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Phytochemical Screening and Antimicrobial Activity of *Phyllanthus niruri*

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Abstract: *phyllanthus niruri* is an Indian herb used for various ailments by traditional healers. In this study carried out phytochemical analysis and antimicrobial investigation of methanolic extract of the leaves *phyllanthus niruri* against a panel of clinically significant bacterial and fungal strains. Phytochemical studies revealed the presence of Phenolic compounds, Saponins, Flavonoids, Terpenoids, Alkaloids, Tannins, Cardioglycosides, Steroids, Reducing Sugars, Anthraquinones and Resins. Susceptibility testing by disc diffusion assay revealed significant antimicrobial activity of methanol extract of leaves against *Coney lunata* and *Salmonella typhi*. The leaf extract exhibited better antimicrobial activity. The study findings provide supportive evidence for the use of *phyllanthus niruri* in traditional medicines.

Key words: *phyllanthus niruri*, Phytochemicals and Antimicrobial activity.

Introduction

India is one of the major countries, having 40 per cent of the global biodiversity and availability of rare plant species. Medicinal and aromatic plants constitute a major segment of the flora, which provides raw materials for use in the pharmaceuticals and drug industries. The indigenous systems of medicines, developed in India for centuries, make use of many medicinal herbs. These systems include Ayurveda, Siddha, Unani and many other indigenous practices. More than 9,000 native plants have been established and recorded for their curative properties. In one of the studies made by the World Health Organisation, it was estimated that 80 per cent of the population of developing countries relies on traditional plant based medicines for their health requirements.

Even in many of the modern medicines, the basic composition is derived from medicinal plants and these have become acceptable medicines for many reasons that include easy availability, least side effects, low prices, environmental friendliness and lasting curative property. In India, the use of herbal medicine can be traced back from the Vedic period and the first written reports are timed to 600 BC with Charaka Samhita.

India is a varietal emporium of medicinal plants and it is one of the richest countries in the world as regards genetic resources of medicinal plants. Many environmental factors like precipitation, mean temperature, soil, wind speed, low and high temperature extremes, duration of snow-cover, length of the vegetation period and the intensity of radiation also known to influence the biochemistry of medicinal plants. However, biochemistry of *P. niruri* growing in different geographical regions of India and environmental factors is fluctuating at various altitudes. In view of the importance of this species, its large

scale multiplication and cultivation of quality planting material (based on the content of active ingredients) is urgently required.

A wide range of plant species belonged to the genus *Phyllanthus* has been phytochemically investigated. Among the studied species, *P. niruri, P. urinaria, P. emblica, P. flexuosus, P. amarus*, and *P. sellowianus*have received the most phytochemical and biological attention. According to the available literature, research has either been focused on isolating all the substances in these plants, or on determining a specific class of natural products.

Materials and methods

The medicinal plant *Phyllanthus niruri* .L were collected from Lalgudi, Tiruchirappalli District, Tamil Nadu, India. The leaf parts were carefully collected from the plant. These cleaned leaf parts were placed separately in polythene bags. Then , these are shade dried in a clean environment to avoid the contamination for 10 days and leaf parts In the present study 25g of dried leaf powder was extracted using soxhlet apparatus with 150 ml of methanol for about 6 hours.

Preliminary phytochemical analysis was carried out for extracts as per standard methods described by Brain and Turner 1975 and Evans 1996.

Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids.

Mayer's test: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Mayer's reagent: Mercuric chloride (1.358g) is dissolved in 60ml of water and potassium iodide (5g) is dissolved in 10ml of water. The two solutions are mixed and made up to 100ml with water.

Detection of Flavonoids

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

Detection of Steroids

Liebermann- Burchard test: 2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H_2SO_4. The color changed from violet to blue or green in some samples indicate the presence of steroids.

Detection of Terpenoids

Salkowski's test: 0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the inner face was indicates the presence of terpenoids.

