

ANTIMICROBIAL ACTIVITY OF PHYLLANTHUS NIRURI AGAINST DIFFERENT HUMAN PATHOGENIC BACTERIAL STRAINS

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ABSTRACT

Use of herbal medicine over synthetic drugs has become increasingly popular due to the various side effects and pathogen resistance after long term uses of synthetic drugs. In order to overcome with these issues alternative drugs from natural resources need to develop. *Phyllanthus niruri* (Linn) member of family Euphorbiaceae is one of the potential medicinal plant, however very less known about its medicinal usage and active ingredients. In this study antimicrobial activity of ethanolic and methanolic extract of *Phyllanthus niruri* was tested against six major human pathogenic microbial strains viz; *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Streptococcus mutans* and *Salmonella typhae*. The results indicate that both the organic extracts (Ethanol and Methanol)

of *Phyllanthus niruri* showing broad spectrum of antimicrobial activity.

KEYWORDS: Antimicrobial activity, Ethanolic and Methanolic extract, Major human pathogenic microbial strains, *Phyllanthus niruri*.

INTRODUCTION

Phyllanthus niruri (Linn), member of family Euphorbiaceae is a winter weed occurs across the hotter place in India. The *Phyllanthus* genus contains over 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical areas (Paithankar, 2011). This plant grows up to 30 to 60 cm in height considered as an herbaceous

weed found near to the cultivated lands, waste places and roadsides. The plant is native to the rain forests in the Amazon and tropical areas including India, China, Pakistan and Bahamas, (Jones and Kenneth, 1995; Grewal, 2000).

P. niruri has several benefits as a herbal medicine. The plant has been found to have hepatoprotective, antilithic, pain-relieving, anti-fungal, diuretic, antispasmodic, hypoglycemic, antiviral and anti-bacterial actions (Paithankar, 2011). The therapeutic action has been investigated with respond to following diseases: diarrhea, dysentery, dropsy, mouth and throat infection, venereal diseases, pimples, eczemas, gangrene, malaria, syphilis, ulcer, urethral secretion, hepatic diseases and gastro-intestinal disorders (Tona et al; 2004).

However there is limited data available on antimicrobial activities of *P. niruri*. Present study focus on investigation and evaluation of antimicrobial potential of *P. niruri* on six major human pathogens.

MATERIAL AND METHODS

P. niruri leaves were collected from the campus of G.B Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India (29.0500° N, 79.5167° E).

Around 30-50 grams of shade dried plant leaves were ethanolic and methanolic extracted by soaking in 50 mL of 90% ethanol and methanol for overnight at room temperature. The mixtures were filtered and filtrates were concentrated on rotary evaporator at 45°C to eliminate the ethanol and methanol. Extract were stored at 4°C in sterile bottle until use. Four gram of each extract (ethanol and methanol) was dissolved in 10 mL of distilled water to obtain the final concentration of 0.4g/mL or 400mg/ml stock solution. Two fold serial dilutions were done to get four different concentrations such as 200 mg/ml, 100 mg/mL, 50 mg/ml and 25 mg/ml of extracts.

Test pathogens includes *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Streptococcus mutans* and *Salmonella typhae* were obtained from IMTECH, Chandigarh in lyophilized form and further subcultured and stored at 4°C.

Antimicrobial activity of leaf extracts was evaluated using agar well diffusion method. 100 µl of nutrient broth having each test pathogen at concentration of 1.6×10^8 was pipetted into 20 ml of nutrient agar in a sterile bottle and poured into the Petri plates. Six wells were bore with the help of cork borer and 1 ml of different dilutions of the ethanolic and methanolic extracts

were introduced to the well and kept one hour to diffuse. Gentamycin (50mg/ml) was used as a control in seventh well. Plates were incubated at 37°C for 24 hrs and observed to evaluate the zone of inhibition to determine the antimicrobial activity.

