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Research Article

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Antimicrobial studies on ethanolic extracts of *Phyllanthus niruri*

Nivetha S.[a]* and Vetha Roy D.[b]

[a]*Department of Chemistry, Arignar Anna College, Aralvoimozhy, Tamilnadu, India*
[b]*Department of Chemistry and Research Centre, Scott Christian College (Autonomous), Nagercoil, Tamilnadu, India*

ABSTRACT

The toxic side effect of the drugs of modern medicine and the lack of medicines for many chronic ailments has led to the reemergence of the herbal medicine, with possible treatments for many health problems. Phyllanthus niruri studied for its antimicrobial properties proved to be effective against all the eight bacterial and fungal strains E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis, Candida albicans, Candida tropicalis, Candida cruzi and Candida parapsolis at the three concentrations 2500µg/ml, 5000µg/ml and 10000µg/ml. The ethanolic root and leaf extracts shows remarkable inhibitory action against E.coli bacteria and Candida tropicalis fungus. The root extract records greater zone of inhibition against Candida parapsolis (22mm at 10000µg/ml) comparable to that of the control (20mm) and the leaf extract exhibits greater activity to that of the control against Proteus mirabilis (26mm at 5000µg/ml against 23mm).

Keywords: *Phyllanthus niruri*, antimicrobial, ethanolic extracts.

INTRODUCTION

Plants have been used for centuries as remedy for human diseases because they contain components of therapeutic values [1, 2]. They are natural sources of antimicrobial agents primarily because of the large biodiversity of such organisms and the relatively large quantity of metabolites that can be extracted from them [3]. The systemic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with the potential to act against multiresistant pathogenic bacteria and fungi.

Phyllanthus niruri is widely used against a variety of ailments including hepatic disorders [4 - 6]. This herb has a potent free radical scavenging activity and could scavenge superoxides and hydroxyl radicals and can inhibit lipid peroxides [6]. Free radicals, from both endogenous and exogenous sources, are implicated in the etiology of several degenerative diseases, such as coronary artery diseases, stroke, rheumatoid arthritis, diabetes and cancer [7].

Its root, leaves, fruits, milky juice, and whole plants are used as medicine. The herb is stomachic and good for sores and useful in chronic dysentery. Fruits are useful for tubercular ulcers, wounds, sores, scabies and ring worm [8,9]. The fresh root is believed to be an excellent remedy for jaundice. A poultice of the leaves with salt cures scabby and without salt applied on bruise and wounds. The milky juice is applied for offensive sores. The infusion of the root and leaves is a good tonic and diuretic when taken cold in repeated doses [10 - 12]. In many parts of India, it is commonly used for the treatment of snake bite. It is a major component of many popular liver tonics in India including Liv.-52. Fresh juice and powder of dried plant are used most frequently in Ayurvedic preparations [13].

The plant is used as a fish poison. In many parts of India especially in deserts, the roots mixed with *Commiphora mukul* are given to camels to cure indigestion. The decoction of leaves and stem are used for dyeing cotton black. [14]

P.niruri was reported to contain variety of phytoconstituents like lignans namely phyllanthin, hypophyllanthin, nirphyllin and phyllnirurin; flavanone glycosides like niranthin, nirtetralin, phyltetralin and lintetralin; a steroidal hormone estradiol; flavanoids like quercetin and astragalin; triterpenes like phyllanthenol, phyllanthenone and phyllanthenol, phyllthenone and phyllanthenol[15 - 17].

Phyllanthus niruri was evaluated *invitro* for their antimicrobial effectiveness on *Escherichia coli, staphylococcus aureus, salmonella typimurium, Asperillus nigar, Asperillus flavus* and *candida albicans. P.niruri* was a good antimicrobial agent for bacterial and fungal pathogens of human and plants. *P.niruri* has bactericidal effect on four pathogens namely *vulgaris, micrococcus luteus, bacillus polymyxa* and *Escherichia coli*. It was also indicated that the extract might not be toxic to the kidney and the liver [18].

A number of pathogens have developed resistance to multiple antibiotics (Multiple Drug Resistance), which threatens to develop complete immunity against all antimicrobial agents which would otherwise be untreatable. Thus, the search for antimicrobial agents is of the utmost importance. The present work aims at analyzing the antibacterial as well as antifungal activities of the ethanolic extract of *Phyllanthus niruri* root and leaves.

