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# Studies on Antibacterial Effect of The Leaves Of *Phyllanthus Niruri* on Some Enteric Pathogens

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**Abstract**
Studies were carried out on the antibacterial effect of *Phyllanthus niruri* (Linn) leaves using ethanol and aqueous extracts of the leaves at concentrations 400mg/ml, 200mg/ml, 100mglml, 50mg/ml and 25mg/ml. Gentamycin was employed as control at concentration of 50mg/ml. Pure isolates of *Escherchia coli, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa* and *Klebsiella aerogenes* were employed as test organisms. Results showed that both the *ethanolic* and aqueous extracts had antibacterial activities against test bacteria but to varying degrees. *P. aeruginosa* was the most susceptible bacterium to the ethanolic extract, followed by *S. typhi. E. coli* was the least susceptible. For the aqueous extract, *K. aerogenes* was most susceptible followed by *P. aeruginosa. S. typhi* was the least susceptible. The minimum inhibition concentration (MIC) of the ethanolic extract for *E. coli* was found to be 50mg/ml while *S. aureus, P. aeruginosa and K.* aerogenes had a common MIC of 12.5mg/ml. However, *S. typhi* was inhibited in all concentrations used. For the aqueous extract; *E. coli* was inhibited in all concentrations used while *S. typhi,* and *P. aeruginosa* had the same MIC of 25mg/ml. *S. aureus* with MIC 12.5mg/ml was the most susceptible. Results of the Minimum Bactericidal concentration (MBC) of the ethanolic extracts showed that it had bacteriostatic effect on test bacteria in all the concentrations tested. The aqueous extracts had bacteriostatic effect on only *K. aerogenes* and had bactericidal effect on all other test bacteria. Statistical analysis revealed that the mean diameter zones of inhibition for gentamycin was significantly higher than those of the plant extracts at 0.05 level of probability though, 400mg/ml concentration exhibited high antibacterial activities in both extracts. This study has established the antibacterial effect of *P. niruri* leaves.

**Keywords:** *Phyllanthus niruri; antibacterial; Jos; Enteric pathogens*
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## Introduction

*Phyllanthus niruri* Linn. belongs to the genus *Phyllanthus* and the tribe Phyllanthae which is in the family of Euphorbiaceae. The genus contains over 600 species of trees and biennial herbs distributed throughout the tropical and subtropical regions of the World (Grewal, 2000).

*P. niruri* is a small erect annual herb growing up to 30 to 60cm in height (Plate 1). It is a herbaceous weed found by the roadside, cultivated lands, waste places of the forest and savanna. The plant is indigenous to the rain forests in the Amazon and tropical areas including Bahamas, India, Pakistan and China (Jones and Kenneth, 1995; Grewal, 2000).

The plant has several tribal names based on it uses or the arrangement of the flower on the leaves. Chanca piedra is the Spanish name meaning "stone breaker" or shatter stone", it stems from its use as elimination agent for gall and kidney stones by the indigenous people of Amazon, (Balee and William,1994). *P. niruri* has several uses in herbal medicine. The plant has been reported to have liver protective antilithic, pain-relieving, hypotensive, antispasmodic, antiviral, anti-fungal, diuretic, antimutagenic, hypoglycemic and anti-bacteria actions (Barros et al., 2003). The therapeutic action has been reported in the following pathologies: pimples, eczemas, gangrene, malaria, syphilis, ulcer, urethral secretion, diarrhea, dysentery, dropsy, mouth and throat infection, veneral diseases, hepatic diseases and gastro-intestinal disorders (Tona et al., 2004).

Data on *P. niruri* antibacterial activities in Nigeria is limited. This, therefore, formed the basis of this study. The study was designed to investigate the antibacterial effect of the leaf extracts of *P. niuriri* on five enteric pathogens.

Plate 1: The mature plant *Phyllantluis niruri*
The Experimental Plant

**Materials and Methods**

*Plant collection and Preparation of Extract*: Fresh leaves of *P. niruri* were obtained from a farmland near Agip Estate, Port Harcourt in River's State of Nigeria. Crude ethanolic and aqueous extracts of the plant were obtained with the aid of soxhlet extractor using the method described by (Sofowora, 1982; Trease and Evans, 1989). The stock and working solution of the leaf extracts were prepared by using the standard method of the National Committee for Clinical Laboratory Standards. 2g each of the extracts (Aqueous and ethanol) were dissolved in 10ml of distilled water to give a concentration of (0.4g/ml. 400mg/ml) stock solution. A two fold serial dilution was carried out to obtain four different dilutions of 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml.

