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Full Length Article

Antibacterial Activity of *Phyllanthus niruri* growing near mobile towers

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ABSTRACT

Phyllanthusniruri Linn. plants growing near the vicinity of mobile towers were analysed for their antibacterial potential against twelve bacterial strains which are the main cause of many common diseases seen in Kanyakumari district. *Phyllanthus* species is known for their potent medicinal properties, but the study on the influence of mobile tower radiations on the medicinal principles of this plant has not been reported. Hence the present work holds great importance. In this work it was found out that the plants growing near mobile towers exhibited higher antibacterial activity than the control plants growing far away from mobile towers. This was evident in the zone of inhibition from the methanol extracts of mutant plants growing near mobile towers of different areas in Kanyakumari district of Tamil Nadu. This workhowever needs more research into the gene level of such *Phyllanthusniruri* plants to confirm whether this elevated antibacterial activity will benefit humans in a positive manner.

Key words: Antibacterial Activity of *Phyllanthus niruri*, mobile towers

INTRODUCTION

India is a varietal emporium of medicinal plants and is one of the richest countries in the world in regard to genetic resources of medicinal plants. It exhibits a wide range in topography and climate, which has a bearing on its vegetations and floristic composition (Parekh, 2007). The discovery of medicinal plants are important both to the agricultural and medicine sectors, helping in establishment of new directions towards propagation of alternative medicinal crops that offer better economic and social benefits (Barbour *et al.,* 2004). Plants are known to contain numerous biologically active compounds which possess curative properties. Since long ago plants have remained the basis for the development of modern drugs for human health (Lawrence, 2009). More than 80,000 species of plants are utilized by different systems of Indian medicine (Prajapati *et al.,* 2006). According to World Health Organisation, 80% of the population of developing countries relies on traditional medicines mostly plant drugs for their primary health needs. Though the recovery is slow, man has used various parts of plants in the treatment and prevention of various ailments because of its inactivity to cause side effects (Tanaka *et al*., 2002; Rawat & Uniyal, 2003).

The compounds from plants inhibit bacterial growth by different mechanisms than those presently used chemical antimicrobials and may have a significant clinical value in the treatment of resistant microbial strains (Eloff, 1998; Barbour *et al.,* 2004). *Phyllanthus niruri* L. (Syn. *Phyllanthus fraternus* Webster) of the family Euphorbiaceae is a herb rich in plant chemicals including alkaloids like astragalin, brevifolin, corilagin, ellagic acid, ellagitannins, phyllanthin, phyllanthenol, phyltertralin, saponins, tricontanal etc. The plant is of medicinal importance for numerous ailments like dysentery, diuretics, kidney stones, influenza, antibacterial, antihyperglycaemic and antiviral activities (Chopra *et al.,* 1986).

Antibacterial activity of *Terminalia thorelli*, *Emblica officinalis* and *Cassia occidentalis* had been reported by Koche *et al*., 2011. Phytochemical analysis and antibacterial activity of *Kedrostis foetidissima* had been reported by Vasantha *et al*., 2012, and revealed that all parts of this plant showed inhibitory activity against *E. coli*, *P. aeruginosa* and *K. pneumonia*. Antimicrobial activity in *Phyllanthus niruri* has been reported by Hoffman *et al.,* 2004 against pathogenic bacterial and fungal strains. Verpoorle and Dihal, 1987 and Muzuneder *et al.,* 2006 has stressed that antibacterial potentiality of the plant extract against resistant pathogens. Herbs that have tannins as part of their work conducted by Mathur *et al.,* (2012) revealed that the methanolic extracts of various parts of *Phyllanthus niruri* have antibacterial activity against five bacterial strains- *E. cloacae, S. aureus, P. aeruginosa, E. coli* and *S. viridians*. Although research on the health effects of plants of the *Phyllanthus* genus is very limited, there´s some evidence that the *Phyllanthus amarus* and *Phyllanthus niruri* species may offer certain benefits (Wong, 2014).