EXPERIMENTAL SECTION

Collection of plant material

Phyllanthus niruri plant sample was collected from the locality of kadiapattanam. The whole plant was air-dried at room temperature for two months. Roots and leaves were powdered separately using mortar and pestle and stored in air-tight containers.

Preparation of root and leaf extract

Ethanolic extract from the root and leaf sample was collected by using soxhlet apparatus by repeating for around 10 cycles. The excess ethanol was drained off by vacuum evaporator. Further the extracts were cooled, weighed and tested for its antimicrobial activity at three different concentrations 2500µg/ml, 5000µg/ml and 10,000µg/ml .

Tested microorganisms

Antibacterial activity of the ethanolic extract of *Phyllanthus niruri* root and leaf was tested against four different bacterial strains *E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Proteus mirabilis*. Similarly the inhibitory action against four different fungal strains *Candida albicans, Candida tropicalis, Candida cruzi and Candida parapsolis* was also analyzed.

Antimicrobial assay

About 0.01ml of extract should be poured into Petri dishes on a flat horizontal surface to a depth of 4mm (25 ml in an 85mm circular dish, 60 ml in a 135 mm circular dish). The poured plates were stored at 4^0C and used within one week of preparation. Before inoculation, plates should be dried with lipids jar so that there were no droplets of moisture on the agar surface. The pH of the medium should be checked at the time of preparation and should be 7.2to7.4.

At least four morphologically similar colonies from an agar medium were touched with a wire loop and the growth was transferred to a test tube containing 0.01 ml of sterile suitable broth. The tubes were incubated for 2 hours at 35 to 37^0C to produce a bacterial suspension of moderate turbidity. Plates were inoculated within 15 minutes of preparation of the suspension so that the density does not change. After the inoculums have dried, single discs were applied with forceps, a sharp needle or a dispenser and pressed gently to ensure even contact with the medium. When fastidious organisms were to be tested touch multiple colonies with a loop and cross streak the appropriate plate for uniform distribution. It was repeated for each antimicrobial agents *E.Coli, Klebsiella pneumonia, P.aeruginosa and Proteus mirabilis* to be used, placing the impregnated discs in their respectively labeled segments. After 24 hours, the diameters of the inhibition zones were measured to the nearest millimeter with vernier calipers (preferably) or a thin transparent millimeter scale. For fungal strains same method was followed but the period of time was 48 hours.

RESULTS AND DISCUSSION

Antibacterial activity of ethanolic extract of *Phyllanthus niruri* root

The ethanolic extract of both *P.niruri* root as well as leaf exhibit significant antibacterial activity against all the bacterial species *E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Proteus mirabilis.*

Table 1 Antibacterial activity of *Phyllanthus niruri*

Bacterial Species	Zone of Inhibition Diameter						
	2500µg/ml		5000µg/ml		10000µg/ml		Control
	Root extract	Leaf extract	Root extract	Leaf extract	Root extract	Leaf extract	(Cefopers/ sulba)
E.coli	7mm	10mm	17mm	13mm	20mm	17mm	22mm
Klebsiella pneumonia	15mm	16mm	12mm	18mm	10mm	13mm	23mm
Pseudomonas aeruginosa	18mm	13mm	14mm	16mm	12mm	11mm	28mm
Proteus mirabilis	11mm	22mm	17mm	26mm	19mm	13mm	23mm

Against *E.coli* and *Proteus mirabilis* the activity was also found to be enhanced, with increase in concentration of the root extract, whereas the activity was found to be lowered against K*lebsiella pneumonia* and *Pseudomonas aeruginosa*. Pronounced activity was observed against *E.coli* as well as *Proteus mirabilis* with zone of inhibition 20mm, 19mm, against the control of 22mm and 23mm at 10000µg/ml. Maximum inhibitory action was observed even at lower concentration against *K.Pneumonia and P.aerugenosa*. Zone of inhibition observed was 15mm and 18mm at 2500µg/ml (Table 1).

