CEDTECH International Journal of Bioscience and Biotechnology
Volume 3, Number 2, June 2022
http: / /www.cedtechjournals.org
ISSN: 2756-4568

A STUDY OF THE ANTIBACTERIAL EFFICACY OF *PHYLLANTHUS NIRURI* STEMFOUND IN SOKOTO STATE OF NIGERIA.

***Aisha Umar. and **Hauwa'u Bello Abubakar**
Department of Biological Sciences,Sokoto State University
Department of Microbiology,Sokoto State University
Email: aishaumar258@gmail.com

ABSTRACT

Phyllanthus niruri is traditionally believed to cure gastroenteritis 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 properties of the extracts of the stem bark of the plant on some common organisms that are widely known to cause these infections. The plant part was sequentially extracted with different organic solvents in increasing polarity. The antibacterial sensitivity test was carried out using the agar well diffusion method. The n-hexane extract did not show any activity on the organisms tested at all concentrations while the ethyl acetate showed activity only on *P. aeruginosa*. The methanol extract showed activities at all concentrations on all the tested organisms.The study justifies the wide use of the plant part as an herb in the study area.

Keywords:*Antibacterial, Phyllanthus niruri,Escherichia coli, ethyl acetate, extraction*

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, the 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 drugs (Srivastava*et. al.,* 1996).

In Sokoto State, Nigeria, *Phyllanthus niruri (L*) locally known as GeronTsuntsayeis an annual herb that belongs to the family Euphorbiaceous. 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 wasteland. It is traditionally believed to boost the 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 bites, malaria and sore throat (Aisha, *etal*., 2019).

Traditional medicine practitioners proclaim that plants such as *Phyllanthus niruri* could provide a natural source of antimicrobial drugs that will control microorganisms and associated infections globally. This is especially important in light of the current COVID-19 pandemic.
Though the plant is widely used for a variety of health management traditionally there is no scientific evidence of its efficacy. Such evidence could provide insight into its widespread 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 activitiesof *Phyllanthus niruri*.

MATERIALS AND METHODS

Collection of Plant Materials

The plant was collected randomly from the 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.

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 then sieved through 0.28 µ mesh sieve before storing the fine powder in polythene bags

Extraction of *Phyllanthus niruri*

Five hundred grams (500 g) of the powdered plant material was extracted using the 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 and antibacterialtests.

Antibacterial screening using Agar well Diffusion Method

Media Preparation

The 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.

Antibacterial Activity (Sensitivity Test)

The extracts were separately dissolved in a sterile distilled water to make solutions of various concentrations by using 1.5 mg, 2.0 mg, 2.5 mg and 3.0 mg of the water samples unto 20 cm^3 sterilized distilled water in a conical flask to obtain a concentration of 15 mg/ml, 20 mg/ml 25 mg/ml and 30 mg/ml and the contents were transferred into a ditch hole of 4 mm in diameter respectively. Surface streaking of the cultures on the media was carried out on the Petri-dishes containing the agar medium. The lid of the Petri-dishes was kept closed to avoid or prevent contamination. The inocular was streaked immediately on the medium with a sterile inoculating loop. The extract was placed with a syringe into the inoculated plate and out in a sufficient area between the plate for each in a petri dish with 15, 20, 25 and 30 mg/ml respectively. Zones of inhibition around the well greater than 4 mm indicated the presence of antibacterial activity against the bacteria. The diameter of the zones of inhibition was measured using a metre rule (Shina 2014).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the micro tube method (Cheesbrough, 1991). The nutrient broth was prepared and sterilized in an autoclave at 121°C for 15 minutes. Twelve test tubes were prepared. 1 cm^3 of the broth was dispensed into tubes 2-12. A concentrated extract solution in sterilized water 3000 cm^3 was prepared. 1 cm^3 of the solution was dispensed in tube 1 and tube 2, from tube 2 serial dilution was carried out by transferring 1cm^3 up to tube 10 serially and from tube 10, 1cm^3 was taken and the content was discarded. Twenty-four hours (24hrs.) culture of each test organism was prepared in a sterile nutrient broth 1:100 dilution was transferred into each tube, from the dilutions 1cm^3 was transferred into each tube from tube 1-12 with the expansion of tube 2 to which (1 cm^3) of the sterile nutrient broth was added to make the final volume of each tube to be (2 cm^3). Tube 1 contained 1cm^3 of the extract solution and 1cm^3 of the inoculum; tube 2 contained 2 cm^3 of the nutrient broth only while tube 12 served as the control for the viability of the culture. The MIC was taken as the lowest concentration of the test compound that inhibits bacteria growth after 24 hours of incubation at 27 °C.

