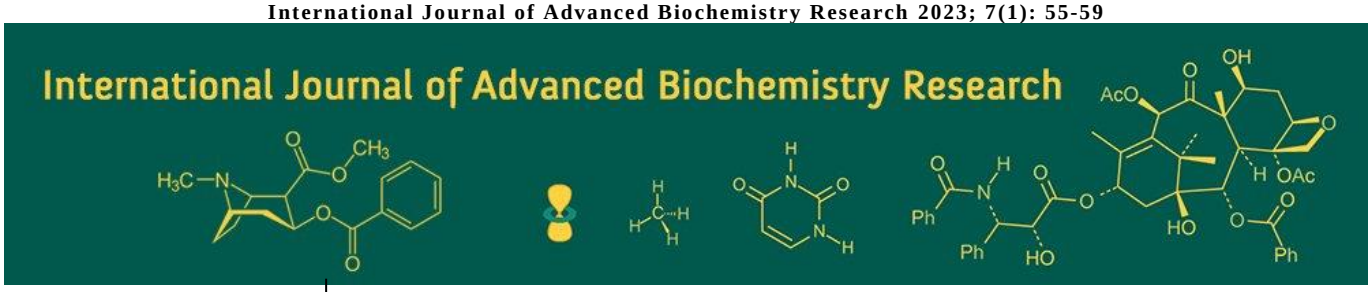

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2023; 7(1): 55-59
www.biochemjournal.com
Received: 22-03-2022
Accepted: 26-04-2022

Nwachoko Ndidi
Department of Biochemistry, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

Goodluck Samuel
Department of Biochemistry, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

Tetam Jack Gbenenee
Department of Biochemistry, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

Corresponding Author:
Nwachoko Ndidi
Department of Biochemistry, Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria

Phytochemical analysis and anti-diarrhoea potential of ethanol leaves extract of *Nypa frutican* in albino rats

Nwachoko, Ndidi, Goodluck, Samuel and Tetam Jack Gbenenee

DOI: https://doi.org/10.33545/26174693.2023.v7.i1a.168

Abstract
Diarrhoea is a leading cause of morbidity and mortality in children under the age of 5 years. Health Organization has encouraged studies that will bring about the desired treatment and prevention of diarrhoea. *Nypa frutican* commonly known as Nipa palm or Mangrove palm is used in traditional medicine for the treatment of various ailments and the leaves are locally used for making roofing mats, hats and brooms. This study was designed to evaluate the phytochemical constituents using Gas Chromatography- Flame Ionization Detector and anti-diarrhoea potential of ethanol leaves extract of *Nypa frutican* in albino rats. Fecal count, measurement of gastrointestinal charcoal meal distance and intestinal fluid electrolyte concentrations procedures were used. The quantitative phytochemical analysis revealed the presence of flavonoids, alkaloids, cyanogenic glycosides, oxalate, saponin, steroids, cardiac glycoside and tannin (35.53, 30.55, 16.88, 10.31, 9.79, 8.71, 4.86 and 1.95) µg/ml respectively. Extract of the leaves of *Nypa frutican* (200, 300 and 400) mg/kg in comparison with 5 mg/kg standard drug (loperamide hydrochloride), inhibited the production of diarrhoea stool, degree of gastrointestinal motility, and inhibited the concentration of intestinal fluid electrolyte (Na^+, K^+, Cl^- and HCO_3^-). The result obtained showed that ethanol leaf extract of *Nypa frutican* may contain some pharmacologically active substance with anti-diarrhoea potentials due its phytochemical components.

