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A Study of the TLC and Acute Toxicity of *Phyllanthus niruri* Found in Sokoto State of Nigeria

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ABSTRACT

Phyllanthus niruri is traditionally believed to cure gastro enteritis infections, respiratory infections, sore throat and malaria in Sokoto state of Nigeria. This traditional practice, however, is not backed by any scientific findings. The present study investigates the antibacterial and acute toxicity of the extracts of the stem bark of the plant on some common organisms that are widely known to cause the ailments they are used to cure. The plant part was sequentially extracted with different organic solvents in increasing polarity. The acute toxicity of the methanol extract showed no sign of toxicity (e.g convulsion tremor, redness of the eye, scratching of the fur or mortality etc.) in the tested animals at all doses of the extracts up to 5000 mg/kg. The absence of any deaths at doses up to 5000 mg/kg of the crude extract showed that the LD₅₀ of the extracts is greater than 5000 mg/kg thus indicating the safety of the extracts in the treatment of various ailments locally in Sokoto.

Keywords: antibacterial, *Escherichia coli*, ethyl acetate, methanol. Bioactive compounds

1.0 INTRODUCTION

Traditional medicine has been used to treat myriads of ailments long before the advent of conventional drugs. Plants possess natural products known as phytochemicals which are capable of killing or inhibiting the growth of microorganisms such as fungi and bacteria. The field has witnessed exponential growth over the last few decades (Manpreet *et. al.*, 2012). More people, these days, are turning to new areas when it comes to health care and medication partly due to the high cost of conventional drugs, environmental issues, side effect problems, efficacy of the existing drugs to cure certain ailments and resistance of some diseases to some drugs (Srivastava *et. al.*, 1996). It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava *et. al.*, 1996).

In Sokoto State, Nigeria, *Phyllanthus niruri* (*L*) locally known as Geron Tsuntsaye is an annual herb which belongs to the family Euphorbiaceae. The height varies between 30-60 cm, stem is angular with numerous distichous, flowers are yellow and numerous, leaves are elliptic oblong; monoecism with 1-3 staminate flowers and solitary pistil late (Caius, 1986); Fruit capsule, very small, globule, smooth seeds (Caius, 1986); Agharkar, (1991) and Gupta, (1984). It grows as wild shrubs in the bush or waste land. It is traditionally believed to boost immunity of children. Women are also known to use it as stimulants. In

many parts of India, it is commonly used for the treatment of snake bite, malaria and sore throat (Aisha, *et al.*, 2019).

Traditional medicine practitioners proclaim that plants such as *Phyllanthus niruri* could provide a natural source of antimicrobial drugs that will control microorganism and associated infections globally. This is especially important in light of the current COVID-19 pandemic.

Though the plant is widely used for variety of health management traditionally there is no scientific evidence of its efficacy and toxicity. Such evidence could provide insight into its wide spread use and provide safety guidelines on its dosage. This outcome of this study is therefore significant as its findings can stimulate the local consumption and consequently local production of drugs based on the plants in Sokoto in particular and Nigeria in general. This has motivated the researcher to investigate the antibacterial activities and toxicity of *Phyllanthus niruri*.

2.0 MATERIALS AND METHODS

2.1 Collection of Plant Materials

The plant was collected randomly from Bado area of Wamakko Local Government of Sokoto State. The plant was identified and authenticated at the Herbarium of the Botany unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto. The vouchered specimen (UDUH/ANS/0202) was deposited in the herbarium.

2.2 Plant Processing

Stems of the plant were carefully removed, cut into small pieces, washed with clean tap water and air dried in the laboratory of the Sokoto state University. The dried samples were then crushed using mortar and pestle, sieved through 0.28 μ mesh sieve before storing the fine powder in polythene bags

2.3 Extraction of *Phyllanthus niruri*

Five hundred grams (500 g) of the powdered plant material was extracted using Serial Exhaustively method using methanol, ethyl acetate and n-hexane. The weights of the crude extracts were recorded, and the fractions obtained were subjected to phytochemical analyses, antibacterial and toxicity tests.

2.4 Identification of Compounds

The plant extracts with the highest antibacterial activities were subjected to thin-layer and column chromatography to isolate and identify specific compounds present.