Determination of Minimum Bactericidal Concentration (MBC)

Tubes showing no visible growth from the MIC test were subcultured 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)

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

RESULTS AND DISCUSSION

Results

Antibacterial sensitivity test

The n-hexane extract of *P. niruri* showedno zone of inhibition on any of the test organisms. The ethyl acetate extract produced increasing zones of inhibition with *P. aeruginosa* with increasing extract concentrations while no zone of inhibition was observed with *S. aureus*, *B. subtilis,* and *E.coli*

as shown in Table 1. These were significantly different ($P < 0.05$) from the activity shown by the control (Ofloxacin) for all the organisms studied.

Table 2 shows that antibacterial activities were observed at all concentrations and with all the organisms for the methanolic extract of *P. niruri*. Though the zones of inhibition with *P. aeruginosa, B. subtilis*and *E. coli* increased with increasing concentration of extracts from 15 to 30 mg/ml, this trend was not observed with *S. aureus*. Also, except for extract concentrations of 15 and 25 mg/ml with *S. aureus*which was significantly not different ($P<0.05$), all the other activities were significantly different ($P<0.05$) from one another and were significantly different ($P<0.05$) from the control (Ofloxacin).

Table 1,Antibacterial activity of ethyl acetate extract of *Phyllanthus niruri* stem bark

Plant extract	Extract conc. (mg/ml)	Zone of inhibition (mm)			
		P. aeruginosa	*S. aureus*	*B. subtilis*	*E. coli*
	15	-	-	-	-
Phyllanthus	20	2.14±0.17	-	-	-
niruri	25	8.30±0.37	-	-	-
	30	11.62±0.44	-	-	-
Ofloxacin	10	38.30±0.12	32.47±0.59	37.33±0.18	34.28±0.39

[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);

Table 2. Antibacterial activity of methanol extract of *Phyllanthus niruri*.

Plant extract	Extract conc. (mg/ml)	Zone of inhibition (mm)			
		P. aeruginosa	*S. aureus*	*B. subtilis*	*E. coli*
	15	15.22±0.11	14.11±0.20*	10.13±0.08	12.19±0.10
Phyllanthus	20	20.16±0.04	8.08±0.03	12.09±0.04	16.12±0.01
niruri	25	30.20±0.01	14.15±0.05*	14.12±0.01	18.07±0.05
	30	34.21±0.04	20.13±0.02	20.13±0.04	22.13±0.02
Ofloxacin	10	38.23±0.08	32.50±0.53	37.25±0.06	34.22±0.06

[a] = values are significantly different from one another across row and column ($P < 0.05$); values asterisked (*) in the same column are significantly not different at ($P < 0.05$); values are reported as mean ± standard deviation (n = 3);

Minimum Inhibitory Concentration (MIC)

The MIC of the extracts is presented in Tables 3 and 4. The ethyl acetate and methanolic extracts of *Phyllanthusniruri* showed MIC of 15 mg/ml for all the test organisms..

Table 3. Minimum Inhibitory Concentration (MIC) of ethyl acetate and methanol extracts of *Phyllanthus niruri* against bacteria species.

Plant extract	Organism	Concentration (mg/ml)								
		30	15	7.5	3.75	1.88	0.94	0.47	0.23	MIC
EPN	P.a	-	-	+	+	+	+	+	+	15
MPN	P.a	-	-	+	+	+	+	+	+	15
	S.a	-	-	+	+	+	+	+	+	15
	B.s	-	-	+	+	+	+	+	+	15
	E.c	-	-	+	+	+	+	+	+	15

EPN = ethyl acetate extract *Phyllanthus niruri* stem bark; MPN = Methanol extract of *Phyllanthusniruri* # P.a = *Pseudomonas aeruginosa;* S.a = *Staphylococcus* aureus; B.s = *Bacillus subtilis;* E.c = *Escherichia coli;* - = no growth of test organism; + = growth of test organism

Minimum Bactericidal Concentration (MBC)

The MBC against bacteria species of the extracts from *Phyllanthusniruri*is shown in Table 4 . The ethyl acetate extract of *Phyllanthusniruri* showed MBC of 1.88, 3.75, and 3.75 mg/ml against *P. aeruginosa, S. aureus,* and *E. coli* respectively. While MBC of 1.88, 15, 7.5, and 7.5 mg/ml were observed against *P. aeruginosa, S. aureus, B. subtilis,* and *E. coli* respectively for the methanolic extract.