Test Organisms

The test organisms included *Staphylococcus aureus, Escherichia coli, Pseudomonas, aerognosa Salmonella typhi* and *Klebsiella aeruginosa*. The organisms were obtained from the Microbiology Department of Jos University Teaching Hospital (JUTH). They were sub-cultured on nutrient agar slant and kept at $4^0$C.

*Susceptibility Testing:* The antibacterial effects of the leaf extracts were determined using the agar well diffusion method. 0.lml of nutrient broth containing each of the test microorganisms at concentrations $1.6x10^8$ were pipetted into 20ml of freshly prepared nutrient agar in a sterile bottle. It was then poured into a plate and allowed to set. Six wells, were bored using 8mm cork borer into which 1ml of the different dilutions of the extracts (400mg/ml; 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml) were introduced and allowed to diffuse for one hour. They were incubated at $37^0$C for 24 hours and later observed to determine the diameter of zones of inhibition. Gentamycin (50mg/ml) was used as a control in the sixth well.

The experiment was repeated for both water and ethanolic extracts. The diameters of the zones of inhibition were measured with metre rule to the nearest millimeter. The minimum inhibitory concentration (MIC) of the aqueous and ethanolic extracts were determined by the broths dilution technique (Puyvelde, 1986) or the doubling dilution method. A volume of one hundred milligram per milliliter (100mg/ml) standard was used for all the extracts. Two sets of seven sterile test tubes were used.

A test tube served for each of the six concentrations and another served for Gentamycin, which was the control. 5mls of the extract was mixed with 5mls of medium (Nutrient broth) and serially diluted to $10^{-5}$ dilution.

Aliquots of 0.1ml inoculum of the test microorganisms were added to each of the five test tubes. The bottles were thoroughly mixed by gentle shaking and incubated for 24 hours at $37^0$C. The tubes were observed visually for growth by comparing the turbidity with the

control. The highest dilution or lowest concentration, which produced no growth, was taken as the minimum inhibitory concentration.

Tubes showing no visible growth from the MIC test were sub-cultured on the fresh nutrient agar and incubated at $37^0$C for 24 hours. The lowest concentration of the extract that yielded no growth was recorded as the minimum bactericidal concentration (MBC). Diameters of zones of inhibition given by leaf extracts were compared with the one produced by the antibiotics Gentamycin (50 mg/ml) used.

*Statistical Analysis***:** Data generated were analysed using 2-way ANOVA.

**Results**

The results of the investigation showed that both ethanolic and aqueous extracts of *Phyllanthus niruri* had activities against the test bacteria but to varying degrees (Tables 1 and 2).

The diameter of zones of inhibition of the ethanolic extract was found to be wider at 400mg/ml concentration (3.72mm), than 200mg/ml concentration (3.46mm). The other concentrations were found to have similar diameter of zones of inhibition 9.0mm (Table 1). Statistical analysis revealed that the mean diameter of zones of inhibition for 400mg/ml extract concentrations was significantly higher than all other concentrations at 0.05 level of probability while 100mg/ml, 50mg/ml and 25mg/ml concentrations did not differ from each other at P > 0.05 (Table 1). *P. aeruginosa* was found to be the most susceptible bacterium in terms of the effects of the ethanolic extract of the experimental *P. niruri*. It was followed by *S. typi* while E*. coli* was found to be the least susceptible. *Klebsiella aerogenes* was the most susceptible bacterium in terms of the effects of the plant's aqueous extract, followed by *P. aeroginosa* and *E. coli* while *Salmonella typhi* was the least susceptible (Table 2).

As for the aqueous extract of the plant the zone of inhibition was found to be most significant for 400mg/ml concentration (22.8mm). It was followed by 200mg/ml concentration (18.8mm) while the least zone of inhibition was recorded for 25mg/ml concentration (8.0mm). The details are given in Table 2. The control (Gentamycin) exhibited the greatest zone of inhibition.

Statistical analysis revealed that the zone of inhibition exhibited by 400mg/ml concentration was significantly higher than all other concentrations at 0.05 level of probability while 100mg/ml, 50mglml and 25mg/ml concentrations did not differ from each other at P < 0.05. The diameter of zone of inhibition exhibited by gentamycin was most significant as compared to those exhibited by experimental plant extracts at P < 0.05. The details are presented in Table 2.

