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**Preliminary phytochemical investigation and antibacterial activity of  
*Phyllanthus amarus* Schum & Thorn.**

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R.Dhandapani<sup>a</sup>, D. Lakshmi<sup>a</sup>,  
V. Balakrishnan<sup>b</sup>, S.Jayakumar<sup>c</sup>,  
Anandhakumar<sup>d</sup>

<sup>a</sup> Medicinal Plants Research Unit, P.G. &  
Research Department of Microbiology,  
Thanthai Hans Roever College,  
Perambalur 621 212, India

<sup>b</sup> Laboratory of Plant Science, Department  
of Biotechnology, K.S.R. College of  
Technology, Tiruchengode, India

<sup>c</sup> Department of Biotechnology, Thanthai  
Hans Roever  
College, Perambalur 621212, India

<sup>d</sup> Department of Botany and  
Microbiology, A.V.V.M. Sri Puspam  
College, Poondi, India

**Abstract**

The aqueous extract of *Phyllanthus amarus* Schum & Thorn. was selected for preliminary phytochemical and antibacterial activity against human pathogens. Minimum inhibitory concentration was performed by broth dilution method and the zone of inhibition was studied by agar disc diffusion method at concentrations of 10 and 20 mg/ml in DMSO (Dimethyl Sulfoxide). The results revealed the antimicrobial activity of extract against

the the strains of microorganisms concentrations in ranges of 60 and 500 µg/ml. Aqueous extract of leaves and roots of *Phyllanthus amarus* exhibited significant antibacterial activity against eight test bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus albus*, *Streptococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus vulgaris*. The plant under study revealed the presence of

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alkaloids, phytosterols, phenolic compounds and tannins, proteins and amino acids, lignins and saponins. *Keywords:* Antimicrobial activity; Alkaloids; Human pathogen; *Phyllanthus amarus*; Phytochemical

## Introduction

*Phyllanthus amarus* (Euphorbiaceae) is native probably to West Pakistan and western India and has been introduced in to Africa and the West Indies. A herb, up to 60 cm in height, occurring as a winter weed throughout the hotter parts of India, particularly on cultivated land (Anonymous, 1969). The compost mixture consisting of loamy soil, dried cow manure, silver sand, powdered charcoal and brick is very suitable for the cultivation of this crop and the plants are propagated by seeds (Supriya Kumar Bhattacharjee, 1998). The dried leaves contain 0.4 percent of a toxic bitter principle, phyllanthin, traces of a tasteless substance, hypophyllanthin and about 5 percent of a colourless wax made up mostly of esters (85%) of long chain fatty acids and alcohols, free fatty acids and hydrocarbons. Phyllanthin is toxin to fish and frog. In frogs it causes depigmentation of the skin, but the colour is regained after about 20 hours. The leaves are rich in potassium (0.83%, fresh basis) which is considered responsible for their powerful diuretic effect. Stem contains saponin. A decoction of the stem and leaves dyes cotton black; sometime it is substituted for ink (Anonymous, 1969). Three new lingans - niranthin, nirtetralin and phyltetralin have been- isolated from leaves, estradiol in

bark and roots have been detected by TLC; estradiol content of 155-350 ug /100g in plant samples; kaempferol-4<sup>1</sup>-rhamnopyranoside and eriodictyol-7- rhamnopyranoside have been isolated from roots, lup-20(29)-en-3beta-ol and its acetate have also been isolated from roots (Rastogi,1990).The herb is bitter in taste, and is reported to possess astringent, deobstruent, stomachic, diuretic, febrifugal and antiseptic properties. It is used in stomach troubles such as dyspepsia, colic, diarrhoea and dysentery and is also employed in dropsy and diseases of urinogenital system. Fresh roots are said to be beneficial in jaundice. They are taken with milk as a galactagogue. In Rajasthan, the roots are used for treating camels suffering from digestive troubles. A decoction of the leaves is used as a refrigerant for the scalp. Leaves and roots are made in to a poultice with rice water for application on oedematous swellings and ulcers. The latex is also applied to offensive sores and ulcers, and mixed with oil it is used in ophthalmia (Anonymous, 1969).

The aim of this paper is to investigate preliminary phytochemical and antibacterial activity of aqueous extract of leaves and roots of *Phyllanthus amarus*.

## Materials and Methods

### Plant material

*Phyllanthus amarus* Schum & Thorn. was the plant material of the present investigation. Fresh leaves were collected from the plant in Cuddalore district, Tamil Nadu, India. Herbarium

specimen was deposited in the Rapinaet Herbarium, St. Joseph's college, Trichy, Tamil Nadu, India. The plant herbarium number was obtained (Voucher No.RHT.1652).

