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The challenging perspective antimicrobial activity of *Phyllanthus reticulatus* against clinical microbes

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Abstract
Traditional medicine is an important source of potentially useful compounds for the development of phytotherapetic agent. Antimicrobials of plant origin have enormous therapeutic potential in the treatment of infectious diseases while simultaneously migitigiting many of the side effects that are often associated with synthetic antimicrobials. In the present investigation suggested that the effect of antimicrobial activity of *Phyllanthus reticulatus* against clinical microbes were performed. In the experiments of the test plant *Phyllanthus reticulatus* leaf and flower extract with different solvent of aqueous, ethanol, ethyl acetate and petroleum ether were treated against the bacteria like *E. coli*, *Enterococcus* sp., *K. pnemoniae*, *Staph. aureus*, *Salmonella* sp., *Vibrio cholera*, and *Proteus* sp. and fungi such as *Aspergillus flavus*, *A. niger*. *A. terreus*, *Penicillium* sp., *P. chrysogenum*, *Rhizoctonia solani*, and *Trichoderma* sp. were performed respectively. However the antimicrobial properties of *Phyllanthus reticulatus* leaf with methanolic and ethyl acetate extract of maximum zone inhibition and excellent performance when compared to other solvent of aqueous and petroleum ether extract. It can be concluded that the plant used to discover natural products that may serve as lead for the development of new biomedical applications.

Keywords: *Phyllanthus reticulatus* bacteria, fungi, antimicrobial

Introduction
Plants are an integral part of human life. Humans depend on plants for various purposes such as food, shelter, cloth, construction, dyes and medicine. Traditional medicinal practitioners from various parts of the world and used plants for treating several diseases and disorders. It is estimated that about 2 and 3rd of population of world depends on traditional medicine to meet the primary healthcare. Plants are used singly or in certain formulations. Traditional medicinal practices have been widespread in various countries. Plants play a crucial role in traditional medicinal practices such as Ayurveda, Unani and Siddha. Therapeutic potential of plants are ascribed to the presence of various secondary metabolites such as alkaloids, polyphenolic compounds and terpenes. Extracts and purified constituents of plants have shown to exhibit a wide array of biological activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer, larvicidal and analgesic activity. Natural products including 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 (Poojari *et al.*, 2009) [13] and (Koohsari *et al.*, 2015) [8].

Medicinal plants have become part and parcel of our day to day life. These medicinal plants are commercially important and if managed properly they will earn more revenue to the country where they grow. One such medicinal plant is *Phyllanthus reticulates*, a multipurpose plant, providing a range of medicinal uses and other commodities for the local people as well as a possibly edible fruit. Commonly this plant grows as a weed.

Plant-based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials is needed enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects of synthetic antimicrobials (Iwu *et al.*, 1999) [7]. They may act as lead compounds for the pharmaceutical industry or as the base for the development of new antimicrobials (Aiyelaagbe, 2001, Aiyoro *et al*, 2008) [2]. *Phyllanthus niruri* Linn belongs to family Euphorbiaceae, commonly known as Stonebreaker (Eng.) due to its antilithic property. Various bioactivities such as antidiabetic (Okoli *et al*, 2011) [11], anti-hepatotoxicity (Ravikumar *et al*, 2011) [14]. Antilithic, anti-hypertensive, and anti-hepatitis B (Bagalkotkar *et al.*, 2011, Naik and Juvekar, 2003) [3, 10] have been reported. Several studies have confirmed

the antimicrobial efficacy of different *Phyllanthus* species. However, there is insufficient information regarding the antimicrobial activities of methanolic extracts of *P. niruri*, leaves have been reported to show diverse medicinal properties. In the present investigation methanolic extract of plant parts of *P. niruri* has been studied for their antimicrobial efficiency.

Materials and Methods

Agar well – diffusion method

Agar well – diffusion method was followed for determination of antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old broth culture of respective bacteria and fungi were determined agar wells (5mm diameter) were made in each of these plates using sterile cork borer. About different solvent leaves and flower extracts of *Phyllanthus reticulatus* added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of preincubation diffusion in order to minimize the effects of variation in time between the applications of different solutions of the plates were incubated in an upright position at 37± 2 °C for 24 hrs for bacterial and 28± 2 °C for fungi. The organic solvents (aqueous and methanol) were acted as a negative control results were recorded, as the presence or absence of inhibition zone. The inhibitory zone around the well indicated absence of tested organism and it was reported as positive and absence of zone is negative. The diameters of the zones measured using diameter measurement scale. Triplicates were maintained and the average values were recorded for antimicrobial activity.