Keywords: phytochemical, anti-diarrhoea, *Nypa frutican*, electrolyte

Introduction
The plant kingdom contains a vast reservoir of biologically active substances with a variety of chemical structures, as well as qualities that can either guard against or treat disease. These physiologically active substances are referred to as phytochemicals. These phytochemicals are present in smaller amounts or concentrations in higher plants as secondary metabolites (Nonita *et al.*, 2010) [10]. These phytochemicals (secondary metabolites) can serve as building blocks for the production of novel medicines (Ajay *et al*., 2018) [1]. Some plants are referred to as medicinal plants because they have therapeutic properties or have a significant pharmacological impact on the human or animal body (Ajay *et al*., 2018) [1]. *Arecaceae* is the family name for *Nypa frutican* also called Nipaplam or Mangrove palm. It has an underground stem that sprouts into more above-ground plants (Udofia *et al*., 2005) [19]. Mangrove plant such as *Nypa frutican* produce secondary metabolites with varieties of biological uses and activities. These secondary metabolites are produced in response to the harsh settings and conditions in which the plant grows. (Nugroho *et al.*, 2020) [11]. Several studies have revealed that leaves of *Nypa frutican* contain phytochemicals. And majority of bioactive chemicals with antioxidant activity, such as flavonoids, phenolics, tannins, saponins, steroids, and triterpenoids have been found in *Nypa frutican* leaves, according to studies by Gazali and Nufus (2019) [5]. Diarrhoea is described as the frequent passage of abnormal liquid or unformed feces, and it is derived from the Greek words *dia* (through) and *rrhoea* (flow), which signify flowing through (Ahlquist, 2001 as cited in Nwachoko and Jack, 2015) [12]. The main causes of diarrhea are either when the digestive system produces too much fluid or when the contents travel through the digestive system too quickly for the stomach to absorb the fluid. Diarrhea is typically a sign of bowel infection (gastroenteritis) (Judit and Tama, 2009) [6]. Experiencing three or more loose or watery bowel movements per day is considered to be diarrheoa, which is one of the most significant health issues in underdeveloped or developing nations (Nwachoko *et al*., 2016) [14]. Chronic diarrhoea last more than four weeks and is distinguished from acute diarrhoea by the duration (Mullhaupt, 2002) [9]. Fever, thirst-related disorientation, gastrointestinal pain,

and weight loss are some typical diarrhoea symptoms and signs (Dolin, 2009) [2]. Most herbal extracts have been shown to be useful in treating diarrhea by one or more of the following mechanisms: improved intestinal absorption, anti-secretory activity, anti-motility or anti-peristaltic action, anti-microbial effect, and anti-spasmodic function (Rawat *et al*., 2017) [17]. Since orthodox medicine is expensive and not accessible to all patients, the World Health Organization (WHO) encourages the use of plants to treat diarrhoea patients (WHO 1999) [20].

Materials and Methods

Collection of plant sample

The fresh leaves of *Nypa frutican* were collected from the river bank of New Calabar River at Eagle Island in Port-Harcourt Local Government Area, Rivers state, Nigeria. The Coordinate of Eagle Island is 4˚47ˈ 20" N and 6˚58ˈ 41" E. The plant was identified and registered with the registration number RSUPb046.

Preparation of plant extract

Fresh leaves of *Nypa frutican* were air-dried to constant weight at room temperature and ground into fine powder. It was further extracted with the Soxhlet apparatus.

Soxhlet Extraction

Crude plant extract was prepared by Soxhlet extraction method. About 20 grams of powdered plant material was packed into a thimble and extracted with 500 ml of ethanol. The process of extraction continued for 24 hours or till the solvent in siphon tube of the soxhlet extractor become colourless and another powdered plant extract was weighed into a thimble and the process continued till significant grams was extracted. After that, the extract was taken in a beaker and kept on water bath and heated to about 40-50 ˚C till all the solvent evaporated. The dried extract was kept in the refrigerator 4˚C for future use in phytochemical analysis and treatment of experimental animals.

Extraction of Phytochemicals

One gram of plant sample was weighed using a weighing balance and it was transferred to a test tube and 15ml of 50% m/v potassium hydroxide (KOH) was added. The test tube was allowed to react in a water bath at 60 °C for 60 minutes and the product was transferred to a separatory funnel and 3ml of hexane were transferred to the funnel. The extracts were combined and washed three times with 10ml of 10% u/v ethanol aqueous solution. The solution was dried with dehydrated sodium sulfate and the solvent was evaporated. The sample was solubilized in 1000µl of pyridine of which 200 µl was transferred to a vial for analysis.

