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Phytochemical screening and antimicrobial activity of *Phyllanthus niruri* Linn

Manas Mathur, Rashi Sharma, Jaya Sharma, Ruchi Pareek and Raka Kamal

Laboratory of Medicinal Plant Biotechnology, Department of Botany, University of Rajasthan, Jaipur-302004, India.

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ABSTRACT

The *in vitro* antimicrobial activity of crude methanolic extracts of various plant parts of *Phyllanthus niruri* was investigated. The extracts exhibited antimicrobial activities with zones of inhibition ranging from 6 to 16 mm. All the extracts exhibited appreciable activity against all the clinically important bacterial and fungal species clinically investigated. Maximum Inhibition zone (16mm) was observed in seeds against *Staphylococcus aureus* and minimum in roots (5mm) against *E. coli.* Phytochemical screening revealed the presence of carbohydrates, proteins, alkaloids and flavonoids in the extracts. The antimicrobial activity of the extract was compared with the standard drugs. The ability of the crude extracts of *P. niruri* plant parts to inhibit the growth of various bacteria and fungi showed its broad spectrum antimicrobial potential, which may be employed in the management of microbial infections.

Introduction

Plant-based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials is needed as antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects of synthetic antimicobials (Iwu *et al*, 1999). They may act as lead compounds for the pharmaceutical industry or as the base for the development of new antimicrobials (Aiyelaagbe, 2001; Aiyoro *et al*, 2008).

Phyllanthus niruri Linn belongs to family Euphorbiaceae, commonly known as Stonebreaker (Eng.) due to its antilithic property. Various bioactivities such as antidiabetic (Okoli *et al*, 2011), anti-hepatotoxicity, (Ravikumar *et al*, 2011) antilithic, anti-hypertensive, anti-HIV and anti-hepatitis B (Bagalkotkar *et al*, 2011; Naik and Juvekar , 2003) have been reported.

Several studies have confirmed the antimicrobial efficacy of different *Phyllanthus* species; however, there is insufficient information regarding the antimicrobial activities of methanolic extracts of *P. niruri,* however, leaves have been reported to show diverse medicinal properties. In the present investigation methanolic extract of various plant parts of *P.niruri* has been studied for their antimicrobial efficiency.

Materials and Methods

Plant Material

Various plant parts of *P. niruri* (leaves, stem, seeds and roots) were collected from the fields at Jaipur and authenticated. The voucher (RUBL* No. 30247) of experimental plant was deposited in the Herbarium of Department of Botany, University of Rajasthan, Jaipur. Plant parts were separated, cleaned and oven dried at 35ºC for 30 min and then at 25^0C till constant weight was achieved and powdered.

Phytochemical analysis of the plant extract

All the sequentially extracted fractions obtained from various organic solvents were subjected to phytochemical tests for the presence of different metabolites following methods of Harborne (1998) and established protocols.

Tele: 91-9828014141;

E-mail address: rakakamal@hotmail.com

Antimicrobial Activity

Methanolic extract was used for determination of antimicrobial activity of *P. niruri.* Five bacterial and two fungal strains were selected for the antimicrobial screening.

Microorganisms Used

Clinical laboratory isolates of bacteria viz *Streptococcus viridians, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Enterobacter cloacae* and fungi viz *Trichoderma viridae* and *Aspergillus niger* were procured from the Microbiology Laboratory, SMS Medical College, Jaipur.

Preparation of Extract

The methanolic extract was obtained by macerating 100 g of dried powder of different plant parts in 95% methanol and kept on a rotary shaker for 24 h, separately. Each of the extract was filtered, centrifuged at 5000rpm for 15 min, dried under reduced pressure and stored at 4 ºC in airtight bottles.

