



Assessment of the Antimicrobial Activity of *Landolphia owariensis* and *Nuclea latifolia* Used in the Treatment of Diarrhoea in Walijo

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Abstract

Despite the success of antibiotic discovery, infectious diseases are consistently ranked second among causes of death worldwide. The antidiarrhoeal activities of the crude extracts from the leaves *Landolphia owariensis* and *Nuclea latifolia* were carried out using standard methods to assess new active compounds with therapeutic potential for infectious diseases. The results of the antidiarrhoeal screening of the crude extracts showed that Methanol, petroleum ether and ethyl acetate extracts of *L. owariensis* gave 9.3 g, 8.2 g, and 6.3 respectively, while extracts of *N. latifolia* gave 8.4 g, 5.5 g, and 4.5 g respectively. The extracts were tested against selected enteric pathogens of different concentration. *Nuclea latifolia* in ethyl-acetate and methanol was highly effective against *S. typhi* with a resistance observed when tested against *S. dysentriae* at some conditions and *E. coli* respectively. The minimum inhibitory concentration was 250 $\mu\text{g}/\text{cm}^3$ for *S. typhi*. Petroleum ether showed moderate effectiveness against *Salmonella typhi* and highly effective against *S. dysentriae*. While for *E. coli*, a complete resistance with minimum inhibitory concentration (MIC) is 500 $\mu\text{g}/\text{cm}^3$ for *S. typhi* and 1 mg/cm^3 for *E. coli*. The degree of effectiveness is observed to be increasing with increased in the concentrations of solvent extract. However, *N. latifolia* extract has shown to be more effective on *S. typhi* followed by *shigella dysentriae*. The minimum inhibitory concentration (MIC) for *Salmonella typhi* and *Shigella dysentriae* is 2 mg/cm^3 respectively. But the wide range of inhibitory properties shown by *L. owariensis* extract against the tested organisms indicates its potential against the diarrhoea causing microbes.

Keywords: Antidiarrheal, *Landolphia owariensis*, *Nauculia latifolia*, Infectious Disease, Antidiarrhoeal Screening

Introduction

Diarrhea remains a global health challenge, significantly affecting morbidity and mortality rates, particularly in developing countries (Walker et al., 2015). It is primarily characterized by the frequent passage of loose or watery stools, often accompanied by abdominal discomfort and dehydration, leading to critical health impacts, especially among children under five years of age (World Health Organization, 2013; Njume and Goduka, 2012). Diarrhea is defined as an increase in the daily stool weight of more than 200 grams. It is one of the most common illnesses in all age groups and is second only to the common cold to cause lost days at work or at school. Diarrhea and related complications can cause severe illness especially in high-risk groups, such as patients with severe morbid conditions underlying immunosuppression and advanced age (Owolabi et al., 2010). Poor sanitation and hygiene are thought to be the root cause of 88% of deaths associated with diarrhea (Webb & Cabada, 2018). The quest for effective therapeutic agents has led researchers to explore traditional medicine, where biologically active compounds from plants have demonstrated potential antidiarrheal properties (Sharma et al., 2012; WHO, 2013; Rout and Panda, 2017). Two plants within the family Apocynaceae that have gained attention in ethnopharmacology for their medicinal properties are *Landolphia owariensis* and *Nuclea latifolia*. *Landolphia owariensis*, commonly known as the Owari rubber vine, is a liana plant found predominantly in West Africa. Of all species of *Landolphia*, *Landolphia owariensis* is the commonest; it is commonly called vine rubber and known locally by various names in Nigeria: Ibo-Eso/Utu, Yoruba-Mba and Hausa-Ciwo (Galadima et al., 2010). Its latex and other parts are traditionally used in treating a variety of ailments, including diarrhea (Obitte & Chukwu, 2011). The plant's constituents, such as flavonoids, alkaloids, and tannins - bioactive compounds known for their antidiarrheal activity, potentially mitigating the effects of

gastrointestinal disturbances (Maroyi, 2011; Maroyi, 2016). Tannins, in particular, are recognized for their astringent properties, which can help reduce intestinal motility and thereby relieve diarrhea (Ajayi et al., 2024; Fagbamigbe et al., 2021). Similarly, *Nuclea latifolia*, a species indigenous to tropical Africa, is also utilized in traditional medicine for treating diarrheal disorders. Its phytochemical profile suggests the presence of active compounds that may effectively inhibit gastrointestinal motility and secretion, mechanisms critical for managing diarrhea (Rahman et al., 2012).

