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Antibacterial Activity of Chanca Piedra (*Phyllathus niruri*) Against Some Oral Bacterial Pathogens

O.J. Egbere,[1,*] F.C. Onwuliri [4],K Musa,[1] Steve Oyero,[2] and U.I. Henry[3]

[1] Department of Microbiology, University of Jos, Plateau State, Nigeria [2] Department of Histopathology, University of Jos Teaching Hospital (JUTH), Jos, Nigeria [3] Federal College of Forestry, University of Jos, Plateau State, Nigeria [4] Department of Botany, University of Jos, Plateau State, Nigeria

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Abstract

The antibacterial activity of the ethanolic and water extracts of Chanca piedra (*phyllanthus niruri*) leaves were demonstrated vivitro against some oral bacteria pathogens; *Streptococcus viridans, Staphyloccocus aureus* and *Lactobacillus sp* isolated from the oral cavities of patients in Plateau State Oral and Dental Health Clinic, Jos, Nigeria. The inhibitory effects of both Minimum Inhibitory Concentration (MIC) results showed that the ethanolic extract had a higher antibacterial activity than the water extract of the plant. The MIC values for the test bacteria were 62.50, 31.25 and 31.25mg/ml for *Lactobacillus sp*, Staph aureus and S. viridans respectively for the water extract while the values for the ethanolic extract were 15.6, 15.63 and 3.91mg/ml for *Lactobacillus sp*, S. Viridans and Staph. Aureus respectively. The results also showed that both extracts were found to be bactericidal against all the test organisms with S. viridans being the most susceptible and *Lactobacillus sp* the least sensitive. The results from this study has provided evidence for the medicinal values of the tested plant and thus its possible utilization as an alternative to chemical mouthwash solution.

Keywords: Chanca piedra (*phyllanthus niruri*), *Streptococcus viridans*, *Staphyloccocus aureus and Lactobacillus sp.*

1.0 Introduction

The World Health Organization (WHO) has defined medicinal plants as plants whose part or whole components contains substances that can be used as therapeutic or are precursors for the synthesis of useful drugs (WHO, 2000). Studies have shown that the plant *Chanca piedra (Phyllanthus niruri)* has a long history in herbal medicine practice in every tropical country where it grows and has successfully been used in the treatment of various ailment including the elimination of kidney and gall bladder stones, hepatitis, urinary tract infections, malaria, chronic dysentery etc, (Taylor, 2005). An invitro study conducted by a group of researchers at the San Marcos University in 1993 indicated that water extracts of *Chanca piedra* was found to have antibacterial activity against *E. coli Klebseilla pneumoniae* and *Proteus mirabilis* (Calixto *et al.* 1998). According to some researchers, dental problems and infections have become a major problem in many developing countries including Nigeria. A number of bacterial, especially members of the normal flora of the mouth are associated with oral infections (Obiajuru *et al.* 2006). The bacterial organisms associated with such infections include the following; *Streptococcus viridans, Staphylococcus aureus, Lactobacillus sp* and *Klebsiella sp* (Topazian, 2002).

Studies have shown that antimicrobial resistance which is now making it difficult to treat some infectious diseases, is due to the emergence and survival of resistant strains of microorganisms (Cheesbrough, 2000). Drug resistance strains are common among staphylococci, streptococci and meningococci (Cheesbrough, 2000).

The aims and objectives of this study are to test both water and ethanolic extracts of *Chanca piedra* against some oral pathogens such as *Streptococcus viridans*, *Staphylococcus aureus* and a *Lactobacillus sp* with the hope that if the plant extractx were found effective they could serve as alternative to the synthetic mouth wash solution found in the market.

* Corresponding author e-mail: egbere@yahoo.com

2.0 Materials and Methods

2.1 Plant Material

Leaves of *Chanca Piedra (phyllanth niruri*) plants used in this study were collected at Makurdi in Benue state, Nigeria. It was identified as C. piedra by Prof. S.W.H. Husseni, of the Department of Botany, University of Jos.