Culture and Maintenance of Bacteria

Above mentioned pure cultures of *S. viridians, S. aureus, P. aeruginosa, E. coli, E. cloacae* and fungal isolates *T. viridae* and *A. niger* were used as indicator organisms. These bacteria were grown in nutrient agar medium prepared by autoclaving 8% Nutrient Agar (Difco-Laboratories, Detroit, USA) in distilled water at 15 lbs psi for 25-30 min and incubating at 37°C for 48 h. Each bacterial culture was maintained on the same medium after every 48 h of sub-culturing. A fresh suspension of test organism in saline solution was prepared from a freshly grown agar slant before every antimicrobial assay.

Determination of Antibacterial Assay

In vitro antibacterial activity of the crude methanol extract was studied against gram +ve and --ve bacterial strains by the agar well diffusion method (Perez *et al*, 1990). Mueller Hinton Agar No.2 (Hi Media, India) was used as the bacteriological medium. The extracts were diluted in 100% dimethylsulphoxide at the concentrations of 5 mg mL^{-1}. The Mueller Hinton agar was melted and cooled to 48-50 ºC and a standardized inoculum (1.5×108 CFU mL^{-1}, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petridishes to give a solid plate. Wells were prepared in the

seeded agar plates. The test compound (100 µl) was introduced in the well (6 mm). The plates were incubated overnight at 37°C. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotic streptomycin and ampicillin. For each bacterial and fungal strain, controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed in triplicate to minimize the error and the mean values are presented.

Determination of Antifungal Assay

Anti fungal activity of the experimental plant was investigated by agar well diffusion method (Bonjar *et al*, 2005). The yeasts and saprophytic fungi were subcultured on Sabouraud's Dextrose Agar (SDA; Merck, Germany) medium and respectively incubated at 37 °C for 24 h and 25 °C for 2 - 5 days. Suspensions of fungal spores were prepared in sterile PBS (phosphate buffered saline) and adjusted to a concentration of 106 cells mL^{-1}. Dipping a sterile swab into the fungal suspension was rolled on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 10 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 mL of several dilutions of fresh extracts was administered to fullness for each well. Plates were incubated at 37 °C. After incubation of 24 h, bioactivities were determined by measuring the diameter of inhibition zone (mm). The diameters of zone of inhibition produced were compared with those of standard clotrimazole used as standard antifungal agent. All the experiments were performed in triplicate and mean values were taken.

Results

Phytochemical screening

Investigations on the phytochemical screening of *P. niruri* extracts revealed the presence of carbohydrates, proteins, alkaloids and flavonoids, which are known to be biologically active. These metabolites can exert antimicrobial activity through different mechanisms (Table 1).

The antimicrobial activity of methanolic extracts of different plant parts of *P. niruri* were tested against 5 bacterial strains (*E. cloacae*, *S. aureus*, *P. aeruginosa* , *E. coli* and *S. viridians*) and 2 fungal strains (*A. niger* and *T. viridae).* The Inhibition Zone (IZ) was measured by antibiotic zone reader (Table 2). Individually against *E .coli* maximum IZ was observed in extract of leaves, which was at par with that of seeds (12mm) and minimum was in roots. In case of *S. aureus* maximum IZ was observed in seeds (16mm) and minimum in stem (7mm), in *P. aeruginosa* maximum IZ was in leaves (15mm) and minimum in roots (10mm) and against *S. viridians and E. cloacae* also leaves gave maximum IZ (14mm and 15mm, respectively) and roots had minimum (8mm in both). Among the fungal strains against *T. viridae*, it was observed that only leaves (12mm) and roots (10mm) showed IZ while stem and seeds did not show any activity and in *A. niger* case only leaves (6.5mm) and seeds (10mm) gave IZ while stem and seeds did not show any activity.

