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Research Article

Studies on antimicrobial activity of leaves of *Phyllanthus niruri* Linn.

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Article Info

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Abstract

The present study investigates the phytochemical composition and antibacterial potential of *Annona squamosa* and *Withania somnifera* using methanolic and aqueous extracts. Qualitative phytochemical screening revealed the consistent presence of tannins, saponins, terpenoids, phenolic compounds, flavonoids, and alkaloids in both plant species, regardless of the solvent used. Antibacterial efficacy was assessed against six human pathogenic bacteria (*Staphylococcus aureus*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Escherichia coli*) using the agar well diffusion method. Methanolic extracts of both plants exhibited notably higher zones of inhibition compared to their aqueous counterparts, with *Proteus vulgaris* showing the greatest sensitivity. However, the standard antibiotic streptomycin sulphate demonstrated superior antibacterial activity across all test organisms. The results highlight the potential of *Annona squamosa* and *Withania somnifera* as sources of bioactive compounds with moderate antimicrobial properties, supporting their traditional use in herbal medicine and indicating their relevance for further pharmacological exploration.

Keywords: *Annona squamosa*, *Withania somnifera*, phytochemicals, antibacterial activity, methanol extract, pathogenic bacteria.

Introduction

The antimicrobial activity of the extracts of *Phyllanthus niruri* Linn. was studied against four gram negative and one gram positive bacteria.[9,10] The results showed that the minimum inhibitory concentration (MIC) of *Phyllanthus niruri* Linn. leaf extract was 50 µg/ml against Salmonella typhi and Staphylococcus aureus *Phyllanthus niruri* Linn. on five selected bacterial strains. *Phyllanthus niruri* Linn. is an herb which belongs to family Euphorbiaceae. Infusion of the plant is used in the treatment of jaundice, dysentery, skin diseases and fever. [11]

In particular, the 2022 Global Antimicrobial Resistance and Use Surveillance System (GLASS) study reported that third-generation cephalosporin-resistant *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* had very high rates of resistance and accounted for substantial suffering globally [4]. That report also correlated the high global use of β-lactams/cephalosporins (ceftriaxone and ceftazidime) and fluoroquinolones (ciprofloxacin and levofloxacin) with substantial increases in the incidence of AMR *E. coli* and *Klebsiella pneumoniae* infections [4]. Another study highlighted the development of resistance mechanisms towards vancomycin, daptomycin, ceftaroline, and linezolid in *S. aureus* [5]. Pakbin et al. (2021), reported substantially increased rates of multi-drug-resistant *Shigella* species infections that are now relatively unaffected by conventional antibiotics, including sulfamethoxazole/trimethoprim (83%), amoxicillin (67%), streptomycin (67%), tetracycline (61%), ampicillin (50%), amoxicillin–clavulanic acid (50%), azithromycin (50%), and chloramphenicol (50%) [6].

Unfortunately, the development of new antibiotics is slow, and <25% of current antimicrobials in the development pipeline represent novel mechanisms [7]. Additionally, none of the novel compounds highlighted in that report are active against the WHO critical threat pathogens *Enterococcus faecium, S. aureus, K. pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* species (ESKAPE). A recent report by the WHO showed a rise in antibacterial agents from 80 in 2021 to 93 in 2023, although those therapies lacked novel mechanisms that target critical pathogens. Only two of the thirteen approved antibiotics since 2017 represent a new chemical class [8].

Phyllanthus niruri Linn. belongs to the genus Phyllanthus and the tribe Phyllanthae which is in the family of Euphorbiaceae. The genus contains over 600 species of trees and biennial herbs distributed throughout the tropical and subtropical regions of the World [1]. The plant is indigenous to the rain forests in the Amazon and tropical areas including Bahamas, India, Pakistan and China [1,2]. The plant has several tribal names based on it uses or the arrangement of the flower on the leaves. Chanca piedra is the Spanish name meaning “stone breaker” or shatter stone”, it stems from its use as elimination agent for gall and kidney stones by the indigenous people of Amazon, [3]. P. niruri has several uses in herbal medicine. The plant has been reported to have liver protective antilithic, pain-relieving, hypotensive, antispasmodic, antiviral, anti-fungal, diuretic, antimutagenic, hypoglycemic and anti-bacteria actions [15]. The therapeutic action has been reported in the following pathologies: pimples, eczemas, gangrene, malaria, syphilis, ulcer, urethral secretion, diarrhea, dysentery, dropsy, mouth and throat infection, veneral diseases, hepatic diseases and gastrointestinal disorders [16].