Despite their historical use, scientific validation of the antidiarrheal properties of these plants remains limited. Previous studies have established the pharmacological potential of various plants in treating diarrhea, yet comprehensive investigations on *L. owariensis* and *N. latifolia* are sparse (Iheagwam et al., 2020; Oladeji et al., 2024). Therefore, exploring the efficacy of these plants through systematic pharmacological assessment is warranted. Evaluating their antidiarrheal effects not only contributes to the ethno pharmacological knowledge base but also provides a foundation for developing novel therapeutics that can address the limitations of conventional treatments, including side effects and antibiotic resistance (Cohen et al., 2017). In many developing countries especially in Nigeria, the plants are commonly used as a remedy for diarrhea and diabetes (Gidado et al., 2004). Other uses include malaria, hypertension and sleeping sickness (Kerharo, 1974). The infusion or a decoction of the root is usually used as a remedy for stomach upset such as diarrhea in adults (Gill, 1992). In the present study, we evaluated the methanol, petroleum ether and ethyl acetate extracts of the plant parts for possible antidiarrheal properties of *Landolphia owariensis* and *Nuclea latifolia* through both in vitro and in vivo approaches, thereby elucidating their potential mechanisms of action and identifying the active phytochemicals responsible for their therapeutic effects. By integrating ethno medicinal insights with modern pharmacological techniques, this study seeks to provide a comprehensive understanding of these plants' roles in managing diarrhea, ultimately informing future research and therapeutic applications.

Materials and Methods

Materials

The following chemicals were used for extraction: Petroleum ether, (Analar grade), Methanol, Jinhuada (JHD) Sci-Tech, Ethyl-acetate, (Fisher Scientific), Ethanol Jinhuada, (JHD) Sci-Tech, Sodium Trioxocarbonate, (Analar grade), Standard Tannic acid solution, Folin Denis reagent, Alkaline Picrate, Acetone, Jinhuada (JHD) Sci-Tech, Ammonium hydroxide, (Analar grade) and Distilled water

Plant material

The arial parts of *L. owariensis* and *N. latifolia* were collected from Waliyo Area of Kachia LGA of Kaduna State Nigeria, in May, 2009. Botanical authentication was confirmed at Biological department of thenigerian defence Academy Kaduna, where a herbarium specimen (No. FHI) was deposited for future reference. The plants parts were washed, air-dried and pulverized by the use of a wooden mortar and pestle coarse powder and stored in polythene bag for use in analysis.

Plant Extraction

A portion (200 g) of each of the powdered plants (*Landolphia owariensis* and *Nauclea latifolia*) was percolated separately into 450 cm³ of methanol for two weeks, and then filtered. The filtrate was evaporated at 40 °C using rotary evaporator and the extract allowed to dry and weighed.

The crude extract of *L. owariensis* was dissolved in 10 cm³ of distilled water and transferred into a separating funnel. Equal volume (200 cm³) of petroleum-ether and methanol were added, and the resulting mixture was shaken thoroughly, and allowed to stand overnight for the two layers to separate. The two layers was separately drained off, and the procedure was repeated until the petroleum ether soluble fraction is completely removed. The two layers were evaporated separately using a rotary evaporator. The ethyl acetate and methanol fractions were partitioned using the same procedure. Three crude extracts were collected at the end of the extraction processes (petroleum-ether, ethyl-acetate and methanol crude extracts, respectively). Thereafter, the percentage yield was calculated and the procedure repeated on *Nauclea latifolia* plant sample (Garba et al., 2009).

Bioassay

The bioassay of the crude petroleum ether, methanol and ethyl acetate extract were performed using the following micro-organisms. *Salmonella typhi*, *Escherichia coli*, and *Shigella dysenteriae* using method described by (Sofowora et al., 2013).

Preparation of Test Organisms

Clinical bacterial isolates were used in this study and are obtained from the Department of Medical Microbiology, Barau Diko Teaching Hospital Kaduna, 44 Army Reference Hospital Kaduna. The organisms were subculture in freshly prepared Petr-dishes. The organisms were *S. typhi*, *E. coli*, and *S. dysenteriae*, all the subcultures were checked for purity and maintain in nutrient agar.

Preparation of McFarland Standard

About 1.0 cm³ of concentrated H₂SO₄ was added to 99 cm³ of distilled water and 1.0 g of BaCl₂ was dissolved in 100 cm³ of distilled water, thus, tag as solution A and B.

About 0.6 cm³ of the prepared solution B was added to 99.4 cm³ of the prepared solution A to give a McFarland turbidity standard solution.