MATERIALS AND METHODS

For analyzing antibacterial activity of *Phyllanthusniruri*, four groups of plants were selected such as control or wild plant growing very far from mobile towers (1km), Mutant 1- *Phyllanthusniruri* plants growing near mobile towers from site-1 (10m-100m) situated at Edaicode of Kanyakumari district, Mutant 2- plants of *Phyllanthus niruri* growing near mobile tower from site -2 (10m-100m) from Pathukani of Kanyakumari district and mutant 3-plants of *Phyllanthus niruri* growing near mobile tower from site -3 (10m-100m) from Thengapattinam of Kanyakumari district. The plants were collected and shade dried. It was ground into a powder form using mortar and pestle. The dried powder was weighed and extracted in the soxhlet using four successive solvents. The solvents used were acetone, petroleum ether, ethanol and methanol (successive solvent method). The extract was then filtered and allowed to dry and stored in air tight container at 4°C in a refrigerator for further analysis. Each plant extract was analysed to detect their antimicrobial properties against few common pathogenic bacterial strains by agar diffusion method (Bauer *et al.,* 1966).

Twelve strains of bacteria viz. *Escherichia coli, Bacillus subtilis, Salmonella enterica, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Streptococcus pyogenes, Corynebacterium pseudotuberculosis, Enterobacter aerogenes, Vibrio cholera, Lactobacillus casei* were used for antibacterial analysis of the plant extracts. The Mueller Hinton Agar medium (MHA) was prepared by pouring 15ml of molten media into sterile petri plates and sterilized at 121° C for 15 minutes. The sterile filter paper disc (5mm) was prepared and impregnated with each plant extract solution and left to dry at room temperature. The media was poured in sterile petri plates. After solidification microbial suspension was spread by a sterile swab, evenly, over the face of a sterile agar plate. The plant extract was applied to the center of the agar plate and incubated at 37°C for 24 hours. The results were read by measuring the zone of inhibition.

RESULTS AND DISCUSSION

The antibacterial activity of *Phyllanthus niruri* from the extract of control plants and plants growing near mobile tower vicinity (within 10m-100m radius) were assayed *in vitro* by agar disc diffusion method. Acetone, petroleum ether, ethanol and methanol extract of *Phyllanthus niruri* were tested against 12 bacterial strains. Table 1-4 summarizes the microbial growth inhibition zones of the extracts of the screened plants. In the methanol extract of the wild plant, the growth of *Salmonella enterica* was inhibited to the maximum (13mm). While low levels of inhibition was seen in the growth of *Corynebacterium pseudotuberculosis, Streptococcus pyogenes* and *E.coli* (6mm). An inhibition zone of 11mm was seen against the multiplication of colonies of *Vibrio cholera* and *Lactobacillus casei* (Table.1). The acetone extract of the control plant inhibited the growth of *Lactobacillus casei, Staphylococcus aureus, Proteus mirabilis* and *E. coli* exhibiting an inhibited zone 10 mm. While medium levels of inhibition was seen in the growth of *Enterobacter aerogenes, Vibrio cholera, Pseudomonas aeruginosa, Corynebacterium pseudotuberculosis* (Table. 1). *Growth of Klebsiella pneumoniae* and *Bacillus subtilis* exhibited very low inhibition zone of 7mm. Low levels of inhibition was also seen in the multiplication *of Streptococcus pyogenes,*