Discussion

Plants synthesize variety of phytochemicals as part of their normal metabolic activities. Chemical profile of a single plant may vary over a time, as it reacts to changing conditions. Plant scientists and natural products chemists are combing the flora for the phytochemicals and lead compounds, which could be developed for treatment of various diseases. In 2010 a survey of 1000 plants was done out of which, 156 clinical trials for evaluation of their pharmacological activities and therapeutic applications gave encouraging results (Cravotto *et al*, 2010). This led to the new search for drugs and dietary supplements derived from plants. During the last 10 years pace of development of new antimicrobial drugs has slowed down, while prevalence of resistance has increased multifold (Akinpelu & Onakoya, 2006). The problem of microbial resistance of growing and outlook for the use of antimicrobial drugs in future is still uncertain therefore, action must be taken to reduce this problem, such as controlling the use of antibiotics and carrying out research for better understanding of genetic mechanism of resistance. This prompted to evaluate plants as source of potential chemotherapeutic and antimicrobial agent along with their ethnomedicinal use (Prashanth *et al*, 2006).

Earlier attempts on antimicrobial activity on other species of *Phyllanthus* (Akinjogunla *et al*, 2010; Rajeshwar *et al*, 2008) have shown promising results against variety of microbial flora.

In the present investigation initial screenings of the experimental plant for possible antimicrobial activities was done using crude methanolic extracts. Nearly all of the identified components from plants that are active against microorganisms are aromatic or saturated organic compounds and most often obtained through ethanol or methanol extractive. In the present study *P. niruri* showed antimicrobial potent activity against bacterial strains as compared to fungal strains.

Conclusion

By virtue of high activity indices of various standard used, the above unitary value even in crude forms of the phytoextracts, when refined to produce antibiotics, have more promising therapeutic value/advantages over the known antibiotics like streptomycin, ampicillin and clotrimazole run in parallel. The present findings can be of commercial interest to both pharmaceutical companies and research institutes in the production of new antimicrobial drugs. More importantly, there have been no side effects or toxicity reports from many years on this plant. There is still a lot of scope for further research, especially towards the mechanism of biological activity of phytochemicals from this plant.

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Table 1. Phytochemical evaluation from different plant parts of *P. niruri*

Parameter		Organic Solvents Used				
		Pet. Ether	Benzene	Chloroform	Alcohol	Water
Physical Appearance	Plant Parts	Yellow Green Sticky	Bright Red Oily	Yellowish orange oily	Red Brown Sticky	Brown dusty viscous
Carbohydrates	Leaves	-	+	++	++	++
	Stem	-	-	+	++	+
	Seeds	+	++	+	+++	+++
	Roots	-	++	+	-	+
Proteins	Leaves	+	+	+	++	+
	Stem	++	-	+	++	++
	Seeds	+	+	+	+++	+
	Roots	+	-	+	++	+++
Flavonoids	Leaves	+	++	+++	+++	+++
	Stem	+	++	+++	++	+++
	Seeds	+	++	+	++	++
	Roots	-	+	+	++	+
Alkaloids	Leaves	-	-	+	+	+
	Stem	+	+ +	+	+ +	+
	Seeds	-	-	+	++	+
	Roots	+	+ +	+	+ ++	-

- absent; + trace amount; ++ moderate amount; +++significant amount

Table 2. Antimicrobial activities of methanolic extracts of *P. niruri*

Test organisms	Plant parts and inhibition zones of growth inhibition (mm)				Standard
	Leaves	Stem	Roots	Seeds	S/A/C
E. coli	12 ±0.77	7±0.41	5±0.23	12±0.61	27.66±0.38 (S)
S. aureus	15±0.91	7±0.39	9±0.43	16±1.01	26.66±0.94 (A)
P. aeruginosa	15±0.91	13±0.77	10±0.61	14±0.87	20.50±0.70 (S)
S. viridians	14±0.82	9±0.45	9±0.41	13±0.83	26.66±0.94 (A)
E. cloacae	15±0.77	9±0.44	8±0.31	10±0.62	27.66±0.38 (S)
A. niger	6.5±0.37	NA	NA	10±0.61	28±0.01 (C)
T. viridae	12± 0.53	NA	10±0.50	NA	28±0.01 (C)

IZ = Inhibition zone (mm) excluding the diameter of disc (6mm) Mean± SE, NA= No Activity,