Results and Discussion

In the present investigation suggested that the antibacterial activities of aqueous, ethanol, petroleum ether and ethyl acetate extract of *P. reticulatus*. Using disc diffusion method. The promising antibacterial activity at one from plant extract regardless to the type of ($Gram^-$ and $Gram^+$) bacteria. The petroleum ether extract and ethyl acetate extract also inhibited the growth of all bacteria but their efficiency was less in ethanol extracted. Ethanol extract produced the highest zone of inhibition, consequently, lowest MIC and MBC value against *S. aureus* among all the studied bacteria. This is intriguing since positive control (Tetracycline) produced much lower inhibition zone than that of ethanol extract. Petroleum ether and ethyl acetate extract also produced highest inhibition zone against *S. aureus* among other bacteria. In addition, earlier studies reported that methanol and chloroform extracts also effective against *S. aureus* (Shruthi *et al.,* 2010) [15] and (Das *et al.,* 2011) [6].

Table 1: Effect of antibacterial activity of *phyllanthus reticulatus* (flower) against some bacteria

Name of the bacteria	Zone of inhibition (mm)			
	Aqueous	Ethanol	Ethyl acetate	Petroleum ether
E. coli	09.9±3.00	12.3±4.10	06.3±2.10	05.3±1.70
Enterococcus sp.	11.7±3.70	13.7±4.50	06.0±2.00	08.6±2.90
K. pneumoniae	12.0±4.00	14.0±4.70	12.0±4.00	12.0±4.00
Proteus sp	10.0±3.30	15.7±5.20	13.7±4.50	12.3±4.10
S. aureus	11.0±3.70	16.3±5.50	16.7±5.70	13.0±4.30
Salmonella sp.	05.0±1.60	05.6±1.80	09.6±3.20	05.6±1.80
Vibrio cholerae	12.3±4.10	18.7±6.20	14.3±4.70	10.3±3.40

Mean ± Standard error

Table 2: Effect of antifungal activity of *phyllanthus reticulatus* (flower) against some fungi

Name of the fungi	Zone of inhibition (mm)			
	Aqueous	Ethanol	Ethyl acetate	Petroleum ether
Aspergillus flavus	06.0±2.00	12.0±4.00	07.3±2.40	08.0±2.70
A. niger	13.7±4.40	19.3±6.40	17.7±5.80	15.7±5.20
A. terreus	06.7±2.20	11.7±3.80	11.7±3.80	03.7±1.20
Penicillium sp.	05.7±1.80	13.0±4.30	09.0±3.00	06.3±2.10
P. chrysogenum	11.3±3.70	13.3±4.40	12.0±4.00	13.3±4.50
Rhizoctonia solani	05.0±1.7.0	12.3±4.10	07.4±2.50	-
Trichoderma sp.	06.7±2.20	12.0±4.00	11.0±3.70	08.4±2.70

Mean ± Standard error

In the present investigation studied that the effect of antibacterial activity of *Phyllanthus reticulates.* However with ethanolic extract against clinical bacteria like *E. coli*, *Enterococcus* sp, *K. pneumonia*, *Proteus* sp, *S. aureus*, *Salmonella* sp, and *Vibrio cholera* was 12.3±4.10, 13.7±4.50, 14.0±4.70, 15.7±5.20, 16.3±5.50, 05.6±1.80 and 18.7±6.20mm zone of inhibition recoded when compared with other solvent extract respectively. The effect of antifungal activity of *P. reticulatus* flower with ethanolic extract was excellent performance against fungi like *Aspergillus flavus*, *A. niger*, *A. terreus*, *Penicillium* sp., *P. chrysogenum*, *Rhizoctomia solani* and *Trichoderma* sp. with 12.0±4.00, 19.3±6.40, 11.7±3.80, 13.0±4.30, 13.3±4.40, 12.3±4.10 and 12.0±4.00mm zone of inhibition measured respectively. It may be due to the plant extract with extraction solvent was main active principles of plant with recording antimicrobial properties when compared to other solvents

Watkins *et al.,* (2012) researchers are searching alternative therapeutic agents to treat multi-drug resistant *S. aureus*. In this context, findings seem to be promising for investigation. Ethanol extract also produced satisfactory inhibition zone with lowest MIC and MBC value against *P. aeruginosa* whereas petroleum ether and chloroform extract failed to show such inhibition. *P. aeruginosa* is also a multidrug resistant bacterium and associated with serious health hazards and mortality.