2.2 Test Organism

Stock cultures used in this were *Streptococcus viridans, Staphylococcus aureus* and *Lactobacillus sp.* They were isolated from the oral cavities of patients in Plateau State Oral and Dental Health Clinic, Jos, Nigeria. These cultures were checked for viability and purity, then maintained on nutrient Agar slopes at 4°C and sub-cultured in nutrient broth at 37°C prior to each antibacterial testing. Standardization of cultures was done as described by Cheesbrough (2000) by suspending some colonies of an overnight culture in 5ml of nutrient broth and comparing the turgidity to that of 0.5MacFarlane turbidity standard after incubating 37°C for 4-6 hours.

2.3 Extraction Procedures

The leaves of the plant were sun-dried and ground into coarse powder using clean mortal and pestle. Fifty gram (50g of the powder and 25ml of ethanol were used for the ethanolic extraction using the soxhlet extraction method (Vogel, 1987). The extraction was carried out at 60°C for 24 hours using the soxhlet apparatus, after which the ethanol was recovered as the extract was allowed to evaporate to dryness at 60°C in a water bath, and then stored at 4°C until needed. The water extract was obtained by subjecting 50g of powdered sample to warm soaking in 299ml of distilled water in a conical flask for 24 hours. The extract concentrated to dryness on a water bath set at 60°C. The dried water extract of the plant was then stored at 4°C until needed.

2.4 Test Procedure for Anti-microbial Activity

The agar well diffusion method as describes by Lamikanra (1999) was employed. Nutrient agar plates were aseptically seeded with broth culture of test organism using sterile swab sticks. For each organisms, the plates were prepared in duplicates and used for each plant extract (ethanolic and water extracts). The plates were allowed to dry and seven wells each of 6mm in diameter were made on the seeded agar using sterile stainless steel cork borer. The cork borer was sterilized by flaming after each well was made. Five out of the seven wells were filled with various concentrations of the extracts, while the sixth was filled with chloramphenicol solution (250mg/ml) as a positive control and the seventh was filled with water (containing zero mg/ml of the extract) as a negative control. The plates were left to stand at room temperature for about an hour for diffusion of the extracts in to agar to take place. The plates were incubated at 37°C for about 18-24 hours before being examined for zones of inhibition.

The minimum inhibitory concentration (MIC) was determined using the broth dilution method as described by Cheesbrough (2000). In the broth dilution test, a series of nutrient broth tubes containing plant extracts with various concentrations in the range of 0.98 to 250mg/ml were prepared and inoculated with standard numbers of test organisms. The lowest concentration of the plant extracts resulting in no growth after 18-24 hours of incubation was noted as the MIC. The Minimum Bactericidal Concentration (MBC) was determined by sub-culturing the test tubes that showed no growth onto fresh nutrient agar plates lacking the test drug extract. The lowest plant extract concentration from which the test microorganisms were not recovered after 24 hours incubation at 37°C was noted as the MBC.

Table 1: Zones of inhibition (mm) of ethanolic and water extracts of *Phyllanthus niriri*

Concentrations of the extracts (mg/ml)												
	250		125		62.50		31.25		15.65		Control	
											Chloramphenicol	Water
Test Organisms	A	B	A	B	A	B	A	B	A	B	-	-
Strept. Viridans	20	18	17	16	15	17	13	16	09	08	29	+
Staph. aureus	22	21	19	18	17	15	14	11	10	09	23	+
Lactobacillus sp	19	16	15	14	13	10	11	10	08	09	30	+