Antimicrobial Screening of the Crude Extracts

Antimicrobial screening of the crude petroleum ether, ethyl acetate, and methanol extracts was carried out using disc diffusion method. A portion of 0.02 g of the crude extract was weighed and dissolved in 10 cm³ of 10 % DMSO to obtain a stock solution of 2000 µg/cm³. Concentrations of 1000 µg/cm³, 500 µg/cm³, 250 µg/cm³, and 125 µg/cm³ were prepared by serial dilution (By dividing the 10 cm³ stock solution of 2,000 µg/cm³ into two equal parts, and made one part to 10 cm³ with distil water to produce 1000 µg/cm³. This dilution was repeated on 1000 µg/cm³ to produce 500 µg/cm³, and so on to produce the various remaining concentrations). The antimicrobial screening of the crude extracts was carried out using agar well diffusion method described by (Sofowora et al., 2013; Trease & Evans, 2002). Microbial isolates of *S. typhi*, *E. coli*, and *S. dysenteriae* were separately grown in a nutrient broth 37 °C for 18 hrs and standardized using 5 cm³ of normal saline to turbidity of 0.5 McFarland standards (10⁸ cfu/cm³), 25cm³ of sterile Mueller Hinton agar and poured into the sterile petri dishes and allowed to solidify. A sterile cork borer (5 mm diameter) was used to make five equidistant holes in the solidified Mueller Hinton agar. A drop of Mueller Hinton was added to each hole and allowed to solidify to seal well bottoms to avoid seepage of extract solution. About 0.5 cm³ of the microorganism (*S. typhi*) loaded into the hole with the aid of syringe. A portion of 2000 µg/cm³ of the crude extracts was added and incubated for 24 hrs at 37°C. Diameter of zones of inhibition was measured using transparent plastic ruler. This procedure was repeated for the following concentrations of 1000 µg/cm³, 500 µg/cm³, 250 µg/cm³, and 125 µg/cm³ respectively for *E. coli* and *S. dysenteriae*

Results

Despite the success of antibiotic discovery, infectious diseases are consistently ranked second among causes of death worldwide (Pavithra et al., 2010). The pursuit of new active compounds that have therapeutic potential for infectious diseases focuses mainly on plants as the nobel reservoir of drug compounds. The resistance of micro-organisms to antibiotics is among the leading problems in the twenty-first century and others which necessitated the need to a progressive search for more potentially safe and available therapeutic agents (Chikezie et al., 2015).

Extraction of Yields

TABLE 1: Yields of the extracts from 200 g of each plant sample

Plants	Solvents of Extraction	Weight of extracts
<i>L. owariensis</i>	Methanol	9.3 g
<i>L. owariensis</i>	Pet-ether	8.2 g
<i>L. owariensis</i>	Ethyl-acetate	6.3 g
<i>N. latifolia</i>	Methanol	8.4 g
<i>N. latifolia</i>	Pet-ether	5.5 g
<i>N. latifolia</i>	Ethyl-acetate	4.5 g

Table 1 showed the various yields of the crude plants extracts and the quantities obtained from the two plants (*L. owariensis* and *N. latifolia*). Methanol, petroleum ether and ethyl acetate extracts of *L. owariensis* gave 9.3 g, 8.2 g, and 6.3 g respectively, while the methanol, petroleum ether and ethyl acetate extracts of *N. latifolia* gave 8.4 g, 5.5 g, and 4.5 g respectively. The results also showed that methanol gave the highest yield followed by petroleum ether and ethyl-acetate for both *L. owariensis* and *N. latifolia* respectively. *L. owariensis* produced more yield of its extract from all the solvents when compared to *N. latifolia* yield.

Antimicrobial Test of the Crude Extracts for *L. owariensis*

Table 2: Results of the Antimicrobial Test of the Crude Extracts for *L. owariensis*

Plants	Solvent Extracts	Concentrations	<i>Salmonella typhi</i>	<i>Shigella dysenteriae</i>	<i>Escherichia coli</i>
<i>Landolphia owariensis</i>	Ethyl-acetate	2 mg/cm ³	+++	++	+++
		1 mg/cm ³	+++	++	+++
		500 µg/cm ³	++	++	0
		250 µg/cm ³	++	0	0
		125 µg/cm ³	++	0	0
		Pet-Ether	2 mg/cm ³	++	+++
	1 mg/cm ³	++	+++	0	
	500 µg/cm ³	++	+++	0	
	250 µg/cm ³	++	+++	0	
	125 µg/cm ³	+	0	0	
	Methanol	2 mg/cm ³	++	+	0
		1 mg/cm ³	++	+	0
		50 µg/cm ³	0	+	0
		250 µg/cm ³	0	0	0
		125 µg/cm ³	0	0	0