Quantification by GC-FID

The analysis of phytochemical was performed on a Buck M910 gas chromatography equipped with a flame ionization detector. A RESTER 15 meter MXT-1 column (15 m × 250 µm × 0.15 µm) was used. The injector temperature was 280°C with spitless injection of 2µl of sample and a linear velocity of 30cm/s. Helium 5.0pa.s was the carrier gas with a flow rate of 40ml/min. The oven operated initially at 200°C, it was heated to 330°C at a rate of 3cm/min and was kept at this temperature for five minutes. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals expressed in µg/g. the detector operated at a temperature of 320 °C.

Collection of Experimental Animals

Male albino rats were obtained from the animal house of Rivers State University, Port Harcourt, Rivers State. The animals were acclimatized for a period of seven (7) days before to the commencement of the experiment and the animals were grouped into five different groups of four (4) animals per group.

Drug used for the study

The drugs used for this study include castor oil, loperamide hydrochloride and activated charcoal

Castor oil induced diarrhea in rats and fecal counts

The method of Awouter *et al.* (1978) as cited in Nwachoko *et al.* (2016) [14] was adopted in determination of the effect of *Nypa frutican* on castor oil induced diarrhoea.

Male rats weighing 100 -110g fasted for 18 hours were randomly distributed into five (5) different groups of four (4) animals per group and the administration procedure was different for each group. Group 1 (control) received distilled water, group 2 received 200 mg/kg of *Nypa frutican* extract, group 3 received 300 mg/kg of *Nypa frutican* extract, group 4 received 400 mg/kg of *Nypa frutican* extract and group 5 received 5 mg/kg of standard drug (loperamide hydrochloride). The distilled water, extract and standard drug were given during the first hour, followed by oral administration of 1ml of castor oil in the next hour to induce diarrheoa. The time taken for onset of diarrhoea and fecal dropping were recorded and the percentage inhibition was calculated using % inhibition = (Control-test)/control X 100.

Castor oil induced diarrhea in rats and intestinal transit

Male rats weighing 100 -110g fasted for 18 hours were randomly distributed into five (5) groups of four (4) animals per group. After one hour of administration of extract (treatment) to the treatment groups, distilled water was given to group 1 (control) and standard drug to group 5, diarrhoea was induced by oral administration of 1ml of castor oil to the control and test groups. After a period of 1hr later, 1ml of charcoal meal was given to each of the animals.

The animals were sacrificed after 1hr following the administration of the charcoal meal from the pyrolus to the caecum were measured and expressed as a percentage of the total length of the intestine from the pyrolus to the caecum of each animal (Nwachoko *et al.*, 2016) [14]. The peristaltic index was calculated using the formula, PI = CML/IL X 100. Where; PI=Peristaltic index, CML = distance travelled by charcoal meal, IL = Length of small intestine.

Results

Table 1: Phytochemical components of ethanol leaves extract of *Nypa frutican*

Class of phytochemical	Components	Concentration µg/ml
Alkaloids	Spartein	17.2035
	Epihedrine	8.0615
	Lunamarine	2.8765
	Ribalinidine	2.4103
Total =		30.5518
Flavonoids	Naringenin	9.4990
	Epicatechin	6.5050
	Flavone	5.6559
	Anthocyanin	3.8487
	Catechin	2.9624
	Kaempferol	2.7186
Flavononones		2.0348
Resveratrol		1.6324
Proanthrocyanin		0.6758
Total =		35.5326
Cyanogenic glycoside		16.8874
Oxalate		10.3147
Saponins	Sapogenin	9.7996
Steriods		8.7109
Cardiac glycoside		4.8623
Tannin		1.9583

Table 2: Effect of ethanol leaves extract of *Nypa frutican* on the fecal count of castor oil-induced diarrhoea albino rats.

Group	Treatment	OD (MIN)	MWF	% I
1	Control	60	3.17	-
2	200 mg/kg	60	3.83	0
3	300 mg/kg	60	2.00	36.9
4	400 mg/kg	60	2.17	31.5
5	5 mg/kg	60	2.33	26.5

Key: OD = Onset of diarrhoea, MWF = Mean wet faeces after 6 hours, I= Inhibition.