Leaf extract exhibited peak activity at 5000µg/ml against *Klebsiella pneumonia (18mm), Pseudomonas aeruginosa (16mm)* and *Proteus mirabilis (26mm).* Above and below the concentration the inhibitory action was suppressed. Higher concentration was required against E.coli which showed pronounced activity (17mm) at 10000µg/ml

Antifungal activity of ethanolic extract of *Phyllanthus niruri*

Against all the fungal species, significant activity was recorded for the *phyllanthus* root and leaf extract. With increase in the root extract concentration, antifungal activity almost remained the same except for C*andida parapsolis* species. Zone of inhibition was found to be enhanced from 14mm to 22mm towards C*andida parapsolis*. For the other three species maximum inhibitory activity was observed at the lower concentration (2500µg/ml).

Table 2 Antifungal activity of *Phyllanthus niruri*

Fungal Species	Zone of Inhibition Diameter						
	2500µg/ml		5000µg/ml		10000µg/ml		Control
	Root extract	Leaf extract	Root extract	Leaf extract	Root extract	Leaf extract	(Flucanazole)
Candida albicans	13mm	18mm	12mm	16mm	12mm	15mm	30mm
Candida tropicalis	19mm	15mm	18mm	20mm	16mm	9mm	18mm
Candida cruzi	15mm	11mm	14mm	14mm	13mm	19mm	30mm
Candida parapsolis	14mm	13mm	19mm	14mm	22mm	14mm	20mm

The most significant observation is that the ethanolic root extract (at 2500µg/ml) exert antifungal activity against *Candida tropicalis* species comparable to that of the control (Figure 10). Zone of inhibition for the control was 18mm whereas the extract recorded 19mm at 2500µg/ml. Again at 10000µg/ml, the extract imposed maximum inhibition against *Candida parapsolis* species similar to that of the control. The zone of inhibition obtained for the extract was 22mm and for the control is 20mm (Table 2).

The leaf extract showed an increase in activity against *C.cruzi* with respect to increase in the concentration (Figure 10). Zone of inhibition at 5000µg/ml was 14mm whereas at 10000µg/ml it was observed to be 19mm. A greater inhibitory effect (20mm) compared to the control (18mm) was obtained at 5000µg/ml against *Candida tropicalis.* Above and below the concentration, the activity got reduced. For the other two species *Candida parapsolis and Candida albicans,* the activity remained almost the same with increase in concentration of the extract.

Antimicrobial activity recorded here may be attributed to the bioactive compounds present in their root and leaves. The bioactive compounds present has been reported to be alkaloids, astragalin, brevifolin, carboxylic acids, corilagin, cymene, ellagic acid, ellagitannins, gallocatechins, geraniin, hypophyllanthin, lignans, lintetralins, lupeols, methyl salicylate, niranthin, nirtetralin, niruretin, nirurin, nirurine, niruriside, norsecurinines, phyllanthin,

phyllanthine, phyllanthenol, phyllochrysine, phyltetralin, repandusinic acids, quercetin, quercetol, quercitrin, rutin, saponins, triacontanal, tricontanol [15,19].

The study well proves that the ethanol extract of *Phyllanthus niruri* root and leaf is a natural agent imparting both antibacterial as well as antifungal activity. The root extract shows inhibitory action especially against *E.coli* bacterial strain and comparable activity to that of the control against *Candida Tropicalis* and *Candida parapsolis*. Similarly, the leaf extract imparts pronounced activity compared to that of the control against *Proteus mirabilis* and *C.tropicalis*. Comparable activity is achieved against *E.coli* and *K.pneumonia* species with that of the control. *Phyllanthus niruri* could therefore be advocated as a promising antimicrobial agent with potential application in pharmaceutical industry for controlling pathogenic organisms especially against *Proteus mirabilis* and *C.tropicalis*.

CONCLUSION

The discovery of active bio-compounds is a matter of urgency as most of the pathogens are getting resistance against number of drugs. The study proves that significant activity can be achieved against all the eight tested microbial species by using *P.niruri* root as well as leaf extract. The finding of this study could therefore justify the use of this plant especially against the multidrug resistant *E.coli* bacteria as well as for *C.tropicalis* fungal infections. The leaf extract can be specially advocated for the treatment of *Proteus mirabilis* infections and the root extract for *Candida parapsolis* infections.

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