

Antibacterial Activity of *Phyllanthus Narayanaswamii* Gamble

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Summary

Antibacterial activity of *Phyllanthus narayanaswamii* leaf extract (absolute alcohol) extract was evaluated against five pathogenic bacterial strains. Leaf extract have shown the highest inhibitory potential against *Pseudomonas aeruginosa* (21±1.0 mm) and it was followed by *Bacillus subtilis* (19±1.5 mm). Glycosides, alkaloids, flavonoids, and tannin were identified from the *Phyllanthus narayanaswamii* leaves.

Keywords: Euphorbiaceae; *Phyllanthus narayanaswamii*; leaves; Glycosides; antibacterial activity

Introduction

The genus of *Phyllanthus* is a member of the Euphorbiaceae family (Spurge family), which groups over 6500 species in 300 genera. Euphorbiaceae is a large family of upright or prostrate herbs or shrubs, often with milky acrid juice, and is mainly a pan-tropical family with some species either more or less temperate (1). The genus *Phyllanthus* can be found around all tropical regions of the world: from Africa to Asia, South America and the West Indies. *Phyllanthus* contains about 550 to 750 species in 10-11 subgenera (2), and have long been used in traditional medicine to treat chronic liver diseases (3). *Phyllanthus* appears to be promising in treatment of patients with chronic hepatitis B virus (HBV) infection (4-5).

Bioactive principles like alkaloids, tannins, flavonoids, lignans, phenols and terpenes have been isolated from various species of *Phyllanthus* and their compounds showed antinociceptive activity (6). According to Liu *et al.* reported the effectiveness and biosafety of genus *Phyllanthus* for chronic HBV infection (7). In this present study, a preliminary antibacterial screening of *Phyllanthus narayanaswamii* Gamble (family: Euphorbiaceae), endemic to KMTR (Kothaiyar) region of Tirunelveli District, Tamil Nadu, India. The medicinal importance of this species is not yet evaluated in detail.

Materials and Methods

Collection of Plant Materials

Fresh leaves of *Phyllanthus narayanaswamii* were collected from Upper Kothaiyar, Kalakad-Mundanthurai Tiger Reserve (KMTR), Tirunelveli. A voucher specimen was deposited in the Centre for Biodiversity and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India.

Extraction

Air-dried and powdered leaves of *Phyllanthus narayanaswamii* were exhaustively extracted with absolute alcohol for 5h. The solvent was removed under reduced pressure, to give a final concentration of 2.56% (v/w) with green colour.

Qualitative phytochemical identification tests

The tests were done to find the presence of the active chemical constituents such as alkaloids, glycosides, terpenoids and steroids, flavonoids, reducing sugar and tannin by the following procedure:

Alkaloid

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagents are added. Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid.

After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for the presence of turbidity or yellow precipitation.

Glycoside

Glycosides are compounds which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration at the junction of two layers and the bluish green color in the upper layer.

Terpenoid and steroid

4mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids.

Flavonoid

4 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.

Tannins

To 0.5 ml of extract solution 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue color was observed for gallic tannins and green black for catecholic tannins.

Reducing Sugar

To 0.5 ml of extract solution, 1 ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

Disc diffusion method for antibacterial activity

The bacterial stock cultures were maintained on nutrient agar (NA) following the procedure prescribed (8). The leaf extract was tested against five strains of bacteria:

Bacillus subtilis, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*. Inoculated Petri plates were incubated at 37°C for 24 hours. After incubation, zone of inhibition around the discs were measured, average values were calculated and tabulated (Table-1). Each strains were separately revived and maintained in nutrient broth at 30°C for 24 h. MIC was determined by the dilution (both micro and macro) method (9).

Results and Discussion

The maximum inhibitory effect was observed *Pseudomonas aeruginosa* (zone of inhibition =21mm; MIC 45.78 µg/ml) and then followed by *Bacillus subtilis*, *Escherichia coli*. *Staphylococcus aureus* (Table-1&2). According to the earlier reports in this aspect, ethanolic extract of the roots of *Nerium oleander* exhibited moderate activity against *Bacillus pumillus* and *Staphylococcus aureus* while with *Escherichia coli* it was high and lower against *Bacillus subtilis* (10). The observations of this present study is corroborative one and also authenticate the findings of Hussain and Gorski (10). As alcohol was used as a solvent system in this study, the major group of active phytochemicals present in this plant species could have a broad and probably a uniform solubility pattern. Further fractionation and chromatographic studies could yield further information in this regard.

In case of other species of *Phyllanthus* like *P.nururi*, *P. burchellii* and *P. parvulus sond* var. *garipensis* a potent antibacterial activity against the pathogenic bacterial strains of *P. aeruginosa*, *E.coli*, *S.aureus* and *S.typhi* was reported (11-12). But in case of this experimental species i.e., *Phyllanthus narayanaswamii* Gamble, there is no such earlier attempts were made to scientifically screen the active phytochemicals responsible for its antibacterial activity (Table-3). In this present study, such a preliminary attempt was made to authenticate the utilization of this species in tribal/folk medicine.

From the qualitative analysis, it is observed that glycosides could be the more abundant group of chemicals (Table. 3). Further, glycosides and alkaloids also present. Earlier reported same active principles were found to be both species are *P. burchellii*

and *P. parvulus sond* var. *garipensis* (12). These kind of constituents may contribute towards the utilization of this plant in tribal or folklore medicine for the treatment of chronic liver diseases like (2,7).

Table 1: Antibacterial activity of specific concentration of alcoholic extract of *Phyllanthus narayanaswamii* compare to control by disc diffusion method

Sl. No	Test organisms	Zone of Inhibition (mm)
1	<i>Escherichia coli</i>	19±1.0
2	<i>Salmonella typhi</i>	15±1.0
3	<i>Bacillus subtilis</i>	19±1.5
4	<i>Pseudomonas aeruginosa</i>	21±1.0
5	<i>Staphylococcus aureus</i>	15±1.0

Values are expressed as Mean ± S.D

Table 2: Minimum inhibitory concentration (MIC) of alcoholic extract of *Phyllanthus narayanaswamii* by dilution method

Sl.No	Bacteria	MIC µg /ml
1	<i>Escherichia coli</i>	≤45.78 µg/ml
2	<i>Salmonella. typhi</i>	≤35.56 µg /ml
3	<i>Bacillus subtilis</i>	≤ 23.33 µg /ml
4	<i>Pseudomonas aeruginosa</i>	≤43.34 µg /ml
5	<i>Staphylococcus aureus</i>	34.84 µg /ml

Values are expressed as Mean ± S.D

Table-3: Phytochemical constituents identification from *Phyllanthus narayanaswamii*

Constituents	Present/Absent
Alkaloids	+
Glycosides	++
Terpenoids	-
Tannins	+
Reducing Sugar	+
Flavonoids	+

Conclusion

Alcoholic extract of *Phyllanthus narayanaswamii* Gamble leaves were exhibited a strong antibacterial activity against *Pseudomonas aeruginosa* and moderately active against *Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis*. The bioassay-guided fractionation and further characterization of active phytochemical principles responsible for the antibacterial potential of this medicinally important plant species is under way in our laboratory.

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