Table 3: Effect of ethanol leaves extract of *Nypa frutican* on intestinal transit of castor oil induced diarrhoea in albino rats.

Groups	Treatment	IL(CM)	CML(CM)	PI (%)	I (%)
1	Control	29.5	21.75	73.7	-
2	200mg/kg	30.0	17.0	56.6	23.2
3	300mg/kg	30.3	20.0	66.0	10.5
4	400mg/kg	32.0	18.0	56.25	23.7
5	5mg/kg	31.5	17.0	53.9	26.8

Key: IL= Intestinal length, CML= Charcoal meal length, PI = Peristaltic Index, I= Inhibition

Table 4: Effect of ethanolic extract of *Nypa frutican* leaves on intestinal fluid electrolyte concentration of castor oil induced diarrhea in albino rats.

Groups	Treatment	K^+	Na^+	Cl^-	HCO_3^-
1	Control	3.17 ± 0.0^{bdfh}	94.13 ± 0.15^{adfh}	73.03 ± 0.15^{bdfh}	27.2 ± 0.20^{bdfh}
2	200 mg/kg	$2.23\pm0.21^{*aceg}$	85.03 ± 0.25^{acfh}	$55.03\pm0.15^{*adeg}$	$21.17\pm0.15^{*adh}$
3	300 mg/kg	$2.50\pm0.30^{*acfg}$	$83.27\pm0.15^{*acfh}$	$63.14\pm0.21^{*bcfh}$	$24.13\pm0.15^{*bcfh}$
4	400 mg/kg	$1.97\pm0.35^{*adeg}$	$71.0\pm11.0^{*bdeg}$	$54.50\pm3.50^{*adeg}$	$21.07\pm0.21^{*adeh}$
5	5 mg/kg	$2.10\pm0.10^{*aceg}$	$68.67\pm4.04^{*bdeg}$	$57.67\pm2.51^{*adeg}$	$22.97\pm0.15^{*adfg}$

Values are express as mean ± standard deviation. * the mean difference is significant at the 0.05 level when comparing group 1with other groups and different superscripts (a'b), (c'd), (e,f), (g'h) differ significantly at 0.05 level when comparing group 2, group 3, group 4 and group 5 respectively with other groups.

Discussion

Diarrhoea is characterized with having more loose or liquid bowel movement per day and it has long been recognized as one of most important health problem in developing countries with increased death rate in children under five years. *Nypa frutican* has been reported to possess pharmaceutical effects used for treatment of different diseases either in combination with other plant materials or singly, thus the need to investigate its phytochemical component and anti-diarrheoa potential. Table 1 showed the phytochemical constituent of *Nypa frutican* ethanol leaves extract which showed that flavonoids has the highest concentration followed by alkaloids and tannin has the least concentration. This conforms to earlier reports on the phytochemical constituent of *Nypa frutican* extracts which revealed the presence of alkaloids, flavonoids, cardiac glycoside, anthranoid and polyphenols (Ebana *et al*., 2015) [4]. Similar result was obtained from research done by Lestari *et al.* (2016) [7] that crude extract of *Nypa frutican* leaves contain polyphenols, flavonoids, steroids, saponin and alkaloid. These phytochemicals have various biological effects on living organisms and research by Pier-Giorgio (2000) [16] reported that flavonoids function as anti-inflammatory, anti-carcinogenic, anti-mutagenic and anti-oxidant agents. Nwiloh *et al.* (2016) [15] reported that bioactive substances with anti-malarial, anti-inflammatory, and anti-microbial properties in animals include quinolone alkaloids like lunamarine, spartein, and ribalinidine. However, the therapeutic effects of herbal remedies employed by traditional healers to treat diarrhea may be supported by the presence of several secondary metabolites, primarily alkaloids, flavonoids, saponins, phenols, tannins, steroids, and terpenoids, in various medicinal plant extracts

(Manzo *et al*., 2017) [8]. Similar study by Nwachoko *et al.* (2016) [14] reported that the anti-diarrheal properties of *Aframomum chrysanthum* seed extract are due to flavonoids and tannins, which account about 44% of the total phytochemical components of the seed.