RESULT AND DISCUSSION

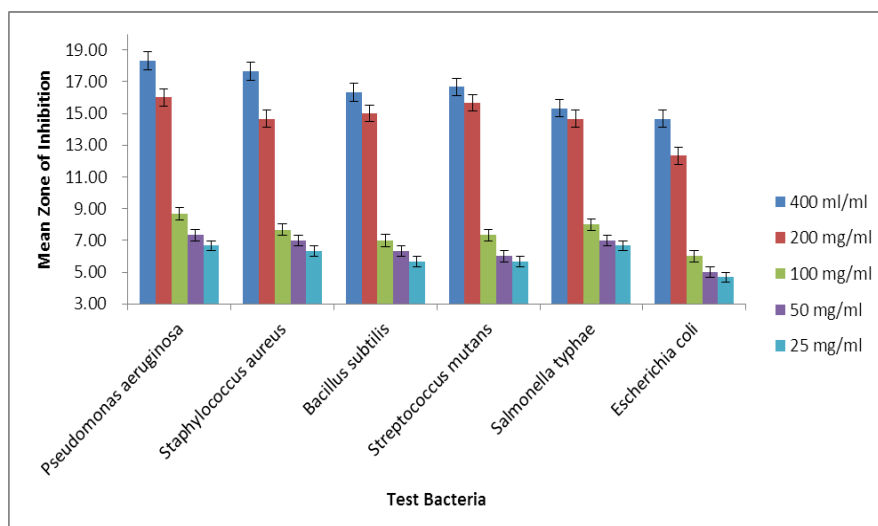
The results of the experiment suggest antimicrobial activity of *Phyllanthus niruri* for both methanolic and ethanolic extracts against the test bacteria with the varying extent (Table 1 and Table 2).

The diameter of zone of inhibition of the methanolic extract was recorded broader at 400 mg/ml concentration and there is gradual reduction at 100 mg/ml, 50mg/ml and least at 25 mg/ml in each tested bacteria. As per table no. 1, *Pseudomonas aeruginosa* shows maximum zone of inhibition of about 18mm and *E.coli* shows minimum zone of inhibition of about 15mm against the concentration of 400 mg/ml of the methanol extract of *Phyllanthus niruri*. *Pseudomonas aeruginosa* was recorded to be the most susceptible bacterium on account of effect of methanolic extract of *Phyllanthus niruri*. It was followed by *S.aureus*, *S.mutants*, *B.subtilis*, *S.typhae* and least in *E. coli* in almost every used concentration.

As of the ethanolic extract (Table No.2) *Pseudomonas aeruginosa* shows the maximum zone of the inhibition of about 17mm and *E.coli* shows minimum zone of inhibition of about 13mm against the concentration of 400 mg/ml of the ethanol extract of *Phyllanthus niruri*. *Pseudomonas aeruginosa* was recorded to be the most susceptible bacterium on account of effect of ethanolic extract of *Phyllanthus niruri*. It was followed by *S.aureus*, *B.subtilis*, *S.mutants*, *S.typhae* and least in *E. coli* in almost every used concentration.

Table 1: Antimicrobial effect of methanolic extract of *P.ninuri* leaf on different pathogens

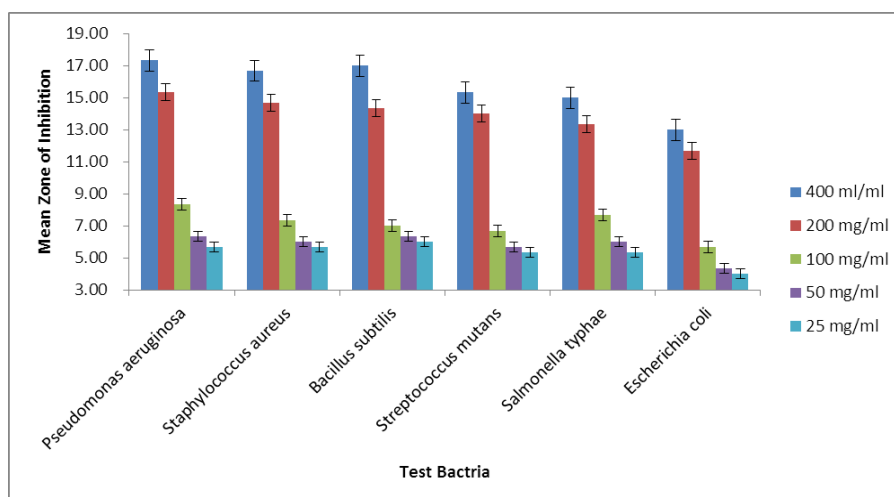
Test Bacteria	Mean Zone of Inhibition (mm) at different concentrations					
	400 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	Gentamycin (50mg/ml)
<i>Pseudomonas aeruginosa</i>	18.33 ±0.67	16.00 ±1.15	8.67 ±0.88	7.33 ±0.67	6.67 ±0.33	34
<i>Staphylococcus aureus</i>	17.67 ±0.88	14.67±1.20	7.67±1.45	7.00 ±0.58	6.33 ±0.67	39
<i>Bacillus subtilis</i>	16.33 ±0.88	15.00 ±1.53	7.00 ± 1.15	6.33 ±0.67	5.67 ±0.67	37
<i>Streptococcus mutans</i>	16.67 ±1.20	15.67 ±1.20	7.33 ± 0.33	6.00 ±0.58	5.67 ±0.33	38
<i>Salmonella typhae</i>	15.33 ±0.88	14.67 ±1.33	8.00 ± 0.58	7.00 ±0.58	6.67 ±0.33	39
<i>Escherichia coli</i>	14.67 ±1.20	12.33 ±0.88	6.00 ± 0.58	5.00 ±0.58	4.67 ±0.33	36



