



Analysis of Phytochemical Constituents and Antibacterial Activity of *Phyllanthus rheedei* Against Human Pathogens

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ABSTRACT

Antibacterial properties of leaf, stem and root extract of *Phyllanthus rheedei* were evaluated for activity against medically important bacteria such as *Staphylococcus aureus*, *Streptococcus pneumonia* MTCC-655, *E.coli* MTCC-1583, *Klebsiella pneumoniae* MTCC-39, *Shigella* MTCC-2957 and *Salmonella typhi murium* MTCC-98. Antibacterial activity was performed by agar well diffusion method. The dry powder was extracted in Ethanolic solvent. The inhibition diameters obtained with bacteria are between 6 to 12 mm. Gram positive bacteria were most inhibited then gram negative bacteria. The preliminary phytochemical analysis revealed the presence of different phytoconstituents such as Tannins, Saponins, Flavonoids, Steroids, terpenoids, Glycosides, Alkaloids and Anthraquinones. It was observed that the leaves possess numerous phyto-constituents rather than stem and root. *Phyllanthus rheedei* can be used to source antibiotic substances for possible treatment of bacterial infections skin and urinary tract infections.

Keywords: *Phyllanthus rheedei*, human pathogens, Antibacterials activity, preliminary phytochemical analysis.

INTRODUCTION

Plants have been used in the preparation of traditional medicine for a long time and most of these folk medicines were prepared from locally grown wild plants. Knowledge about the uses of plants was compiled by trial and error and passed down from one generation to another orally. Now days, world markets are turning to plants as the sources of ingredients in healthcare products. Plant secondary metabolites were found to be sources of various phytochemicals that could be used directly or as intermediates for the production of pharmaceuticals, as additives in cosmetic, food or drink supplement. In recent years, there has been a resurgence of interest in the discovery of new compounds from plants with the aim of finding novel treatment against a variety of illnesses. Many medicinal plants that reported to have the potential for medicinal propose were investigated for useful active compounds.

The genus *Phyllanthus* (Euphorbiaceae) has between 550 to 750 species and several of them produce useful secondary metabolites which have been extracted from whole plants. *Phyllanthus* species are traditionally used in the treatment of a variety of ailments including jaundice, asthma, ulcer, hepatitis, tuberculosis, malaria, dysentery, gonorrhoea, flu, diabetes, dropsy, syphilis, cough, diarrhoea, vaginitis and urinary diseases and other hepatic disorders¹⁻³. Several compounds such as alkaloids, tannins, flavonoids, lignans, phenols and terpenes have been isolated and identified in various species of *Phyllanthus* and have shown antinociceptive action in mice and other therapeutic activities⁴. Antiviral effects against hepatitis B virus and possibly against the

reverse transcriptase of retroviruses have also been reported⁵⁻⁷.

Phyllanthus rheedei Wight belongs to the family Euphorbiaceae, small herb 75(100)cm its grown in hills 900-1200 m in fallow fields and river banks, distribution in Tropical Africa, India, New Guinea⁸. It is used an oriental folk medicine in diabetes mellitus. *Phyllanthus rheedei* used in traditional medicine to treat liver diseases. Therefore, an attempt was made for the screening of phytochemical compounds in *in vivo* plant parts of *Phyllanthus rheedei*.

MATERIALS AND METHODS

Collection of plant materials

The Plant of *Phyllanthus rheedei* was collected from its natural habitats, in Yercad hills and was identified by Botanical Survey of India Southern Regional Center at Coimbatore, Tamilnadu, India.

Extraction procedure

Plant part like leaves, stem and root were dried under shade condition. The samples were ground to fine powder using an electric blender and dissolved separately in 100 mL of solvent. The solution was kept in at room temperature for seven days to allow the extraction of compounds from leaf, stem and root. The solution of each sample was stirred after every 24 hrs sterile glass rod. After 7 days, the solution was filtered through what man No-1 paper. The solvent was evaporated and sticky substances obtained that was stored in the refrigerator and suspended in 10 % dimethylsulfoxide prior to use.

Chemical testes were carried out the ethanolic extracts and on the powder specimens using standard procedures



to identify the constituents as described previously⁹⁻¹⁰. The specific procedure involved for the evaluations of a particular group of chemicals. Then the extract was stored under refrigeration at 4°C for further studies.

Human pathogenic bacterial species

The human pathogenic bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae* MTCC-655, *E.coli* MTCC-1583, *Klebsiella pneumoniae* MTCC-39, *Shigella* MTCC-2957 and *Salmonella typhimurium* MTCC 98 were obtained from Microbial Type Culture Collection from Chandigarh and were maintained in Nutrient agar slant at 4°C for experimental studies.

Antibacterial activity

The antibacterial activity of different plant part extracts was determined by agar well diffusion method. The Mueller Hinton Agar (HiMedia) was inoculated with 24 hour old culture. A sterile 5 mm bore was used to cut 4-5 wells of equidistance in each of plates. The concentration of 100µl ethanolic extraction are poured in to the each well and the sample extracts were allowed to diffuse properly by keeping the petri plates in refrigerator at 4°C for 2 hours followed by incubation at 37°C for 24 hours. Solvent used for extraction (methanol) was used as control. After incubation the diameter of inhibitory zones formed around each well was measured (mm) recorded.