Detection of Anthroquinones

Borntrager's test: About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink color indicates the presence anthraquinones.

Detection of Phenols

Ferric chloride test: Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black color indicates the presence of phenol.

Lead acetate test: Extract was treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of phenol.

Detection of Saponins

Froth test: About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

Detection of Tannins

Ferric chloride test: A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green color formation indicates the presence of tannins.

Detection of Carbohydrates

Fehling's test: 0.2gm filtrate is boiled on water bath with 0.2ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

Fehling's solution A: Copper sulphate (34.66g) is dissolved in distilled water and made up to 500ml using distilled water.

Fehling's solution B: Pottassium sodium tartarate (173g) and sodium hydroxide (50g) is dissolved in water and made up to 500ml.

Detection of Oils and Resins

Spot test: Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

Antibacterial activity - Preparation of bacterial inoculums

Stock cultures were maintained at 4 oC on slopes of nutrient agar. Active cultures for experiment were prepared by transferring a loop full of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hours at 37 oC. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0 X 106 colony forming units (CFU/ml) for bacteria.

Antifungal activity - Preparation of fungal inoculums

The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 hours and the suspensions were checked to provide approximately 105 CFU/ml.

3.8.4. Fungal susceptibility test by disc diffusion assay

All the tests were performed according to Esteban *et al.* (2005). The inoculums was evenly spread on the surface of 10 cm petridishes containing Sabouraud's dextrose agar medium (Merck, Germany) and exposed to dry. Standard antibiotic (Fluconazole, concentration 1µg/ml) was used as positive control. Then, the antifungal discs were kept in the plates, after which the plates were incubated at 37 oC for 72 hours. After the colonies grew, the diameters of zone of inhibition observed were measured.

Results and Discussion

Phytochemical screening of *P.niruri* in methanolic extract

In the present investigation, analysis of chemicals and bioactive compounds were made in the plant collected from allur regions of Tirucirappaalli. *P. niruri*, phytochemical screenings were performed to test the presence of different secondary metabolites. The phytochemical screening of *P. niruri* methanolic extract revealed the presence of Phenolic compounds, Saponins, Flavonoids, Terpenoids, Alkaloids, Tannins, Cardioglycosides, Steroids, Reducing Sugars and Resins, which are known to be biologically active. These metabolites can exert antimicrobial activity through different mechanisms (Table 1). The flavonoids, as an anti-oxidant in this plant may contribute to the effects of this plant as

hepatoprotective and nephroprotective, antimicrobial, anti-inflammatory, and anti-carcinogenic effect. Alkaloids in this plant may be responsible for its effects as anti-malaria, analgesic properties and its use in treatment of stomach disorder. This is consistent with the past works of Okwu and Josiah, (2006). Alkaloids and their synthetic derivatives are used as basic medicinal agents for their antispasmodic and bactericidal effects. Tannins have astringent properties, hasten the healing of wounds and inflamed mucous membranes (Okwu and Josiah, 2006).

Table 1: Phytochemical screening of the methanolic extract of *P. niruri*

S.No.	Phytochemicals	Methanolic Extract
1.	Phenolic Compounds	+
2	Saponins	+
3.	Flavonoids	+
4.	Terpenoids	+
5.	Alkaloids	+
6.	Tannins	+
7.	Cardio glycosides	+
8.	Steroids	+
9.	Reducing Sugars	+
10.	Anthraquinones	-
11.	Resins	+

Table 2. Antibacterial activity of methanolic leaf extract of *P.niruri* at varying concentration against different microbial organisms

S.No.	Name of micro organism	Control (µg/ml)	Zone of inhibition in diameter (mm)			
			10 (µg/ml)	20 (µg/ml)	30 (µg/ml)	40 (µg/ml)
1.	*Coney lunata*	21	08	10	15	18
2.	*Salmonella typhi*	19	10	12	14	17

The antimicrobial activities of methanolic extract of P. niruri were tested against two organisms. The Inhibition Zone (IZ) was measured by antibiotic zone reader (Table 2). Many of the infectious diseases are still a major challenge to health issues all over the world. The emergence of resistance to

antibiotics has further compounded the problem (Alli et al., 2011). The need for new antimicrobial compounds has become imperative. The ethonobotanical importance of the tested medicinal plants has been highlighted and it is used for various diseases. The results of antimicrobial activity of leaf extract of the *P. niruri* and are shown in Tables 2. The different concentrations of crude extracts were tested against the pathogenic organisms (Figures 1 and 2).