Key: Diameter of the Zones of Inhibition (21 mm and above) +++: Highly effective, (11 mm – 20 mm) ++: Moderately effective, (1 mm – 10 mm) +: Less effective, 0: No Inhibition/Resistance

Table 2 showed the result of the antimicrobial screening of *Landolphia owariensis*. The antimicrobial efficacy of the extracts were tested against selected enteric pathogens at different concentration. *Landolphia owariensis* in Ethyl-acetate was shown to be highly effective at 2 mg/cm³, 1 mg/cm³ and moderately effective at 500 µg/cm³, 250 µg/cm³ and 125 µg/cm³ against *S. typhi*, and it was moderately effective at 2 mg/cm³, 1 mg/cm³, 500 µg/cm³ when tested against *S. dysenteriae*. The same was highly effective on *E. coli* at 2 mg/cm³, 1 mg/cm³ and there was complete resistance at 500 µg/cm³, 250 µg/cm³ and 125 µg/cm³. The degree of effectiveness is observed to be increasing with the increase in concentration of the solvent extracts. However, it was observed to be more effective on *Salmonella typhi*, followed by *Shigella dysenteriae* and least effective on *E. coli*. The minimum inhibitory concentration [MIC] of *L. owariensis* in Ethyl-acetate on *S. dysenteriae* is 500 µg/cm³ and 1mg/cm³ for *E. coli*. This observed result corroborates with the previous findings by Galadima et al. (2010) that methanolic and ethanolic extract of root of the plant inhibited growth of *S. aureus* and *S. typhi*. *Landolphia owariensis* in petroleum ether has shown to be moderately effective at 2 mg/cm³, 1 mg/cm³, 500 µg/cm³, 250 µg/cm³ and less effective at 125 µg/cm³ against *Salmonella typhi*. It was found to be highly effective at 2 mg/cm³, 1 mg/cm³, 500 µg/cm³, 250 µg/cm³ and no inhibition at 125 µg/cm³ when tested against *S. dysenteriae*. There was complete resistance at 2 mg/cm³, 1 mg/cm³, 500 µg/cm³, 250 µg/cm³ and 125 µg/cm³ when used against *E. coli*. The degree of effectiveness is observed to be increasing with increase in the concentration of the solvent extract. However, it has shown to be more effective on *S. dysenteriae* followed by *S. typhi*, while *E. coli* exhibited

complete resistance at all level of concentration. The minimum inhibitory concentration (MIC) was observed on *S. dysenteriae* at 250 µg/cm³. This finding completely aligned with the findings of Lombor and Gbeyonron (2018) which shows complete resistance by *E.coli* against extract of fruit pulp of the plant. *Landolphia owariensis* in methanol exhibited moderate effect at 2 mg/cm³, 1 mg/cm³ against *Salmonella typhi*. Complete resistance was shown by *S. typhi* at 500 µg/cm³, 250 µg/cm³ and 125 µg/cm³ of the solvent extract. It has shown to be less effective at 2 mg/cm³, 1 mg/cm³, 500 µg/cm³ while complete resistance was evident at 250 µg/cm³ and 125 µg/cm³ when tested against *S. dysenteriae*. *E. coli* exhibited complete resistance to the extract at 2 mg/cm³, 1 mg/cm³, 500 µg/cm³, 250 µg/cm³ and 125 µg/cm³. The solvent extract has shown to be more effective on *S. typhi* followed by *S. dysenteriae*, while *E. coli* shows complete resistance. The minimum inhibitory concentration (MIC) is 1 mg/cm³, 500 µg/cm³ for *S. typhi* and *S. dysenteriae* respectively. This observed antimicrobial property could be due to the presence of alkaloid, flavonoids, and tannins as reported by (Nwaogu et al., 2008).

Antimicrobial Test of the Crude Extracts for *N. latifolia*

TABLE 3: Results of the Antimicrobial Test of the Crude Extracts for *N. latifolia*

Plants	Solvent Extracts	Concentrations	<i>Salmonella typhi</i>	<i>Shigella dysenteriae</i>	<i>Escherichia coli</i>
<i>Nauclea latifolia</i>	Ethyl-acetate	2 mg/cm ³	+++	0	0
		1 mg/cm ³	+++	0	0
		500 µg/cm ³	+++	0	0
		250 µg/cm ³	+++	0	0
		125 µg/cm ³	0	0	0
	Pet-Ether	2 mg/cm ³	++	+++	+++
		1 mg/cm ³	++	0	0
		500 µg/cm ³	++	++	0
		250 µg/cm ³	0	++	0
		125 µg/cm ³	0	++	0
	Methanol	2 mg/cm ³	+++	++	0
		1 mg/cm ³	0	0	0
		500 µg/cm ³	0	0	0
		250 µg/cm ³	0	0	0
		125 µg/cm ³	0	0	0

Key: Diameter of the Zones of Inhibition (21 mm and above) +++: Highly effective, (11 mm – 20 mm) ++: Moderately effective, (1 mm – 10 mm) +: Less effective, 0: No Inhibition/Resistance.