Pseudomonas aeruginosa and *Bacillus subtilis* (6mm) in the petroleum ether extract of the control plant, while a maximum growth inhibition zone of 7 mm was observed in the multiplication of *Salmonella enterica*. The ethanol extract of the control plant showed the highest inhibition of 10mm against the growth of *Enterobacter aerogenes;* while medium levels of inhibition was seen in the growth *of Streptococcus pyogenes, Klebsiella pneumonia, Pseudomonas aeruginosa, Vibrio cholera*. Least inhibition zone of 6 mm was seen against the multiplication of colonies of *Corynebacterium pseudotuberculosis* and *Proteus mirabilis*. In the methanol extract the growth of *Enterobacter aerogenes* was inhibited to 16mm which was the highest inhibition zone produced in the extract of mutant 1, the least inhibition zone was formed against multiplication of *Streptococcus pyogenes, Klebsiella pneumonia, Vibrio cholera* and *Bacillus subtilis* with the inhibition of 8mm zone. *Corynebacterium pseudotuberculosis, Pseudomona saeruginosa, Proteus mirabilis* and *E. coli* showed average inhibition zone (Table 2). Mutant 1 acetone extract inhibited the growth of *Proteus mirabilis* producing a zone of inhibition of 15mm, while *Vibrio cholera* and *E.coli* showed very little inhibition zone of (6mm). The medium levels of inhibition were shown against the growth of *Lactobacillus casei* and *Bacillus subtilis*. The petroleum ether extract of mutant 1, when exposed to *Pseudomonas aeruginosa*, only 9mm inhibition zone was produced. Least inhibition zone was detected in the growth of *Klebsiella pneumonia, Vibrio cholera, Lactobacillus casei, Salmonella enterica, E. coli* and *Staphylococcus aureus* (Table 2). The ethanol extract of mutant 1, exhibited low inhibitory zone against the growth of *Staphylococcus aureus* (6mm), while *Corynebacterium pseudotuberculosis, Vibrio cholera, Lactobacillus casei, Salmonella enterica, Bacillus subtilis* and *E. coli* were found to be medium in their inhibitory action. The maximum inhibition zone of 13mm was formed against the growth of *Enterobacter aerogenes* and *Proteus mirabilis* (Table 2).

The methanol extract of mutant 2 showed the maximum inhibition zone of 16 mm against the colony formation of *Salmonella enterica*, while the growth of *Corynebacterium pseudotuberculosis, Vibrio cholera, E. coli* and *Staphylococcus aureus* were found to be inhibited in medium levels (Table.3). Low inhibitory zone of 6mm was observed against *Klebsiella pneumoniae* and *Proteus mirabilis* in this extract. An inhibitory zone of 14 mm was seen in the growth of *Pseudomonas aeruginosa* in the acetone extract which was found to be maximum, but low inhibition zone of 6mm could be seen in the growth of *Klebsiella pneumoniae* and *Vibrio cholera*. Medium inhibition zone was formed against the growth of *Lactobacillus casei* and *Bacillus subtilis.* Petroleum ether extract of mutant 2 when exposed to *Salmonella enterica, a* maximum inhibition zone of 9mm was observed; while in mutant 2 extract, *Enterobacte raerogenes* and *E. coli* showed low inhibition zone of 6 mm. The ethanol extract of mutant 2 exhibited high inhibition zone of (16mm) against the growth of *Bacillus subtilis*. While *Streptococcus pyogenes, Klebsiella pneumonia* and *Lactobacillus casei* when exposed to the ethanol extract of mutant 2 showed the medium inhibition zone. The inhibition zone was (6mm) low against *Proteus mirabilis* in this extract.

In the methanol extract of mutant 3, growth of *Streptococcus pyogenes* and*Klebsiella pneumonia* was presented with low inhibition zone of 7mm, while maximum inhibition zone of 14mm was observed against the growth of *Enterobacter aerogenes*. Medium levels of inhibition were seen against the growth of *Corynebacterium pseudotuberculosis, Pseudomonas aeruginosa* and *E. coli*. Maximum inhibition zone (13mm) was found against *Pseudomonas aeruginosa* in the acetone extract. Low inhibition zone (6mm) was observed against *Streptococcus pyogenes*. While medium level of inhibition was seen against *Lactobacillus casei*. In petroleum ether extract of mutant 3, maximum inhibition zone was found to be 9mm against *Pseudomonas aeruginosa*. Low inhibition zone was found against the multiplication of colonies of *Streptococcus pyogenes, Klebsiella pneumonia*, *Enterobacter aerogenes, Proteus mirabilis and Bacillus subtilis*. A high inhibition zone (15mm) was found to be seen against the growth of *Bacillus subtilis* in the ethanol extract of mutant 3.While low inhibition zone (6mm) was observed against *E. coli*. Medium levels of inhibition were found against the growth of *Enterobacte raerogenes* and *Lactobacillus casei* in this extract.