Screening of phytochemical components

Tannins

One mL of water and 1-2 drops of ferric chloride solution were added in 0.5 mL of extracted solution. Blue colour was observed for Gallic tannins and green black for catecholic tannins.

Saponins (Foam test)

Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes it indicated the presence of saponins.

Flavonoids (Alkaline Reagent test)

Extractions were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which became colourless on addition of dilute acid, indicates the presence of flavonoids.

Steroids

Two mL of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 mL H₂SO₄. The colour changed from violet or blue or green in some samples indicating the presence of steroids.

Terpenoids (Salkowski test)

Five mL of each extract was mixed in 2 mL of chloroform, and concentrated H₂SO₄ (3mL) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show the presence of terpenoids.

Cardiac glycosides (Keller-Killani test)

Five mL of each extract was treated with 2 mL of glacial acetic acid containing drop of ferric chloride solution. This was underlaid with 1 mL of concentrated sulphuric acid. A brown ring of interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Alkaloids

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloids solution produces white yellowish precipitated when a few drops of Mayer's reagents are added.

Anthraquinones

Born frager's test was used for detecting the presence of anthraquinones. In this case 0.5 g of the plant extract was shaken with benzene layer separated and half of its own volume of 10% ammonia solution added. A pink, red, or violet colouration in the ammonia phase indicated the presence of anthraquinone.

RESULTS

Antimicrobial activity of Methanol extracts

The antibacterial activity of ethanolic extracts of plant part were investigated using agar well diffusion method against selected human pathogens such as *Staphylococcus aureus*, *Streptococcus pneumoniae* MTCC-655, *E.coli* MTCC-1583, *Klebsiella pneumoniae* MTCC-39, *Shigella* MTCC-2957 and *Salmonella typhi murium* MTCC 98. It is observed significant zone of inhibition against all microorganisms. It showed maximum inhibition activity against *Staphylococcus aureus*, *Streptococcus pneumoniae* MTCC-655 and minimum inhibition activity of *E.coli* MTCC-1583, *Klebsiella pneumoniae* MTCC-39, *Shigella* MTCC-2957 and *Salmonella typhi murium* MTCC 98.

Phytochemical constituents

The preliminary phytochemical test revealed that the *Phyllanthus rheedii* leaves contain the phytochemicals viz., Tannins, Flavonoids, Steroids, Terpenoids, Glycosides and alkaloids. The stem contains Tannins, Steroids, Terpenoids, Glycosides, Alkaloids and Anthraquinones. The root contains Tannins, Flavonoids, Terpenoids, Glycosides and Anthraquinones.

From the observations it is obvious that *Phyllanthus rheedii* leaves and stem possessing numerous phytoconstituents rather than root (Table 1). The phytochemical observation was supported by¹¹ who has reported the phytochemicals present in the *Phyllanthus debilis*.

Table 1: Preliminary phytochemical analysis in various parts of *Phyllanthus rheedei*

Plant Constituent	In vivo Plant Parts		
	Leaf	Stem	Root
Tannins	+	+	+
Saponins	-	-	-
Flavonoids	+	-	+
Steroids	+	+	-
Terpenoids	+	+	+
Cardiac Glycosides	+	+	+
Alkaloids	+	+	-
Anthraquinones	-	+	+

Table 2: Antibacterial activity of *Phyllanthus rheedei* methanol extract against bacterial pathogens

Organism	Concentration of extract (100µl) and zone of inhibition (mm)		
	Leaf	Stem	Root
<i>Staphylococcus aureus</i>	10mm	11mm	12mm
<i>Streptococcus pneumoniae</i>	7mm	9mm	11mm
<i>E.coli</i>	4mm	6mm	7mm
<i>Klebsiella pneumoniae</i>	7mm	8mm	8mm
<i>Shigella</i>	5mm	6mm	8mm
<i>Salmonella typhimurium</i>	6mm	5mm	7mm

DISCUSSION

There are about 45,000 plant species in India with capacity to produce a large number of organic chemicals concentrated hotspot in the region of Eastern Himalayas, of high structural diversity^{12, 13}. This study reports the presence of different phytochemicals and antibacterial activity with biological activity that can be valuable therapeutic index^{14,15}. In the present study, we have found that the biologically active phytochemicals were present in the ethanolic extracts of *Phyllanthus rheedei*. The antibacterial properties of these extracts may be due to the presence of above mentioned phytochemicals.

CONCLUSION

The preliminary phytochemical study on *in vivo* plant parts like leaf, stem, root in medicinal plant *Phyllanthus rheedei* revealed the presence of different phytoconstituents such as Tannins, Saponins, Flavonoids, Steroids, terpenoids, Glycosides, Alkaloids and Anthraquinones in different plant parts. The leaves and stem possess numerous phyto-constituents rather than root. The extracts of these plants can be used as an easily accessible source of natural antimicrobial agent and can be of assistance in some dermatological problems, bacterial infections skin and urinary tract infections.

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