Other species of this genus were also found to demonstrate antimicrobial activity (Taylor, 2003). The findings of this study, could therefore justify the use of this plant in traditional medicine in the treatment of bacterial infections. The present investigation confirms the folkloric use of *P.niruri* stem bark as indigenous medicine for the treatment of some bacteria and fungi associated diseases. The data obtained could serve as an important platform for further study on this plant particularly the isolation and elucidation of the bioactive principles.

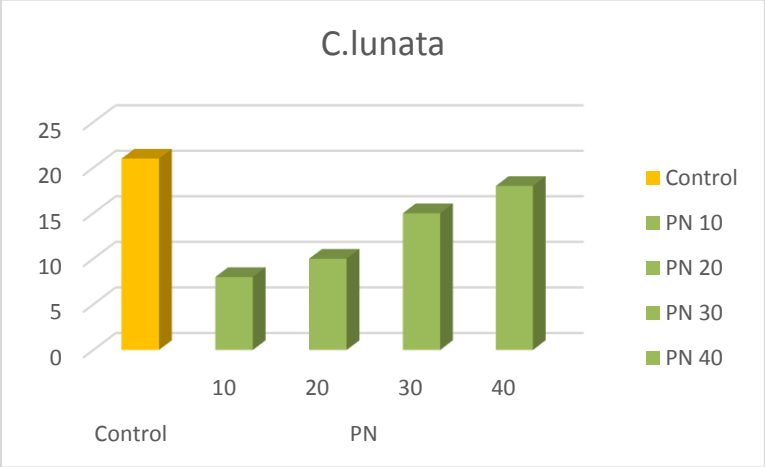

Figure 1: Antibacterial activity against *C.lunata*

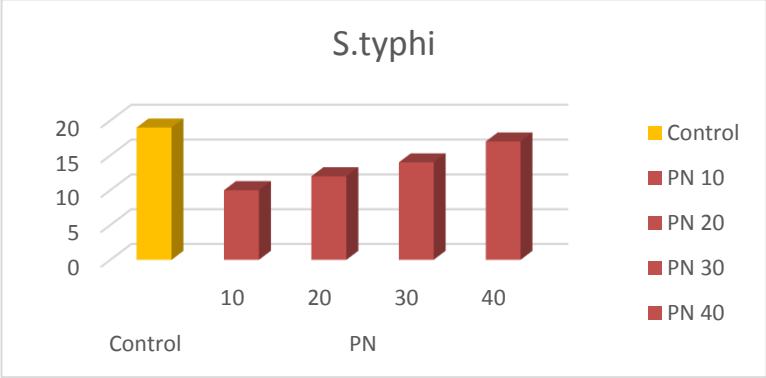

Figure 2: Antibacterial activity against *S. thphi*

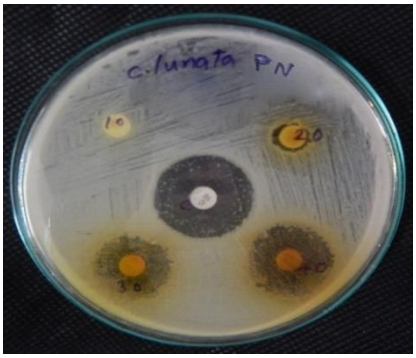

Plate 1: Effect of methanolic leaf extract of *P.niruri* (By disc diffusion method) against *S. typhi*