Table 3 showed the result of the antimicrobial screening of *N. latifolia*. The antimicrobial efficacy of the extracts were tested against selected enteric pathogens of different concentration. *Nuclea latifolia* in ethyl-acetate have shown a high level of effectiveness at 2 mg/cm³, 1 mg/cm³, 500 µg/cm³, 250 µg/cm³ but ineffective at 125 µg/cm³ against *S. typhi*. There was a complete resistance at 2 mg/cm³, 1 mg/cm³, 500 µg/cm³, 250 µg/cm³ and 125 µg/cm³ when tested against *S. dysenteriae* and *E. coli* respectively. The degree of effectiveness is constant for the various concentration. However, it was observed to be only effective on *S. typhi* as there was no inhibition on *S. dysenteriae* and *E. coli*. The minimum inhibitory concentration is 250 µg/cm³ for *S. typhi*. This result differ slightly with the findings of Umeh et al. (2005) which shows that petroleum ether and chloroform extracts of the plant effectively inhibit both gram positive and gram negative bacteria. The resistance showed by *S. dysenteriae* and *E. coli* could be due to dosage or biological variability. *Nuclea latifolia* in petroleum ether has shown to be moderately effective at 2 mg/cm³, 1 mg/cm³, 500 µg/cm³ and ineffective at 250 µg/cm³, and 125 µg/cm³ against *Salmonella typhi*. It was shown to be highly effective at 2 mg/cm³ and moderately effective at 1 mg/cm³, 500 µg/cm³, 250 µg/cm³ and 125 µg/cm³ against *S. dysenteriae*. When tested against *E. coli*, it was observed to be highly effective at 2 mg/cm³ and complete resistance was noticed at 1 mg/cm³,

500 µg/cm³, 250 µg/cm³ and 125 µg/cm³. The degree of effectiveness is observed to be increasing with increase in the concentration of the solvent extract. However, it has shown to be more effective on *S. dysenteriae* followed by *S. typhi* and *E. coli*. The minimum inhibitory concentration (MIC) is 500 µg/cm³ for *S. typhi* and 1 mg/cm³ for *E. coli*. This result is in agreement with the work carried out by El-Mahmood et al. (2008) on *N. latifolia* extract, where they reported that the plant extract effective on pathogenic bacteria such as *S. typhi* and *S. aureus*.

Nuclea latifolia in methanol has shown to be highly effective at 2 mg/cm³ and ineffective at 1 mg/cm³, 500 µg/cm³, 250 µg/cm³ and 125 µg/cm³ against *S. typhi*. *S. dysenteriae* has shown to be moderately sensitive to the solvent extract at 2 mg/cm³ and completely resistance at 1 mg/cm³, 500 µg/cm³, 250 µg/cm³ and 125 µg/cm³. There was complete resistance to all the varied concentrations when tested against *E. coli*. The degree of effectiveness is observed to be increasing with increased in the concentrations of solvent extract. However, *N. latifolia* extract has shown to be more effective on *S. typhi* followed by *shigella dysenteriae*. The minimum inhibitory concentration (MIC) for *Salmonella typhi* and *Shigella dysenteriae* is 2 mg/cm³ respectively. The complete resistance exhibited by *E. coli* was not in agreement with the findings of Omer et al. (1998) who reported that the extracts of *N. latifolia* are effective against gram negative bacteria. This may be as a result of natural resistance, genetic variability or mutational changes which occur even before the introduction of the drug. The resistance can be transferred from one bacterium to another of the same species, and also between different species and sometimes, even between related genera. But the wide range of inhibitory properties shown by *L. owariensis* extract against the tested organisms indicates its potential against the diarrhoea causing microbes.

Conclusion

The results of the bioassay carried out on *L. owariensis* and *N. latifolia* revealed that the plants crude extracts from the two plants were very active against *S. typhi*, *S. dysenteriae* and *E. coli*. The results also showed that the ethyl acetate extract of *Landolphia owariensis* was the most active. Other phytochemicals that are reported to have great antimicrobial properties should be investigated and molecular screening of the plants active components. Further studies on the targeted plants should be expanded to cover additional pathogens not covered by this research work as well as establishing the safety of the compound in the host organism.

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