Material and Method

The plant material was collected from Botanical garden of Yeshwant Mahaviyalaya, Nanded, India, by taking permission from principal.

Preparation of plant extract : The plant parts like leaves and fruits were detached and washed with clean water. Materials were air dried on a clean sheet for one week at room temperature.

Preparation of plant extract :

The dried leaves of *Phyllanthus niruri* Linn., were powdered and sieved through a 40-mesh screen. The fine powder was stored in air tight containers and left in the refrigerator. 1% stock solution was prepared with 0.1% dimethyl sulphoxide solution (1gram powder was soaked in 100 ml of 0.1% dimethyl sulphoxide) for one week. The extract was filtered using membrane filter. The extracts obtained were stored in a refrigerator at 4°C until required for use.

Microorganisms :

Pure isolates of E. coli (3 strains), Klebsiella pneumoniae (2 strains), Salmonella paratyphi A (3 strains), S. typhi (4 strains) and S. aureus (3

strains) are pre cultured in nutrient growth overnight in a rotary shaker at 37° c, centrifuged at 10.000 rpm for 5 minutes. The pellet was suspended in double distilled water and cell density was standardized spectrophotometer at 660 nm.

Standardization of inoculum :

Organisms from the semisolid nutrient medium were inoculated into peptone water. After 6 h of inoculation, a loop full of peptone water with inoculum was streaked on Muller Hinton agar to check the purity. About 3 - 5 pure colonies of each organism were inoculated into normal saline and the turbidity was adjusted to the McFarlands scale (150 ×106 cfu/ml).

Preparation of medium :

Muller Hinton Agar was prepared and bottled in a screw capped (universal) container and autoclaved (121°C) for 20min. Then the medium was allowed to equilibrate in a water bath to a constant temperature (50°C).

Preparation of dilutions of antibacterial agents :

Dilutions of antibacterial agents from selected plant parts were prepared from stock solution (1 ml = 10,000 µg). The concentrations used for the study were 50, 100, 200 and 400 µg/ml. Different concentrations of the plant extracts were mixed thoroughly with Muller Hinton Agar. About 20 ml of medium per plate was poured into each Petri plate and was left to solidify. Control plates without antimicrobial agents were also prepared.

Inoculation of the media : The agar surface of the plates containing the antimicrobial agents was inoculated with replicator with sixteen 4 mm stainless steel screws as prolongs (like "Steer's Replicator"). About 16 holes with equal distance were made on the stainless steel plate. With the help of this device, 16 different samples were inoculated at the same time. Inoculated agar plates were incubated at 37°C for 24 h.

