

Preliminary phytochemical analysis and *In-vitro* antibacterial activity of *Phyllanthus reticulatus* leaf extracts

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Abstract

Medicinal plants are a good alternative to chemical drugs to treat various disease conditions owing to their effective long term curing properties and fewer side effects. The present study aims to investigate the active phytochemicals present in one such rarely investigated medicinal plant *Phyllanthus reticulatus* by performing a preliminary phytochemical analysis and further evaluating the antibacterial activity of the aqueous leaf extracts of *Phyllanthus reticulatus* by performing well diffusion test against two common human bacterial pathogens namely *Escherichia coli* and *Pseudomonas aeruginosa*. The qualitative analysis confirmed that the leaves of *Phyllanthus reticulatus* contain bioactive compounds and the diameter of the zone of inhibition indicated the high inhibitory effectiveness of the aqueous leaf extracts of *Phyllanthus reticulatus* against both the test organisms thereby confirming its antibacterial activity. Further studies can help us to identify potential lead compounds to design a cost effective drug.

Key Words: Bacterial Pathogens, *Escherichia*, Medicinal Plants, Phytochemicals, *Pseudomonas*

INTRODUCTION

In traditional cultures many medicinal plants are used all over the world and recently they are becoming increasingly popular in modern society as an alternative to synthetic chemicals (Verma *et al.*, 2011). Such medicinal plants are good potential source of drug and if the phytochemicals are identified they can turn out to be commercially important increasing the revenue of the country where they grow.

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One such medicinal plant is *Phyllanthus reticulatus*. (Fig. 1). The plant is widely present in Tropical Africa, Srilanka, South East Asia, China, Malaysia and throughout India, mostly in hedges or waste places. Fruiting and flowering season of plant is from July to March (Verma *et al.*, 2011). Various species of *Phyllanthus* have been reportedly used in the treatment of a variety of ailments around the world (Muhammad Farrukh Nisar *et al.*, 2018). *Phyllanthus reticulatus* is commonly known as *Pancoli* or *Karineli*. It is a multipurpose plant with a wide range of medicinal uses, bearing an edible fruit. (Manjula *et al.*, 2017).

In India, decoction of the dried bark and leaves of the plant are used as a natural diuretic and to treat small pox owing to their cooling effect (Verma *et al.*, 2011). Various parts of the plant have been shown to contain tannins, which are partly responsible for its medicinal and dyeing properties (Venkataraju *et al.*, 2012). *Phyllanthus reticulatus* is used to treat syphilis, asthma, inflammation, malaria, gastric complaints including constipation etc. Therapeutic potential of plants are ascribed to the presence of various secondary metabolites such as alkaloids, polyphenolic compounds and terpenes (Begum *et al.*, 2006).

Plants are known to possess several antimicrobial compounds and are used in all traditional medicines. Like any therapeutic agent, when overdosed or incorrectly used they also have the potential to induce adverse effects side effects like stomach upset, diarrhea, nausea, allergic reactions leading to redness, swelling, itching, skin rash, pale skin and gray skin color only in children (Vaghasiya, 2009). Plants may represent a potential source of antibiotics as evidenced by huge number of studies that have dealt with antimicrobial action of medicinal plants. (Shruthi *et al.*, 2010)

Phyllanthus reticulatus is a broad spectrum medicinal plant that has received world- wide recognition. *P. reticulatus* is generally employed to reduce pain and


Domain :	Eukaryota	
Kingdom:	Plantae	
Claudus :	Angiosperm	
Order :	Malpighiales	
Family :	Euphorbiaceae	
Tribe :	Phyllanthae	
Genus :	Phyllanthus	
Species :	<i>P.reticulatus</i>	

Fig 1. *Phyllanthus reticulatus*

Table 1. Phytochemical constituents of leaf extracts of *Phyllanthus reticulatus*

S.No	Test	Aqueous extract of <i>Phyllanthus reticulatus</i>
1	Alkaloids	+++
2	Saponins	+++
3	Reducing sugar	+
4	Steroids	+
5	Phytosterol	++
6	Flavonoids	++
7	Aminoacids and Protein	-
8	Phenol	+++
9	Glycosides	+
10	Tannins	+++

+ indicates presence; - indicates absence

also has anti-microbial, antiseptic, antiviral, anti-diabetic, antipyretic properties, and is also used in the treatment of wound, ulcers and urogenital diseases. Plants contain numerous constituents; some tend to possess some level of toxicity. *P. reticulatus* has been classified among plants with a low potential for toxicity (Begum *et al.*, 2006). Natural products from plants provide lead compounds for synthesis of therapeutic agents by pharmaceutical companies. Compounds such as morphine, quinine, taxol, vincristine, nicotine, vinblastine and digoxin are of plant origin (Ghani, 2003).

This paper gives an account of preliminary phytochemical screening of leaf extracts of *P. reticulatus* and its *in vitro* antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

COLLECTION OF SAMPLE

Plant Materials: Matured leaves of *Phyllanthus reticulatus* were collected from the local area of Coimbatore district, Tamil Nadu, India during November 2019 and were washed thoroughly and shade dried for about two weeks.

Extraction of Plant Material

The dried leaves were ground to a fine powder. The powder was preserved in an air tight container. About five grams of the sample was taken in a conical flask and to this 20ml of water was added. The mixture was subjected to extraction in an incubator shaker for 24 hours at room temperature. The mixture was filtered with Whatmann No1 filter paper and the filtrate was stored for further analysis.

PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening was done according to the phytochemical methods described by Vijayakumar *et al.*, (2017)

Test for Alkaloids (Wagner's test)

About few ml of the extract was treated with 4-5 drops of Wagner's reagent. The formation of reddish brown precipitate confirms the presence of Alkaloids.

Test for Phenol (Ferric chloride test)

About 2ml of the extract was treated with 10% ferric chloride solution and observed for the formation of deep blue / black colour to indicate the presence of phenol.

Test for reducing sugars (Fehling's Test)

To 1 ml of the extract few drops of Fehling's reagent was added and the mixture was boiled in a boiling water bath for 10 minutes and observed for the appearance of blue colour to indicate the presence of reducing sugars.

Test for Saponins (Foam test)

To 2 ml of the plant extract 6ml of water was added in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam for few seconds. The presence of foam confirms the presence of saponins.

Test for Flavonoids (Ferric chloride test)

To about 2ml of the plant extract, few drops of 10% ferric chloride solution was added. The formation of green or blue colour indicates the presence of flavonoids.

Test for Phytosterols (Salkowski's Test)

One ml of the plant extract was treated with 2 ml of chloroform and few drops of acetic anhydride were added. To that mixture equal amount of concentrated sulphuric acid was added. The formation of bluish green colour indicates the presence of phytosterols.

Test for Aminoacids and Proteins (Ninhydrin test)

To a few ml of the plant extract small amount of Ninhydrin reagent was added. A purple or violet colour

Table 2. Zone of inhibition of aqueous leaf extracts of *Phyllanthus reticulatus* against bacteria

S.No	Volume of extract (μ l)	Zone of inhibition against <i>Escherichia coli</i> (in mm)	Zone of inhibition against <i>Pseudomonas aeruginosa</i> (in mm)
1	25	2	2
2	50	2.3	2.2
3	75	2.4	2.4
4	100	2.5	2.6
5	Standard antibiotic (Chloramphenicol-25)	3	2.8

formed indicates the presence of amino acids and proteins.

Test for Steroids

About 2 ml of chloroform and 0.2 ml of concentrated sulphuric acid were added to 1ml of the extract. The formation of red colour precipitate indicates the presence of steroids.

Test for Tannin (Ferric chloride test)

To about 1ml of the extract few drops of dilute ferric chloride solution was added. The presence of tannin is confirmed by the formation of dark green or blue colour or reddish brown colour. (Reddish brown – Condensed tannin).

Test for glycosides

To 1ml of the extract few ml of concentrated sulphuric acid was added. Formation of red colour indicates the presence of glycosides.

PREPARATION OF THE BACTERIAL INOCULUM

Active bacterial culture for the experiment was prepared by inoculating the test cultures *Escherichia coli* and *Pseudomonas aeruginosa* into each test tubes containing 50ml of nutrient broth and the bacterial cultures were incubated with agitation for 24 hours at 37° C in an incubator shaker.

Agar Well Diffusion method :

The antibacterial activity of the extracts was determined by Well Diffusion method. Muller Hinton Agar plates were prepared by pouring sterilized 20ml of molten media into each sterile petriplates. To prepare the antibiotic stock about 30mg of Chloramphenicol was dissolved in 1 ml of ethanol to give a concentration of 30 μ g/ μ l. After solidification of the media, about 20-25 μ l of test culture was swabbed uniformly on the Muller Hinton Agar plates. On each inoculated plate, 10 mm diameter wells (5 wells at equal distance in one

plate) were bored on the agar using a sterile cork borer. A Concentration of 25 μ l, 50 μ l, 75 μ l and 100 μ l of extract was added to each well and was allowed to diffuse for 1 hr. About 30 μ l was added to the well marked as standard. The plates were then incubated at 37°C for about 24 hours and a control was also maintained. The diameter of the zone of inhibition resulting from the activity of the extracts was measured in mm. The antibacterial activity of the plant extract against the selective micro-organism was evaluated by comparing with that of the standard antibiotic (Parekh Jigna, 2007).

RESULTS AND DISCUSSION

Phytochemical analysis of leaf extracts of *Phyllanthus reticulatus*

The phytochemical analysis of the leaf extracts of *Phyllanthus reticulatus* indicates the presence of Flavonoids, Saponins, Tannins, Phenols, Phytosterol and Alkaloids (Table 1).

Antibacterial activity of aqueous leaf extracts of *Phyllanthus reticulatus*:

The results of the disc diffusion method (Kirby Bauer et al., 1966) performed to test the antibacterial activity of the aqueous extracts of *Phyllanthus reticulatus* leaves against the bacterial cultures *Escherichia coli* and *Pseudomonas aeruginosa* using Chloramphenicol as the standard antibiotic are given in Table 2.

The aqueous leaf extracts of *Phyllanthus reticulatus* were prepared and subjected to phytochemical analysis. Such a qualitative analysis confirmed that the leaves of *Phyllanthus reticulatus* contain bioactive compounds such as alkaloids, saponins, phenol and tannins. The antibacterial activity of the leaf extracts of *Phyllanthus reticulatus* was determined by well diffusion method against the test pathogenic organisms namely *Escherichia coli* and *Pseudomonas aeruginosa*. The average diameter of the zone of inhibition indicated the high

effectiveness of the extracts similar to the standard antibiotic used against both the bacteria.

The results of the present study are comparable with that of earlier studies (Begum *et.al.*, 2006; Vaghasiya, 2009; Narisimbudhu, 2012; Manjula, V., 2017; Muhammad Farukh Nisar *et.al.*, 2018).

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