2.4.1 Thin Layer Chromatography (TLC)

TLC was employed both in developing appropriate solvent systems and for confirming the purity of the fractions from the column chromatography. Minimum amount of dried extracts (~10 mg) was dissolve in 1 cm³ of appropriate solvents (methanol or ethyl acetate) and was used for TLC. The extract was spotted on the absorbents (silica gel GF 254 recoated plate) and developed in appropriate solvent mixtures. After several trials, methanol: n-hexane in 7:3 (v/v) solvent system was used for the methanol extract while ethyl acetate: n-hexane in 8:2 (v/v) was chosen for the ethyl acetate extract. After the development, the plate was viewed under a UV box before spraying with 10% of sulphuric acid in methanol and then heated in an oven at 105 °C for five minutes for visualization.

2.4.2 Column Chromatography

120 g of silica gel was mixed with appropriate solvent system [methanol: n-hexane in 7:3 (v/v) solvent system for the methanol extract while ethyl acetate: n-hexane in 8:2 (v/v) for the ethyl acetate extract] and the content was stirred and poured into a column (10 cm long and 1.5 cm in internal diameter), which was plugged with cotton wool at the bottom. More solvent was added with gradual tapping of the side of the column using a rubber rod for proper compaction of the particles. While the silica gel settles and without allowing the column to run dry, the extract (~3 g) was introduced into the column and elution commenced after an hour by opening the tap and the fraction was collected in drops. Several fractions were collected and monitored using TLC after elution. Similar fractions were combined and concentrated in vacuo to give specific compounds. The compounds were further purified by a second column chromatography as described in Letidal *et al.* (2009) before subjecting them to further analysis.

2.5 Antibacterial screening using Agar well Diffusion Method

2.5.1 Media Preparation

Nutrient broth was prepared according to the manufacturer's instructions. Thirty-seven grams (37 g) of the powder agar was poured into a litre of distilled water in a conical flask and the flask was heated to dissolve the content. Aluminum foil was plugged into the mouth of the conical flask and the dissolved agar was sterilized in an autoclave machine at 121°C for 15-20 minutes and then allowed to cool at about 45°C. The mixture was then poured into sterile plates (petri dishes) and was allowed to stand at 37 °C for 20 hours to solidify.

2.5.4 Determination of Minimum Bactericidal Concentration (MBC)

Tubes showing no visible growth from the MIC test were subculture unto Nutrient agar and the plates were incubated at 37 °C for 24 hrs. The lowest concentration of the plant extract produced yielding no growth was recorded as the MBC (Shina 2014)

2.6 Acute toxicity test

The Acute oral toxicity of the extract was determined by the 'Up-and-Down' method using the OECD-425 guidelines (OECD, 2001). A limit dose of 5000 mg/kg was used for the study. The test was carried out with healthy albino rats (males and females) aged 8 to 10 weeks weighing 130 – 150 g of the plant extract. The rats were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. The study was conducted under internationally accepted principles for laboratory animal use and care. The mice were stored in cages and randomly selected ones were marked on the tail for individual identification. They were put on a 12-h light/dark cycle and housed at room temperature (approximately 23 °C) with constant humidity. Before commencing the experiment, the mice were allowed a week to acclimatize to the laboratory conditions. Drinking water and food were provided *ad libitum* throughout the experiment, except for the short fasting period where only drinking water was accessible with no supply of food for 12 h before they were treated with the extracts.

2.7 Statistical Analysis

The numerical data obtained from various determinations are averages of triplicate observations. The data were subjected to statistical analysis using SPSS 17.0 statistical software. One-way Analysis of Variance (ANOVA) using LSD and Turkey's test at $\alpha = 0.05$ was used to compare variables with one another and with controls for any significant difference

3.0 RESULTS AND DISCUSSION

3.1 Results

The fractions were further subjected to antibacterial activity test and the results obtained are presented in Tables 5 For both the methanol and ethyl acetate extracts, the activity shown were observed to be significantly different from one another ($P < 0.05$) and fraction 2 (Met 2 and EA 2) exhibited the highest activity.