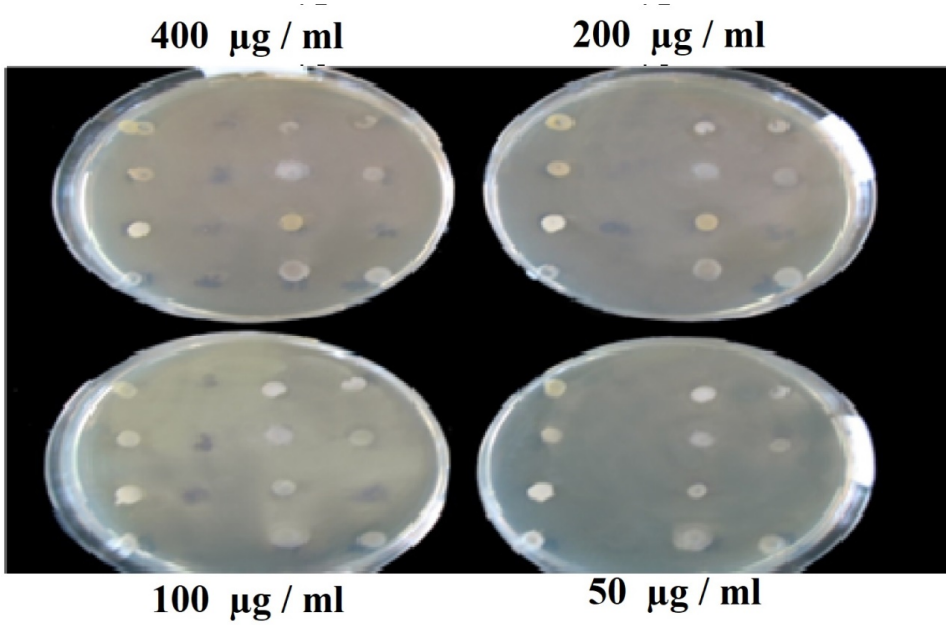
Experimental Results

Phytochemical Analysis : Results of the present investigation were depicted in following table and in Plate. It was noteworthy that the lowest concentration of the leaf extracts of (50 µg/ml) *Phyllanthus niruri* Linn. was found to be very effective in inhibiting the growth of all the selected strains of S. typhi (4 strains) and S. aureus (3 strains), where *Phyllanthus niruri* Linn. has no inhibitory effect on the other 3 bacterial strains of E. coli, K. pneumoniae and S. paratyphi A even at 400 µg/ml.

Plant Name	Part Used	Name Of the organism	No. of (n) isolates	MICs (µg)			
				50	100	200	400
***Phyllanthus niruri* Linn.**	Leaves	E. coli	n = 3	-	-	-	3
		K. pneumoniae	n = 2	-	-	-	2
		S. paratyphi A	n = 3	-	-	-	3
		S. typhi	n = 4	4	-	-	-
		S. aureus	n = 3	3	-	-	-

Table 1: Antimicrobial activity of the plant extract.

Fig1: *Phyllanthus niruri* Linn. showing antimicrobial activity against S. typhi and S. aureus at 50 µg/ml.



The table presents the **Minimum Inhibitory Concentrations (MICs)** of *Phyllanthus niruri Linn.* leaf extract against five bacterial pathogens, based on varying concentrations (50, 100, 200, and 400 µg). The values indicate the number of bacterial isolates (out of total tested) inhibited at each concentration.