### **Preparation of leaf powder**

The collected leaves were washed, shade dried at room temperature and then milled in to coarse powder by a mechanical grinder. This method was followed by Ghosh et al. (2006).

### **Preparation of drugs (aqueous extract)**

Leaf powder was boiled in distilled water and after filtration (using Whatmann No.40 filter paper) the extract was evaporated to dryness by slow heating and continuous drying in a water bath. The residue was collected, which is considered as a drug (Rao et al., 1995).

### **Preliminary phytochemical investigation**

The preliminary phytochemical profile was studied as per the method of Kokate (1994).

### **Microorganisms used**

The standard drugs and the test microorganisms were gram positive *Staphylococcus aureus* NCIM 2079, *Streptococcus faecalis* NCIM 2080, *Staphylococcus albus* NCIM 2178, *Bacillus subtilis* NCIM 2063, and gram negative bacteria such as *Escherichia coli* NCIM 2065, *Pseudomonas aeruginosa* NCIM 2036, *Klebsiella*

*pneumoniae* NCIM 2098, and *Proteus vulgaris* NCIM 2027, were obtained from National Chemical Laboratory (NCL) Pune, India.

### **Antibacterial screening**

The aqueous extracts of leaf and root were screened for their antibacterial activity by disc diffusion method. The aqueous extract of leaves and roots of *Phyllanthus amarus* were found to exhibit antibacterial activity at 10 and 20 mg/ml concentrations. Ciprofloxacin (5 µg/ml) was used as standard drug.

### **Determination of Minimum Inhibitory Concentration (MIC)**

Minimum Inhibitory Concentration (MIC) of the extracts was performed by broth dilution method at concentration of the extract ranging from 60 µg/ml to 500 µg/ml in DMSO against all the test microorganisms.

### **Results**

The preliminary phytochemical screening of aqueous extracts leaf and root *Phyllanthus amarus* are given in (Table1). The leaf extract of *P.amarus* showed the presence of alkaloids, phytosterols, phenolic compound, and tannins, proteins and aminoacids, lignin and saponin. The root extracts of *P.amarus* showed the occurrence of alkaloids, phytosterols, phenolic compounds and tannins, proteins and amino acids, lignins and saponins. The carbohydrates, fixed oils and fats, gums and mucilages are absent in aqueous extracts of leaf and root of *P.amarus*.

Both aqueous extracts of leaf and root showed appreciable antibacterial activity against selected pathogenic bacteria. The results of antibacterial test using disc diffusion method are presented (Table 2). Both extracts had similar antibacterial activity. The values of different inhibitory concentration ranged at 10 and 20 mg/ml. These effects were particularly observed against gram positive bacteria such as *Staphylococcus aureus*, *Staphylococcus albus*, *Streptococcus faecalis*, *Bacillus subtilis* and gram negative bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus vulgaris*

The leaf extract showed maximum activity recorded against gram positive bacteria such as *Staphylococcus aureus*, *Staphylococcus albus*, *Bacillus subtilis* and gram negative *Escherichia coli*. These effects were compared with commercially available antibiotics under the same laboratory condition (Table 2). The results of MIC study revealed the antibacterial activity of extracts against the test strains of microorganisms between concentration ranges of 60 to 500 µg/ml.

## Discussion

*Phyllanthus amarus* is used as diuretic, stomachic and antiseptic in traditional medicine. The fresh leaves and roots are used to treat jaundice and digestive troubles. The study plant is also used in treatment of dental disease in folk medicine. The study plant has not yet been evaluated for antimicrobial activity against dental infectious disease. Hence, the present study was

undertaken to investigate antibacterial activity against dental pathogen.

Moreover, previous studies on other medicinal plants have reported the antibacterial and antifungal activity of phenolic and phenylpropanoic compounds, coumarins, methylated and phenylated flavonoids. Tannins have been reported to exhibit some levels of antibacterial activity.

Our preliminary phytochemical screening of the aqueous extracts (leaves and roots) of *Phyllanthus amarus* have revealed the presence of alkaloids, phytosterols, phenolic compounds and tannins, proteins and amino acids, lignin and saponin. These phytochemical constituents may be responsible for antimicrobial activity against dental pathogens. It is concluded that the antimicrobial activity of *Phyllanthus amarus* and its active phytochemical constituents can be helpful in the therapeutic treatments for dental pathogens.

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