Results obtained in the present study revealed that *Phyllanthus niruri* possess appreciable and potential antimicrobial activity

Table 1: Antibacterial activity of wild *Phyllanthus niruri* extracts

No.	Microorganism	Zone of inhibition of Methanol extract	Zone of inhibition of Acetone extract	Zone of inhibition of Petroleum ether extract	Zone of inhibition of Ethanol extract
1.	*Corynebacterium pseudotuberculosis*	6mm	8mm	-	6mm
2.	*Streptococcus pyogenes*	6mm	-	6mm	8mm
3.	*Klebsiella pneumoniae*	7mm	7mm	-	8mm
4.	*Pseudomonas aeruginosa*	-	8mm	6mm	9mm
5.	*Enterobacter aerogenes*	9mm	9mm	-	10mm
6.	*Vibrio cholera*	11mm	9mm	-	8mm
7.	*Lactobacillus casei*	11mm	10mm	-	7mm
8.	*Salmonella enterica*	13mm	-	7mm	-
9.	*Proteus mirabilis*	7mm	10mm	-	6mm
10.	*Bacillus subtilis*	8mm	7mm	6mm	7mm
11.	*E. coli*	6mm	10mm	-	7mm
12.	*Staphylococcus aureus*	7mm	10mm	-	7mm

Table 2: Antibacterial activity of Mutant 1 of *Phyllanthus niruri* extracts

No.	Microorganism	Zone of inhibition of Methanol extract	Zone of inhibition of Acetone extract	Zone of inhibition of Petroleum ether extract	Zone of inhibition of Ethanol extract
1.	*Corynebacterium pseudotuberculosis*	15mm	-	-	8mm
2.	*Streptococcus pyogenes*	8mm	7mm	-	7mm
3.	*Klebsiella pneumoniae*	8mm	7mm	6mm	7mm
4.	*Pseudomonas aeruginosa*	13mm	12mm	9mm	7mm
5.	*Enterobacter aerogenes*	16mm	7mm	-	13mm
6.	*Vibrio cholera*	8mm	6mm	6mm	10mm
7.	*Lactobacillus casei*	9mm	8mm	6mm	9mm
8.	*Salmonella enterica*	-	7mm	6mm	8mm
9.	*Proteus mirabilis*	10mm	15mm	7mm	13mm
10.	*Bacillus subtilis*	8mm	8mm	7mm	8mm
11.	*E. coli*	10mm	6mm	6mm	8mm
12.	*Staphylococcus aureus*	9mm	6mm	6mm	6mm

against commonly encountered microorganisms in humans. The antimicrobial properties of *Phyllanthus niruri* has been widely reported by Hoffman *et al.,* 2004. The effect of *Phyllanthus niruri* extracts agrees with the work of Contreras and Gamarra (1993) that showed antibacterial effect of *Phyllanthus niruri* over *E. coli*. The present investigation agrees with the results of Uchechi and Njoku (2006) that showed antibacterial effect of *Phyllanthu sniruri* over *E. coli, S. aureus* and *Salmonella typhi*. According to Muzumeder*et al.,* 2006, growth of resistant pathogens was inhibited by *Phyllanthu sniruri* extract. The authors have concluded that the methanolic extract of the plant can fight diarrhoea and dysentery causing micro-organisms. Maximum inhibitory activity of methanolic extract of *Phyllanthus* against multi-resistant strains of *Staphylococcus aureus, S.saprophyticus* and *E. coli* have been observed by Thomas *et al.*, 1999. The higher activity of the methanol extracts may be due to higher solubility of the active compoundsinto this solvent. Methanol had a higher power to extract the active antibacterial compounds in the plant thereby exhibiting higher activity with higher zones of inhibition. However the present work is one of its kinds as it proves that *Phyllanthus niruri* plants growing near mobile tower vicinity express more antibacterial activity. This may be due to the reason that these plants growing near mobile towers produce more secondary metabolites to resist the ionic radiations sent down to Earth from the mobile towers.