Graph A: Graphical representation of antimicrobial effect of methanolic extract of *P.ninuri* leaf on different pathogens

Table 2: Antimicrobial effect of ethanolic extract of *P.ninuri* leaf on different pathogens

Test Bacteria	Zone of Inhibition (mm) at different concentrations					Gentamycin (50mg/ml)
	400 mg/ml	200 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	
<i>Pseudomonas aeruginosa</i>	17.33±1.45	15.33 ±0.88	8.33 ±0.67	6.33±0.33	5.67 ±0.33	35
<i>Staphylococcus aureus</i>	16.67 ±0.88	14.67 ±0.33	7.33 ±1.20	6.00 ±0.58	5.67± 0.33	38
<i>Bacillus subtilis</i>	17.00 ±1.15	14.33± 1.45	7.00 ±0.58	6.33± 0.33	6.00± 0.58	39
<i>Streptococcus mutans</i>	15.33 ±0.33	14.00 ±0.58	6.67 ±0.67	5.67 ±1.20	5.33 ±0.88	38
<i>Salmonella typhae</i>	15.00 ±0.58	13.33 ±0.67	7.67± 0.67	6.00± 0.58	5.33± 0.33	39
<i>Escherichia coli</i>	13.00 ±0.58	11.67± 0.88	5.67 ±0.33	4.33 ±0.33	4.00 ±0.58	40



Graph B: Graphical representation of antimicrobial effect of Ethanolic extract of *P.ninuri* leaf on different pathogens

The antimicrobial activity of the extracts of *Phyllanthus niruri* may be due to the presence of lignans; phyllanthin and hypophyllanthin, flavonoids, triterpenoids, glycosides, and tannins, in the plant extract (Rajesh Kumar et al; 2002). The higher activity of the methanol extracts may be due to higher solubility of the active compounds in these solvents (Parekh et al; 2007 and Boer et al; 2005). Methanol had a higher power to extract the active antibacterial compounds in the plant which exhibited higher activity with higher zones of inhibition (Njoroge et al; 2012).

The results of experiment indicate that the potency of the extract increased with increase in concentration. This further confirms the finding of Kurosaki and Nishi (1983) who reported that higher concentration of antibacterial extracts shows more growth inhibition to some microbial pathogens.

CONCLUSION

In conclusion, results support that leaf extracts of *Phyllanthus niruri* have broad spectrum of antimicrobial activity. The methanolic extract of *Phyllanthus niruri* shows greater antimicrobial activity than ethanolic extract and hence might be useful as strong therapeutic agent for pathological damages.

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REFERENCES

1. Paithankar V. V., Raut K. S., Charde R. M., Vyas J. V. *Phyllanthus Niruri*: A magic herb. *Research in Pharmacy*, 2011; 1(4): 1-9.
2. Jones J.P. and Kenneth I.R. *Immune Power from Rain Forest*. Healing Arts Press, Toronto, Canada, 1995; 54 – 58.
3. Grewal R.C. *Medicinal plants*. Campus Book International, Roshan, Delhi. 2000; 296 – 303.
4. Tona L., Cimanga R.K., Mesia K., Musuamba C.T., De Bruyne T., Apers S. Hernans, N., Van Mient, S., Pieters L., Totte J. and Vlietinck A.J. In vitro antiplasmodial activity of extracts and fractions from seven medicinal plants used in the democratic Republic of Congo. *J. Ethnopharmacol*, 2004; 93(1): 27 – 32.

5. Rajesh Kumar NV, Joy KL, Giriya K, Ramsewak RS, Nair MG, Ramadasan K. Antitumor and anticarcinogenic activity of *Phyllanthus amarus* extract. *Journal of Ethnopharmacology*, 2000; 81: 17–22.
6. Njoroge AD, Anyango B, Dossaji SF. Screening of *Phyllanthus* Species for Antimicrobial Properties. *Chemical Sciences Journal*, 2012: CSJ-56.
7. Kurosaki, F. and Nishi. Isolation and antimicrobial activity of *Phyllanthus* on 6 – metonymy mullein from culture carrot cells. *Phytochem.*; 1983; 22 (3): 666– 672.
8. Parekh J, Chanda SV. Antibacterial phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research*, 2007; 10: 175–181.
9. Boer JH, Kool Mziray WR, Herdberg I, Levenfors JJ. Antifungal and antibacterial activity of some herbal remedies from Tanzania. *Journal of Ethnopharmacology*, 2005; 96: 461–469.