Table 4. Minimum Bactericidal Concentration (MBC) of ethyl acetate and methanol extracts of *Phyllanthus niruri* against bacteria species.

Plant extract	Organism	Concentration (mg/ml)								
		30	15	7.5	3.75	1.88	0.94	0.47	0.23	MBC
EPN	P.a	-	-	-	-	-	+	+	+	1.88
	S.a	-	-	-	-	+	+	+	+	3.75
	E.c	-	-	-	-	+	+	+	+	3.75
MPN	P.a	-	-	-	-	-	+	+	+	1.88
	S.a	-	-	+	+	+	+	+	+	15
	B.s	-	-	-	+	+	+	+	+	7.5
	E.c	-	-	-	+	+	+	+	+	7.5

EPN = ethyl acetate extract *Phyllanthus niruri* stem bark; MPN = Methanol extract of *Phyllanthusniruri* stem bark; P.a = *Pseudomonas aeruginosa;* S.a = *Staphylococcus aureus;* B.s = *Bacillus subtilis;* E.c = *Escherichia coli;* - = no growth of test organism; + = growth of test organism

DISCUSSION

Extraction

The plant materials were sequentially extracted with different organic solvents in order of increasing polarity (i.e. n-hexane, ethyl acetate and methanol). The sequential extraction method ensured the extraction of active compounds from the plant materials according to their polarity and also is likely to reduce the antagonistic actions of compounds in the extracts. Good yields were obtained when compared to other studies (Shina, 2014).

This is further supported by the results of antibacterial studies, which showed that the extracts possess good activity against the tested organisms (see Tables 1 and 2). The highest activity was shown by the methanol extract of *P. niruri* against*P. aeruginosa*(at 30 mg/ml extract concentration)while the least activity was shown by the ethyl acetate extract of *Phyllanthus niruri* against *P. aeruginosa*(at 20 mg/ml extract concentration). The activity of the extract could not be correlated with the polarity of the solvent or the nature of the plants when the results in tables 1 and 2 are compared. However, the varied activity shown by the extracts was consistent with the findings of Shanmugam*et al.* (2014) and provides support for the use of the extracts of this plant by local herbalists for the treatment of one ailment or the other.

Although the ethyl acetate extract of *P. niruri* showed activity with only *P. aeruginosa*, the methanol extract showed antibacterial activities with all the tested organisms and the activity was observed to increase with increasing concentration of the extract. Generally, these results agree with the findings of Kurosaki and Nishi (1983) and Shanmugam (2016) who showed that a higher concentration of some extracts could show appreciable growth inhibition in both bacteriostatic and bactericidal organisms. The results are also consistent with the findings of Prior and Cao (2000) who reported that *P. niruri* is used in the treatment of chronic dysentery, skin problems cure gastroenteritis infections, respiratory infections, sore throat, malaria and jaundice and those of Shina*et al.* (2012) and Shina (2014) who worked on some medicinal plants found around Northwestern Nigeria.

It was observed that the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of*P. niruri* varied with the type of bacteria tested. The most potent activities were against *P.*

aeruginosa, S. aureus and *E. coli.* The MIC and MBC values were less than values reported by Shina (2014) who reported MIC of 30 – 90 mg/ml for aqueous ethanol and ethyl acetate extractsof*Diospyrosmespiliformis* and*Zizipilusspinachristi*. The values were however within the range of values reported by Irobi and Daramola (1993), which showed values of 0.25 – 32 mg/ml. Generally, the results from these studies provide evidence for the medicinal values of the tested plants.

CONCLUSION

The study investigated the efficacy of *Phyllanthus niruri* against some common bacterial organisms that are widely known to cause gastrointestinal and respiratory ailments. Theresults of the study showed that the stem bark of the plant contained some important phytochemicals that can inhibit the growth of the tested bacteria. The plant also showed that it is bactericidal which justifies the plant's wide use in the treatment of some ailments in Sokoto State.

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