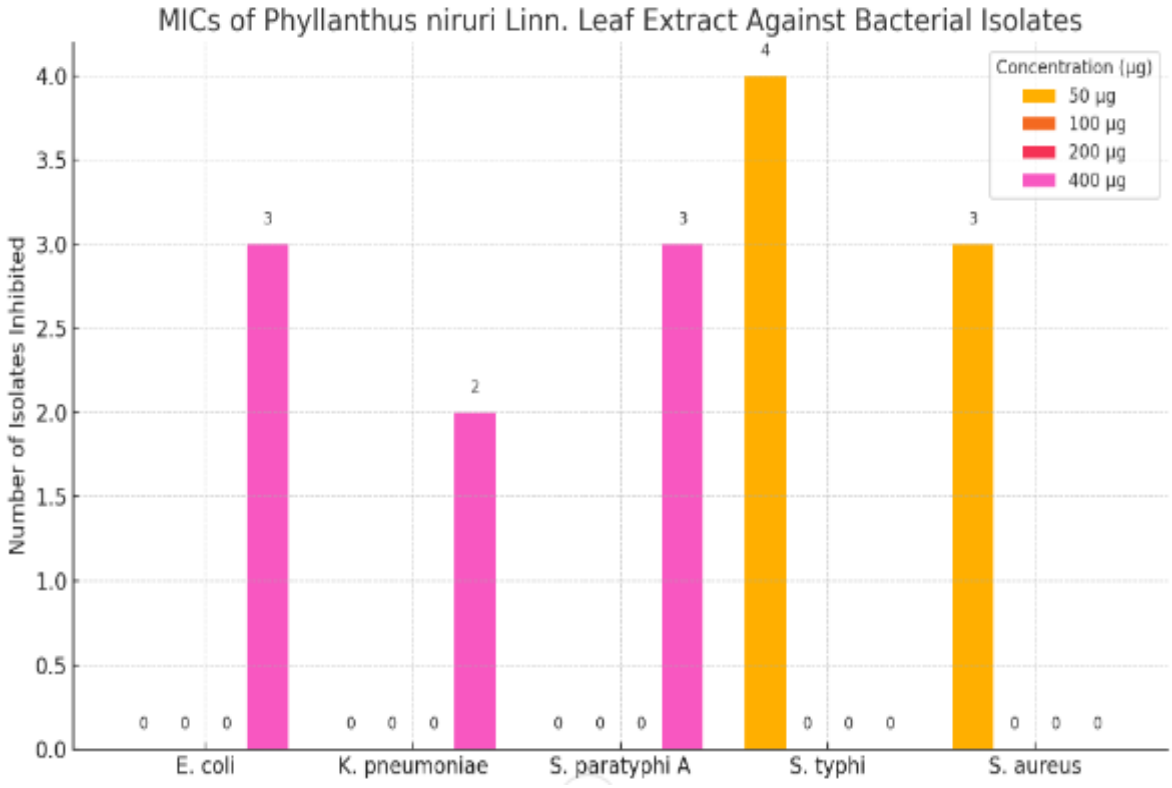


Fig2: bar chart illustrates the number of bacterial isolates inhibited by different concentrations of *Phyllanthus niruri Linn.* leaf extract.

This enhanced bar chart clearly illustrates the number of bacterial isolates inhibited by different concentrations of *Phyllanthus niruri Linn.* leaf extract:

- 50 µg showed effectiveness only against *S. typhi* (4 isolates) and *S. aureus* (3 isolates).
- 400 µg was required to inhibit *E. coli*, *K. pneumoniae*, and *S. paratyphi A.*
- No inhibition was observed at 100 µg and 200 µg for any organisms.

This highlights the extract's selective antibacterial activity, with Gram-positive bacteria showing more sensitivity at lower concentrations.

Findings:

- ***E. coli, K. pneumoniae,* and *S. paratyphi A*** showed no inhibition at 50, 100, or 200 µg. However, all isolates (n = 3, 2, and 3 respectively) were inhibited at **400 µg**, indicating **low sensitivity** to the extract.
- *S. typhi* showed partial sensitivity at **50 µg**, with **4 isolates** inhibited, suggesting **higher susceptibility** at lower concentrations.
- *S. aureus* also showed inhibition at **50 µg** for **3 isolates**, indicating **good antibacterial activity** of the extract at lower concentration.

Conclusion:

Phyllanthus niruri Linn. leaf extract demonstrates **variable antibacterial activity** depending on the microorganism. It is most effective against *S. typhi* and *S. aureus*, showing activity even at low concentrations (50 µg), while higher concentrations (400 µg) are required to inhibit *E. coli*, *K. pneumoniae*, and *S. paratyphi A.* This suggests its **potential as a plant-based antibacterial agent**, particularly against Gram-positive bacteria.

Discussion

The results of the present study revealed that the dimethyl sulphoxide extracts of leaves of *Phyllanthus niruri* Linn. possess appreciable potentiality of inhibiting the growth of all the strains of S. typhi and S. aureus at 50 µg/ml. Results of the present study are in line with the scientific investigations of Ekwenge and Njoku (2006). The aqueous and ethanolic extracts of *Phyllanthus niruri* Linn. showed high inhibition against S. aureus, E. coli and S. typhi. [13]

From the present study, it is evident that the dimethyl sulphoxide leaf extracts of *Phyllanthus niruri* Linn. had profound and highly significant effect on S. typhi and S. aureus at the lowest concentration (50 µg/ml) examined. Thus *Phyllanthus niruri* Linn. may provide a possible cure for typhoid and Staphylococcal diseases. This justifies the need why it is used in folk medicine as curative plant for typhoid fever and as intestinal anesthetic[12,14].

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Conflict of interest: None to declare.

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