotic active substance which draws fluid from the GIT by osmosis hence the stool is left loose or watery. Increase in luminal osmolarity may give rise to osmotic diarrhea which can manifest due to intolerance to certain food. In traditional setting, the powdered X. *aethiopica* seeds is added as spice when cooking certain food in order to prevent food-induced diarrheal.

The preliminary phytochemical screening of the ethanol seed extract of X. *aethiopica* (ESEX) revealed the abundance of alkaloids, terpenoids, saponin, phenols, tannins and cardiac glycosides, while steroid and anthraquinone were moderately present. The anti-diarrhea properties of some of these metabolites have been demonstrated in previous studies and they exhibit anti-diarrheal activity independently or synergistically.[16] Tannins reduce diarrhea by reducing intestinal secretion mediated by the denaturation of secretary proteins and by inhibiting the motility of intestine via altering the intracellular Ca ion levels.[17] Flavonoids are inhibitors of GIT motility and water and electrolyte secretion. The ability of saponins to cause inhibition of histamine release in vitro.[18] could be the mechanism by which it exhibits anti-diarrheal activity since inhibition of histamine (H_1) receptors produces relaxation and H_1 receptors are dominant in the gut and these receptors mediate contraction.[19]

The antioxidant property of *Xylopia aethiopica* seed extract has been reported.[20,21] This property may have played some role in the anti-diarrhea effect of ESEX.

Typical acute toxicity such as changes in general behavior, variations in body weight and mortality were absent after oral administration of ESEX during the given period of testing. Since the maximum dose level recommended by OECD is 2000mg/kg body weight, further dosing to estimate the median lethal dose (LD50) of the plant was not performed.[22] Based on the result, it can be stated that *Xylopia aethiopica's* LD50 value is greater than 2000mg/kg and this plant can be classified under category 5 in accordance with Globally Harmonized System of Classification and Labeling of Chemicals (GHSCL).[23] However according to the report,[24] the median lethal (LD_{50}) dose of *Xylopia aethiopica* fruit ethanol extract is 3000 mg/kg.

Castor oil induces diarrhea through its active compound ricinoleic acid by stimulating secretary processes and intestinal motility secondary to irritation and inflammation.[25] Hence, anti-diarrheal agents act by inhibiting one or more of these pathophysiologic processes. The result of the castor oil induced diarrhea revealed that the extract (250 and 500 mg/kg) caused a significant ($p<0.05$) increase in time of onset of diarrhea, significant ($p<0.05$) reduction of wet feces (WF), and total feces (TF) when compared to control.

This result may justify the addition of *X. aethiopica* seed powder in Okpa (a popular delicacy in Eastern part of Nigeria) when preparing this delicacy so as to prevent

diarrhea that some people experience after eating the Okpa. Though ESEX exhibited dose–dependent anti-diarrhea effects, this inhibition was lower than that of the reference anti-diarrhea drug, loperamide. The ESEX was able to cause a percentage GIT inhibition at both doses but atropine at 1mg/kg produced higher GIT inhibition (90%).

This result also confirms the anti-diarrhea property of *X. aethiopica* seed extract, justifying further its folkloric use in the prevention and treatment of diarrhea. The extract caused inhibition in the growth of the tested micro-organisms especially at higher concentration. Anti-microbial agents are used for the treatment of diarrhea.

4.2 CONCLUSION

This study has elucidated both the relative safety of the seed extract of *X. aethiopica* as well as its anti-diarrheal activity. Accordingly, the study validates the traditional use of the seed extract as anti-diarrhea agent and may be a guide to it being a potential source of new agent for the treatment of diarrhea.

4.3 Recommendation

Further chronic toxicity study on the extract is recommended since the seed is added as spice in preparation of many food delicacies.

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