Table 5. Antibacterial activity of column chromatographic fractions of methanol extract of *Phyllanthus niruri* stem bark.^a

Fractions	Conc. (mg/ml)	Zone of inhibition (mm)			
		<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>
Met 1	15	10.16±0.03	10.03±0.02	8.17±0.04	9.12±0.03
	20	13.21±0.01	14.11±0.03	11.09±0.02	12.15±0.02
	25	15.33±0.02	16.01±0.02	14.20±0.02	15.22±0.01
	30	15.75±2.31	20.25±0.03	17.11±0.02	20.12±0.01
Met 2	15	20.09±0.02	18.14±0.04	16.14±0.01	17.31±0.03
	20	25.40±0.04	20.21±0.03	18.19±0.02	20.30±0.02
	25	32.48±0.05	22.19±0.01	24.19±0.02	23.31±0.02

	30	36.08±0.00	34.10±0.01	28.04±0.02	29.25±0.02
Met 3	15	16.21±0.02	12.07±0.02	16.09±0.07	14.03±0.02
	20	18.13±0.02	15.07±0.02	18.20±0.02	15.02±0.01
	25	21.49±0.01	18.10±0.02	21.22±0.03	18.14±0.02
	30	25.17±0.01	22.04±0.02	23.21±0.03	22.12±0.06
Ofloxacin		38.26±0.07	32.44±0.61	37.27±0.08	34.50±0.45

^a = values are significantly different from one another across row and column (P < 0.05); - = no activity; values are reported as mean ± standard deviation (n = 3); Met 1, Met 2 and Met 3 are Methanol fractions 1, 2, and 3 obtained from column chromatography.

Table 6.. General appearance and behavioural observations for control and treated groups

Observation	6hrs	12 hrs.	5 hrs.	12 hrs.
Skin and fur	normal	normal-	Normal	Normal
Eyes	normal	Normal	Nil	Normal
Lethargy	normal	Normal	Nil	Normal
Membrane	normal	Normal	Nil	Normal
Behavioral	normal	Normal	Nil	Normal
Salivation	normal	Normal	Nil	Normal
coma	normal	Normal	Nil	Normal

Table 7. Acute toxicity of the methanol extract of *P. niruri* on Albino rats.

Group	No. of animals used	Dose (mg/kg)	Sign of toxicity	Mortality
1 (control)	6	-	Nil	0/6
2	6	540	Nil	0/6
3	6	1080	Nil	0/6
4	6	2160	Nil	0/6
5	6	3240	Nil	0/6
6	6	4320	Nil	0/6
7	6	5000	Nil	0/6

3.2 DISCUSSION

Column chromatography enabled separation of some of the compounds present in the extracts. And as noted earlier, fractions 2 (Met 2) was observed to show the highest activities on all the tested organisms: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtiles* and *E. coli*. The fact that fraction 2 of the methanolic extract from *P. niruri* showed higher zone of inhibition on selected microorganisms could be due to the separation of further mixtures from the extract using column (McMurry, 2011).

. No sign of any toxicity effect or mortality was observed in the rats at all doses of the extracts up to 5000 mg/kg during the 14 days of observation. The toxicity signs monitored were calmness, colour eyes, convulsion, stretching of furs, scratching of the mouth or nose. Only calmness was observed at the administration of the extracts. This disappeared within 24 hours and might have been as a result of the introduction of a strange remedy into the system of the rats. The absence of any mortality after 14 days of observation at a dose of up to 5000 mg/kg of the plant extracts indicated that the LD₅₀ of the extracts of *P. niruri* was greater than 5000 mg/kg. These results therefore indicate that the administered extracts had no or negligible toxicity effects on the animals. These are in line with the findings of Jothy *et al.* (2011) and that of Kennedy *et al.* (1986) who noted that substances with LD₅₀ values higher than 5000 mg/kg by oral route are regarded as being safe or practically non-toxic. Some studies have even proposed extracts that did not cause mortality at doses of up to 2000 mg/kg as nontoxic (Ilavarasan *et al.*, 2005 and Sangetha *et al.*, 2008). This result compares very well with doses reported by Shirish (2011) and therefore establishes the safety of the extracts *P. niruri* in their use as remedies in the treatment of ailments locally in Sokoto.

4.0 CONCLUSION

The results from this study have shown that the extracts from *Phyllanthus niruri* bark contained some important phytochemicals which possess antibacterial activities. And this explains why the plant was used in the treatment of some ailments in Sokoto State. The acute toxicity showed LD₅₀ value greater than 5000 mg/kg indicating no or negligible acute toxicity effects on the organisms. Thus, indicating the safety of the use of the extracts of *Phyllanthus niruri* in the treatment of some diseases in Sokoto. Hence the plant can be used as a medicinal agent in known dosages, especially in rural communities where conventional drugs are unaffordable because of their high cost.

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