Table 2 showed the inhibition potential of *Nypa frutican* ethanol leaf extract on the mean wet fecal count of albino rats. Group 3 animals showed highest percentage inhibition followed by group 4. However, there was no inhibition in group 2 and the least inhibition was seen in group 5. The onset of diarrhoea was recorded in all groups after 60 minutes of diarrhoea induced with castor oil. Diarrhoea stimulates peristaltic activity in the small intestine leading to changes in the electrolyte permeability of the intestine, thus increasing faecal output, as a result of increased peristaltic activity. The pharmacological assessment of a putative anti-diarrhea agent is based on a substance's or extract's capacity to lower the faeces output or suppress the mean wet fecal count of an animal or experimental unit brought on by rapid peristaltic activity (Nwachoko *et al*., 2016) [14].

Table 3 showed the inhibitory effect of ethanol leaf extract of *Nypa frutican* on castor oil induced gastrointestinal transit in albino rats. The result revealed the percentage inhibition of 26.8, 23.7 and 23.2 for group 5, 4 and 2 respectively. And the least percentage inhibition was seen in group 2. The highest charcoal meal length and peristaltic index was observed in group 1 (control) and the least charcoal meal length and peristaltic index was observed in group 5, 4 and 2 respectively. However, Group 3 showed lower percentage inhibition, higher charcoal meal length and peristaltic index when compared to other groups. This study revealed that charcoal meal length is directly proportional to peristaltic activity or index and the ability of the extract to reduce peristaltic activity proves its anti-diarrheoa potential, causing a reduction in charcoal meal length. This conform to similar research which have been established that many anti-diarrhea medications work by decreasing gastrointestinal tract transit or motility (Nwachoko and Oghale, 2017) [13].

Table 4 shows the effect of *Nypa frutican* ethanol leaves extract on electrolyte concentration of castor oil induced diarrheoa in albino rats. Numerous crucial biological processes involving organisms involve electrolytes. The conductivity of nerve impulses, the contraction of muscles, hydration, and pH regulation are all facilitated by electrolytes (Downs *et al*., 2020) [3]. Ricinoleic acid, which is present in castor oil, irritates and then inflames the intestinal mucosa. Prostaglandin is thus released, changing the mucosa's permeability to fluid and electrolyte transport. This hinders the reabsorption of Na+, Cl-, and water or fluid, causing an increase in secretion and a loss of electrolytes and fluid as a result of diarrhoea (Teke *et al*., 2007) [18]. The result showed significant decrease in the intestinal fluid electrolyte concentrations in all treated groups (extract and standard drugs) when compared with the control (group 1). Both the groups which received *Nypa frutican* extract and those which received standard drugs showed significant ($p<0.05$) decrease in (K^+, Na^+, Cl^- and HCO^-_3) concentrations. From the result, both groups treated with *Nypa frutican* extract and standard drugs (Loperamide hydrochloride) showed significant reduction in electrolyte loss caused by castor oil induced diarrhoea in albino rats which conforms to Nwachoko *et al.* (2016) [14] that an increase in intestinal fluid electrolyte concentration is linked to diarrhoea, and a decrease in these electrolytes in the treated groups supports an extract's anti-diarrheal properties.

Conclusion

The research revealed that ethanol extract of *Nypa frutican* leaves possess significant anti-diarrheoa properties due to its inhibitory effect on wet fecal count, inhibition of distance travelled by charcoal meal and inhibition of intestinal fluid electrolytes. From the result obtained, *Nypa frutican* leaves are reservoir of pharmacological active compounds and using it for making roofing mats, ceiling mats, hats and brooms is an underutilization of the plant benefits.