Table 3: Antibacterial activity of mutant II of *Phyllanthus niruri* extracts

No.	Microorganism	Zone of inhibition of Methanol	Zone of inhibition of Acetone	Zone of inhibition of Petroleum ether extract	Zone of inhibition of Ethanol extract
1.	*Corynebacterium pseudotuberculosis*	11mm	-	-	7mm
2.	*Streptococcus pyogenes*	8mm	7mm	7mm	11mm
3.	*Klebsiella pneumoniae*	6mm	6mm	-	13mm
4.	*Pseudomonas aeruginosa*	10mm	14mm	8mm	8mm
5.	*Enterobacter aerogenes*	9mm	7mm	6mm	-
6.	*Vibrio cholera*	13mm	6mm	-	7mm
7.	*Lactobacillus casei*	8mm	12mm	-	15mm
8.	*Salmonella enterica*	16mm	-	9mm	9mm
9.	*Proteus mirabilis*	6mm	7mm	7mm	6mm
10.	*Bacillus subtilis*	8mm	11mm	-	16mm
11.	*E. coli*	12mm	9mm	6mm	8mm
12.	*Staphylococcus aureus*	12mm	8mm	-	9mm

Table 4: Antibacterial activity of Mutant III of *Phyllanthus niruri* extracts

No.	Microorganism	Zone of inhibition of Methanol extract	Zone of inhibition of Acetone extract	Zone of inhibition of Petroleum ether extract	Zone of inhibition of Ethanol extract
1.	*Corynebacterium tuberculosis*	13mm	7mm	-	7mm
2.	*Streptococcus pyogenes*	7mm	6mm	6mm	10mm
3.	*Klebsiella pneumoniae*	7mm	7mm	6mm	8mm
4.	*Pseudomonas aeruginosa*	11mm	13mm	9mm	7mm
5.	*Enterobacter aerogenes*	14mm	8mm	6mm	12mm
6.	*Vibrio cholera*	10mm	7mm	-	9mm
7.	*Lactobacillus casei*	9mm	10mm	-	11mm
8.	*Salmonella enterica*	10mm	7mm	8mm	10mm
9.	*Proteus mirabilis*	9mm	8mm	6mm	7mm
10.	*Bacillus subtilis*	10mm	9mm	6mm	15mm
11.	*E. coli*	11mm	9mm	-	6mm
12.	*Staphylococcus aureus*	10mm	9mm	7mm	8mm

Note: Table 1 to 4 disc size 5mm

This fact is supported by the results of this work as mutant plants expressed more antibacterial activity than control plants growing far away from the vicinity of mobile towers.

Earlier reports by Bieza and Lois (2001) conclude that a variety of adaptations have evolved that help plants cope with the exposure to UV but we do not yet understand the exact role many of them play in the overall protection against UV damage. The isolation and characterization of mutants has been a powerful tool to learn about various mechanisms that help to protect plants against different types of UV radiation damage. Mutants showing hypersensitivity to UV radiation have been instrumental in laying down the groundwork for our understanding of some of these mechanisms. Increased levels of flavonoids and other UV-absorbing phenolics have been postulated as important UV defense mechanisms (Landry *et al.*, 1995; Mazz*aet al.*, 2000). Elevated levels of secondary metabolites have been reported by Bieza and Lois in *Arabidopsis thaliana* plants exposed to UV radiations.

However more work into the gene level of *Phyllanthus niruri* plants will confirm the cause of this elevated antibacterial activity from plants growing near mobile tower vicinity will confirm the cause of this elevated antibacterial activity. The present study however needs more substantiate molecular level evaluation of the mutant plants to confirm whether this change will benefit mankind in a positive manner by becoming an alternative to allopathic medicines in the present era of multiple resistant strains of micro organisms.

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