Table 1: The effects of ethanolic leaf extracts of *P. niruri* on the test bacterial species

| Test bacteria | Mean diameter of zones of inhibition (mm) at different concentrations | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 400 (mg/ml) | 200 (mg/ml) | 100) (mg/ml) | 50 (mg/ml) | 25 (mg/ml) | Bactaria Total | Bacte-ria Mean | Gentamycin 50mg/ml |
| *Escherichia coli* | 16.00 | 12.00 | 9.00 | 9.00 | 9.00 | 55.00 | 11.00 | 36 |
| *Staphylococcus aureus* | 16.00 | 14.00 | 9.00 | 9.00 | 9.00 | 57.00 | 11.4 | 40 |
| *Salmonella typhi* | 16.00 | 15.00 | 9.00 | 9.00 | 9.00 | 58.00 | 11.6 | 40 |
| *Psendomonas aeruginosa* | 18.00 | 17.00 | 9.00 | 9.00 | 9.00 | 62.00 | 12.4 | 30 |
| *Klebsiella aerogenes* | 16.00 | 14.00 | 9.00 | 9.00 | 9.00 | 57.00 | 11.4 | 42 |
| *Concentration total* | 82.00 | 72.00 | 45.00 | 45.00 | 45.00 | 289 | | 188 |
| Concentration Mean | 16.2 | 14.4 | 9.00 | 9.00 | 9.00 | | | 37.6 |
| LSD | 1.11 | 1.11 | 1.11 | 1.11 | 1.11 | | | |

Table 2: The effects of aqueous leaf extracts of *P. niruri* on the test bacteria.

| | Mean diameter of zones of inhibition (mm) at different concentrations |
|---|---|

| Test bacteria | 400 (mg/ml) | 200 (mg/ml) | 100) (mg/ml | 50 (mg/ml ) | 25 (mg/ml ) | Bact-eria Total | Bacte-ria Mean | Genta-mycin 50mg/ml |
|---|---|---|---|---|---|---|---|---|
| *Escherichia coli* | 20 | 18 | 16 | 15 | 12 | 87 | 16.2 | 30 |
| *Staphylococcus aureus* | 24 | 20 | 17 | 11 | 0 | 72 | 14.4 | 40 |
| *Salmonella typhi* | 24 | 19 | 16 | 10 | 0 | 69 | 13.8 | 40 |
| *Psendomonas aeruginosa* | 20 | 18 | 17 | 15 | 13 | 83 | 16.6 | 40 |
| *Klebsiella aerogenes* | 26 | 19 | 17 | 16 | 15 | 93 | 18.6 | 42 |
| *Concentration total* | 114 | 94 | 83 | 67 | 40 | 398 | | 192 |
| Concentration Mean | 22.8 | 18.8 | 16.6 | 14.4 | 8.0 | | | 38.5 |
| LSD | 4.83 | 4.83 | 4.83 | 4.83 | 4.83 | | | |

The results of the minimum inhibition concentration (MIC) of the ethanolic extracts of *P. niruri* showed that (*Salmonella typhi check spelling???)* was inhibited at all the concentrations. *E coli* had MIC of 50mg/ml while all other test bacteria had MIC of 12.5 mg/ml (Table 3). The aqueous extract proved more effective against the test bacteria with an MIC of 50mg/ml for *K. (aeruginosa check spelling???)* followed by 25mg/ml for *S. typhi* and *P. (aeruginosa check spelling???) S. aureus* had an MIC of 12.5mg/ml while no MIC value was recorded for *E. coli* as its growth was inhibited in all the concentrations as seen in Table 4. The extract and media sterility control yielded no growth.

Table 3: The minimum inhibitory concentration (MIC) of the ethanolic leaf extracts of *Phyllanthus niruri*

| Test bacteria | Concentration in (mg/ml) 100 | 50 | 25 | 12.5 | 6.25 | Control MSC | ESC | MIC |
|---|---|---|---|---|---|---|---|---|
| *Escherichia coli* | - | - | + | + | + | - | - | 50 |
| *Staphylococcus aureus* | - | - | - | - | + | - | - | 12.5 |
| *Salmonella typhi* | - | - | - | - | - | - | - | - |
| *Psendomonas aeruginosa* | - | - | - | - | + | - | - | 12.5 |
| *Klebsiella aerogenes* | - | - | - | - | + | - | - | 12.5 |

Table 4: The minimum inhibitory concentration (MIC) of the Aqueous leaf extracts of *P. niruri*

| Test bacteria | Concentration in (mg/ml) 10 0 | 50 | 25 | 12.5 | 6.25 | Control MSC | ESC | MIC |
|---|---|---|---|---|---|---|---|---|
| *Escherichia coli* | - | - | - | - | - | - | - | - |
| *Staphylococcus aureus* | - | - | - | - | + | - | - | 12.5 |
| *Salmonella typhi* | - | - | - | + | + | - | - | 25 |
| *Psendomonas aeruginosa* | - | - | - | + | + | - | - | 25 |
| *Klebsiella aerogenes* | - | - | + | + | - | - | - | 50 |

- = No growth
+ = Growth
MSC = Medium sterility control
ESC = Extract sterility control

The results of the minimum bactericidal concentration (MBC) of the ethanolic extract of *P. niruri* revealed that it had bacteriostatic effect on the test bacteria in all the concentrations (6.25mg/ml – 100mg/ml) as seen in Table 5. The aqueous extract however, had a lethal (bactericidal) effect on *E. coli* and *S. aureus*, at concentration of 50mg/ml. *S. typhi* and *P. aeruginosa* had the MBC of 100mg/ml. The extract was bacteriostatic on *K. aerogenes* in all the concentrations tested (Table 6).