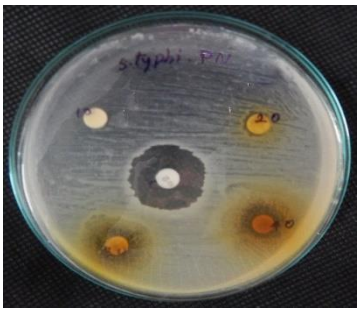

Plate 2: Effect of methanolic leaf extract of *P.niruri* (By disc diffusion method) against *C.lunata*

Conclusion

Over the past decade, herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play a central role in the healthcare system of large proportions of the world's population. This is particularly true in developing countries, where herbal medicine has a long and uninterrupted history of use. Recognition and development of the medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations. Many traditional healers and herbalists have been treating cancer patients for many years using various medicinal plant species.

The crucial factor for the ultimate success of an investigation into bioactive plant constituents is thus the selection of plant material. In view of the large number of plant species potentially available for study, it is essential to have efficient systems available for the rapid chemical and biological screening of the plant extracts selected for investigation.

These active components are of great pharmacological value and further work is required regarding these compounds.

REFERENCES

Achilya, G. S., Kotgale, S. G., Wadodkar, A. K. and Dorle, A. K., 2003. Hepatoprotective activity of *Panckgavya gritha* in carbon tetrachloride induced hepatotoxicity in rats. *Indian J. Pharmacol*., **35**: 311.

Ahmad, I. and Beg, A.Z., 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J. Ethanopharmacol*., **74**: 113-123.

Ahmad, I., Mehmood, Z. and Mohammad, F., 1998. Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethnopharmacol*., **62**: 183-193.

Cabieses, F., 1993. Apuntes de medicina traditional, La racionalizacion de lo irracional. "Notes of traditional medicine", *Consejo Nacional de Ciencia y Technologia,* Concytec, Lima-Peru, p. 414.

Calixto, J.B., Santos, A.R.S., Filho, V.C. and Tunes, R.A. 1998. A review of the plants of the genus *Phyllanthus:* their chemistry, pharmacology, and therapeutic potential. *J. Med. Biol. Res.* **31:** 225-258.

Dwivedi, Y., Rastogi, R., Sharma, S.K., Kapoor, N.K., Garg, N.K. and Dhawan, B.N., 1990. *Indian J. Med. Res*., **92**: 195-200.

Eisei Indonesia, P.T., 1995. *Medicinal Herb Index in Indonesia*, 2(ed.); PT Eisei Indonesia, Jakarta.

Ekwenye and Njoku, 2006. Antibacterial effect of *Phyllanthus niruri* (Chanca piedra) on three enteropathogens in man. *Intl. J. Mol. Med. Adv. Sci*., **2**(2): 184-189.

Segelman, A.B., Farnsworth, N.R. and Quimby, M.D., 1969. False-negative saponins test results induced by the presence of tannins. *Lloydia*, **32**: 52-58.

Tanksley, S.D., Young, N.D., Paterson, A.H. and Bonierbale, M.W., 1989. RFLP mapping in plant breeding: new tools for an old science. *Bio/technology,* **7**: 257-264.

World Health Organisation 1991. *Traditional Medicine and Modern Health Care*, World Health Organisation. Summary of WHO guidelines for the assessment of herbal medicines. Herbal Gram, **28**: 13Ð14, 1993.

Xin- Hua, W., Qngl, C., Bog, X. and Chua, L. F., 2001. A comparative study of *Phyllanthus amarus* compound and interferon in the treatment of chronic viral Hepatitis B, *Southwest J.Trop. Med. Pub.Health*, **32** (1): 40-42.

Zhentian, L., Jervis, J. and Helm, R.F., 1999. C-glycosidic ellagitannins from white oak heart wood and callus tissues. *Phytochemistry*, **51**: 751-756.