Key: A - Ethanolic extract
B - Water Extract of
+ - Growth

3.0 Results and Discussion

The results in Table 1 show that generally, both ethanolic and water extracts of *Phyllanthus niruri* exhibited antibacterial activity against the test organisms with the efficacy increasing with increase in dose concentrations of the extracts. However, the result indicated that the ethanolic extract had greater activity than the water extract on the test isolates. The susceptibility of the test organism to the ethanolic extract was observed to be highest at dose concentration of 31.25mg/ml for *Streptococcus viridans* (with zone diameter of 13mm). On the other hand, the inhibitory concentration of the water extract of the plant was at the concentration of 62.50mg/ml (twice higher) for both *Streptococcus viridans* and *Staphylococcus aureus*. At the concentration of 125mg/ml of both extracts, *Lactobacillus sp* was found to be the least sensitive among the test organisms having zones of inhibition of 15 and 14mm for ethanolic and water extracts respectively. This could be due to the fact that *Lactobacilli* have been frequently reported as normal commensals of the oral cavity, which adheres strongly to the teeth (Pelczar, 1999). Since a lot of plant materials such as chewing sticks and even some astringent food vegetables like bitter leaf are normally chewed by many Nigerians, there is a possibility that the *Lactobacillus sp* may have developed resistances to the astringent phytochemical in this plant, *Chanca piedra*.

Table 2: Results of Minimum Inhibitory Concentrations (MIC) of ethanolic extract of *Phyllanthus niruri*.

	Dilution of extracts (mg/ml)										
Test Organisms	250	125	62.50	31.25	15.63	7.81	3.91	1.95	0.98	MIC	Water
Strept. Viridans	-	-	-	-	-	+	+	+	+	15.63	+
Staph. aureus	-	-	-	-	-	_	-	+	+	3.91	+
Lactobacillus sp	-	-	-	-	-	+	+	+	+	15.63	+

Key: - No growth
+ Growth

When the effect of the control drug (Chloramphenicol) on the test organism is compared with that of the test plant, it could be observed that, at the highest concentration of 250mg/ml, the zone diameters of both plant and the antibiotic are close. This means that the plant extract could be used in treating infections caused by the organisms since research has shown that antimicrobial drug resistance is fast developing among bacterial organisms including members of the genera staphylococci, streptococci and enterococci (Cheesbrough, 2000).

Table 3: Result of Minimum Inhibitory Concentration (MIC) of water extract of *Phyllanthus niruri*.

	Dilution of extracts (mg/ml)										
Test Organisms	250	125	62.50	31.25	15.63	7.81	3.91	1.95	0.98	MIC	Water
Strept. Viridans	-	-	-	+	+	+	+	+	+	62.5	+
Staph. aureus	-	-	-	+	+	+	+	+	+	62.5	+
Lactobacillus sp	-	-	+	+	+	+	+	+	+	125.0	+

Key: - No growth
+ Growth

Table 4: Results of Minimum Bactericidal Concentration (MBC) of ethanolic extract of *Phyllanthus niruri*.

	Dilution of extracts (mg/ml)										
Test Organisms	250	125	62.50	31.25	15.63	7.81	3.91	1.95	0.98	MBC	Water
Strept. Viridans	-	-	-	-	+	+	+	+	+	31.25	+
Staph. aureus	-	-	-	+	+	+	+	+	+	62.50	+
Lactobacillus sp	-	+	+	+	+	+	+	+	+	125.00	+

Key: - No growth
+ Growth

Generally, the results of the Minimum Inhibitory Concentration (MIC) shown in Tables 2 and 3 for the test bacterial followed the same pattern as the results in Table 1 for the inhibitory zone diameters. Staphylococcus aureus has the lowest MIC value.

The Minimum Bactericidal Concentration (MBC) results of both the ethanolic and water extracts of *Phylanthus niruri* in Tables 4 and 5 shows that both extracts exhibited bactericidal activity on the test organisms, with the ethanolic extract exhibiting stronger activity than the water extract. This result also reflects the assertion by Prescott *et al*. (1999) that, a bactericidal drug kills pathogens at levels only 2-4 times the Minimum Inhibitory Concentration (MIC).

In conclusion, both the ethanolic and water extracts of the test plant (*Phyllanthus niruri*) have demonstrated antibacterial activity against the various test organisms. This finding agrees with that of Calixto

et al., (1993) who has earlier reported that the plant was found to be effective against some bacteria invitro. Based on these findings it has been observed that the ethanolic extract of the plant is more efficacious against the oral pathogenic bacteria than the water extract. This implies that ethanolic extract of the plant can be used in preparing an effective alternative to chemical mouth wash solutions to eliminate some common oral pathogens associated with infections of the bucal cavity.

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