Table 5: The minimum bactericidal concentration (MBC) of ethanolic leaf extract of *Phyllanthus niruri* on test bacteria

| Test bacteria | Concentration in (mg/ml) 100 | 50 | 25 | 12.5 | 6.25 |
|---|---|---|---|---|---|
| *Escherichia coli* | + | + | + | + | + |
| *Staphylococcus aureus* | + | + | + | + | + |
| *Salmonella typhi* | + | + | + | + | + |
| *Psendomonas aeruginosa* | + | + | + | + | + |
| *Klebsiella aerogenes* | + | + | + | + | + |

Table 6: The minimum bactericidal concentration (MBC) of Aqueous leaf extract of *Phyllanthus niruri* on test bacteria

| Test bacteria | Concentration in (mg/ml) 100 | 50 | 25 | 12.5 | 6.25 | MBC |
|---|---|---|---|---|---|---|
| *Escherichia coli* | - | - | + | + | + | 50 |
| *Staphylococcus aureus* | - | - | + | + | + | 50 |
| *Salmonella typhi* | - | + | + | + | + | 100 |
| *Psendomonas aeruginosa* | - | + | + | + | + | 100 |
| *Klebsiella aerogenes* | + | + | + | + | + | |

- = No growth
+ = Growth

**Discussion**

The results obtained showed that the ethanolic and aqueous extracts of the leaves of *Phyllanthus niruri* were potent against the test organisms. The susceptibility of these bacteria agrees with the reports of Pelzar, (1998) that Gram-negative bacteria are more resistant than their Gram-positive counterparts. It was also observed that the aqueous extracts exhibited more potency than the ethanolic extracts. This is in line with the use of water as a solvent in preparation of decoction for use traditionally because most of the active biochemicals present in the plant are water soluble, fragile and hence, damaged in alcohol (Cheji, 1998).

The efficacy of the aqueous extracts against the test bacteria agrees with Burkil (1935) who reported that the leaves, of *P. niruri* have several uses especially for stomach ailments, dropsy and urinogenital diseases, in India, Malaysia and Philipines.

The results of this investigation revealed that the potency of the extracts increased with the increase in concentration. This confirms the findings of Kurosaki and Nishi (1983) who reported that higher concentrations of antibacterial substances exhibit more growth inhibition of some microbial pathogens.

The low efficacy of the ethanolic extracts against the test bacteria observed in this study might stem from the difficulty of dissolution of some of the constituent extract in ethanol.

The results of the minimum inhibitory concentration (MIC) of the ethanolic extract and aqueous extract varied with the types of microorganisms tested. The most significant effect was observed on the use of the ethanolic extract against *S. aureus,* with MIC 6.25mg/ml, it was followed by *P. aeruginosa* and *K. aerogenes* with the same MIC 12.5mg/ml. While the least significant effect was observed on *E. coli* with MIC 50mg/ml. The growth of *S. typhi* was inhibited in all concentrations of the ethanolic extract. This is in conformity with the ethnobotanical findings of Jones and Kenneth (1995) who reported that the plant was used in the treatment of several ailments including constipation, flu and typhoid fever caused by *S. typhi* in the Bahamas.

All the concentrations of the aqueous extract inhibited *E. coli*. *K. aerogenes* with MIC 50mg/ml was the least susceptible bacterium to the aqueous extract while *S. aureus* was the

most susceptible bacterium. This is consistent with previous studies, which showed that the plant extract is active on *S. aureus* (Grewal, 2000).

The result of the MBC of the ethanolic extract showed that the test organisms were inhibited but not killed in all the concentrations. This showed that the ethanolic extract has bacteriostatic effect on these organisms. The reason is not far-fetched since most of the active biochemical present in *P. niruri* are water-soluble and more fragile, water-soluble plant chemicals and sterols are damaged in alcohol as reported by Qian-Cutrone, et al. (1996).

The investigation of the MBC of the aqueous extract revealed that most of the concentrations of the extract killed the test microorganisms. It showed that the aqueous extract has lethal/bactericidal effect on most of the bacteria. *E. coli* and *S. aureus* with MBC 50mg/ml had the highest lethal concentration than *P. aeruginosa* and *S. typhi* with MBC 100mg/ml. All the concentrations of the extract had a bacteriostatic effect on *K. aerogenes*.

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