References

1. Ajay S, Vipin SK, Sanjeev ST, Pravesh K, Anil S. A review on hepatoprotective activity of medicinal plants. International Journal of Advanced Research in Pharmaceutical and Biological Sciences. 2018;1(2):31-39.
2. Dolin BJ. Effects of proprietary Bacillus coagulans preparation on symptoms of diarrhea-predominant irritable bowel syndrome. Methods and Findings in Experimental and Clinical Pharmacology. 2009;31(10):655-659.
3. Downs BW, Bagchi M, Morrison BS, Galvin J, Kushner S, Bagchi D. Development and utilization of a novel prodosomed-electrolyte and phytochemical formulation technology to restore metabolic homeostasis. Metal Toxicology Handbook; c2020. p. 67-80.
4. Ebana RUB, Etok CA, Edet UO. Phytochemical screening and antimicrobial activity of *Nypa fruticans* harvested from Oporo River in the Niger Delta Region of Nigeria. International Journal of Innovation and Applied Studies. 2015;10(4):1120.
5. Gazali M, Nufus H, Nurjanah N, Zuriat Z. The Exploration Potency of Bioactive Compounds of Leaves Extract Nipah (*Nypa fruticans* Wurmb) from the Coast of West Aceh as Antioxidant. Journal Pengolahan Hasil Perikanan Indonesia. 2019;22(1):155-163
6. Judit B, Tama B. Diarrhea, from the gastroenterologist's point of view. International Journal of Antimicrobial Agents. 2009;14(1):65-69.
7. Lestari Y, Ardiningsih NP. Gram-Positive and Negative Antibacterial Activity of Nipah Leaf Extract and Fraction (*Nypa fruticans* Wurmb.) Origin of the Kakap River Coast of West Kalimantan. Journal of Equatorial Chemistry. 2016;5(4).
8. Manzo LM, Moussa I, Ikhiri K. Phytochemical screening of selected medicinal plants used against diarrhea in Niger, West Africa. International Journal of Herbal Medicine. 2017;5(4):32-38.
9. Mullhaupt B. Diarrhea. Bulletin of the World Health Organization. 2002;91(42):1749-1756.
10. Nonita PP, Mylene MU. Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. Journal of Medicinal Plants Research. 2010;4(5):407-414.
11. Nugroho GD, Wiraatmaja MF, Pramadaningtyas PS, Febriyanti S, Liza n, Naim DM, *et al*. Phytochemical composition, medicinal uses and other utilization of *Nypa fruticans*. International Journal of Bonorowo Wetland. 2020;10(1):1-15.
12. Nwachoko N, Jack IR. Phytochemical screening and antidiarrhoea activities of *Tetracarpidium conophorum* induce in albino rats. Sky Journal of Biochemistry Research. 2015;4(4):21-24.
13. Nwachoko N, Oghale OV. Evaluation of anti-diarrhoea potential of watermelon seed (*Citrullus lanatus*) in

albino rats. Biochemical Pharmacology. 2017;6(2):1000228.
14. Nwachoko N, Essien EB, Aylogu EO. Antidiarrhoea activities of aqueous extract of *Aframomum chrysanthum* seed in albino rats. International Journal of Original Resource. 2016;2(3):172-177.
15. Nwiloh BI, Uwakwe AA, Akaninwor JO. Phytochemical screening and GC-FID analysis of ethanolic extract of root bark of *Salacia nitida* L. Benth. Journal of Medicinal Plants Studies. 2016;4(6):283-287.
16. Pier-Giorgio P. Flavonoids as antioxidants. Journal of Natural Products. 2000;63(7):1035-1042.
17. Rawat P, Singh PK, Kumar V. Evidence based traditional anti-diarrheal medicinal plants and their phytocompounds. Biomedicine and Pharmacotherapy. 2017;96:1453-1464.
18. Teke GN, Kuiate JR, Ngouateu OB, Gatsing D. Antidiarrhoeal and antimicrobial activities of *Emilia coccinea* (Sims) G. Don extracts. Journal of Ethnopharmacology. 2007;112(2):278-283.
19. Udofia SI, Udo ES. Local knowledge of utilization of nipa palm (*Nypa fruticans* Wurmb) in the coastal areas of Akwa Ibom state, Nigeria. Global Journal of Agricultural Sciences. 2005;4(1):33-40.
20. WHO. WHO Monographs on selected medicinal plants. Bulbus Allii Capae. WHO, Geneva; c1999. p. 289.