Table 3: Effect of antibacterial activity of *phyllanthus reticulatus* (leaf) against some bacteria

Name of the bacteria	Zone of inhibition (mm)			
	Aqueous	Ethanol	Ethyl acetate	Petroleum ether
E. coli	08.7±2.90	12.3±4.00	20.0±6.70	14.3±4.80
Enterococcus sp	13,0±4.30	14.7±4.90	17.0±5.70	17.0±5.70
K. pneumonia	09.0±3.00	10.0±3.00	18.3±6.10	12.3±4.10
Proteus sp	05.0±1.70	7.70±2.00	18.3±6.10	12.7±4.20
S. aureus	11.7±3.80	12.7±4.10	11.7±3.70	05.0±1.70
Salmonella sp.	06.7±2.40	11.7±3.80	12.0±4.00	08.0±2.70
Vibrio cholerae	12.0±4.00	11.7±3.80	15.3±5.10	07.0±2.30

Mean ± Standard error

Table 4: Effect of antifungal activity of *Phyllanthus reticulatus* (leaf) against some fungi

Name of the fungi	Zone of inhibition (mm)			
	Aqueous	Ethanol	Ethyl acetate	Petroleum ether
Aspergillus flavus	06.7±2.20	08.0±2.70	11.0±3.70	09.0±3.00
A. niger	07.3±2.50	12.0±4.00	16.0±5.40	10.3±3.40
A. terreus	-	19.0±6.30	17.7±5.80	06.4±2.10
Penicillium sp	10.0±3.30	17.7±5.80	22.3±7.50	13.7±4.70
P. chrysogenum	06.7±2.30	18.7±6.20	16.3±5.40	14.7±4.80
Rhizoctomiasolani	07.3±2.50	08.4±2.70	08.4±2.70	-
Trichoderma sp	06.7±2.20	11.3±3.80	10.3±3.50	10±3.40

Mean ± Standard error

According to the test plant *P. reticulatus* leaf with ethyl acetate solvent was highly responsible than the aqueous, ethanol, and petroleum ether extract. As per the antibacterial activity of *P. reticulatus leaf* potentiality with ethyl acetate against bacteria *E. coli*, *Enterococcus* sp, *K. pneumoniae*, *Proteus* sp, *S. aureus*, *Salmonella* sp, and *Vibrio cholera* 20.0±6.70, 17.0±5.70, 18.3±6.00, 18.3±6.10, 11.7±3.70, 12.0±4.00 and 15.3± 5.10 mm zone of inhibition recorded respectively. Other solvents like aqueous, ethanol and petroleum ether was moderate activity against bacteria whereas antifungal activity of *P. reticulatus* leaf with ethyl acetate was extraordinary properties against fungi like *Aspergillus flavus*, *A. niger*, *A. terreus*, *Penicillium* sp, *P. chrysogenum*, *Rhizoctomia solani* and *Trichoderma* sp with 11.0±3.70, 16.0±5.40, 17.7±5.80, 22.3±7.50, 16.3±5.40, 08.4±2.70 and 10.3±3.50 mm zone of inhibition recorded respectively.

Though found notable inhibition at least by ethanol extract against *S. aureus* and *P. aeruginosa* this extract also inhibited the growth of other bacteria potentially. On the basis of MIC and MBC value, categorized our studied extracts as bactericidal and bacteriostatic and found, ethanol extract showed bactericidal activity not only against *S. aureus* and *P. aeruginosa* but also against *S. sonnei*, *S. lutea*, *B. megaterium*, *B. subtilis*, *S. aureus*, *B. cereus*, petroleum ether and chloroform extract since petroleum ether extract was bactericidal against *B. cereus* and *S. aureus*. It is important to note that are preliminary in nature that could be a basis for further investigation to identify the active compounds responsible for bacterial growth inhibition. This could dramatically increase antibacterial efficacy than that of the crude extracts. Plants produce different secondary metabolites as a part of their immune system (Bednarek 2012) [4].

This lead to the result that samples only inhibited the activities of two indicators bacteria as *P. aeruginosa* and *S. aureus*. According to the previous study, *P. amarus* a plant related with *Phyllanthus urinaria* when extract with ethanol may inhibit the activity of *Salmonella typhi* (Oluwafemi and Debiri 2008) [12]. Moreover, the *Phyllanthus urinaria* can also inhibit *Pseudomonas aeruginosa*, *Staphylococcus aureus* and another microorganisms *Escherichia coli*, *Bacillus cereus*, *Klebsiella aerogenes*, *Proteus vulgaris*, *Shigellaboydis* when extract of *Phyllanthus urinaria* with acetone or methanol (Daburet. *et al.*, 2007) [5] has found the antimicrobial activities on *S. aureus* and *P. aeruginosa* of the water extract of *P. urinaria*.

Hence, there is a need for new antimicrobial compounds with broad spectrum activity. Which are cheaper and with less toxicity. Household medicinal plant may have some antimicrobial activity against disease.

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