



Review Article

Antimicrobial activity of *Phyllanthus niruri* (Chanka piedra)Chandana G¹, Manasa R², Vishwanath S³, Shekhara Naik R¹, Mahesh MS^{1,*}¹Dept. of Food Science and Nutrition, Yuvaraja's College, Mysuru, Karnataka, India²Dept. of Biochemistry, JSS College for Women, Mysuru, Karnataka, India³Dept. of Nutrition and Dietetics, JSS Academy of Higher Education and Research, Mysore, Karnataka, India

ARTICLE INFO

Article history:

Received 26-11-2020

Accepted 02-01-2021

Available online 06-02-2021

Keywords:

Phyllanthus niruri

Chanka piedra

Antimicrobial

Antiviral

Antibacterial

Herb

ABSTRACT

Phyllanthus niruri Linnaeus (Euphorbiaceae) also known as Chanka piedra is widely grown and used throughout the tropical and subtropical countries of the world. In India it is present in the coastal areas. It is an annual herb and field weed having very short life. *Phyllanthus* comprises of 600-700 species with minor distinguishing features among them. In Indian ayurvedic system, plant extract of *P. niruri* is used as a medicine for asthma, bronchitis, anemia, leprosy, etc., As mentioned in the book Charaka Samhita *P. niruri* is used as an effective treatment for stimulating liver, improving digestion, to increase appetite and produce laxative effects. *Phyllanthus niruri* is a traditional herb with long-standing Ayurvedic, Chinese and Malay ethnomedical records and antimicrobial activity. Antimicrobial activity refers to the process of killing or inhibiting the disease causing microbes. Methanolic extract of *P. niruri* is an effective antibacterial agent to treat bacterial infections since the extract exhibited significant antimicrobial potency. The alcoholic extract of leaves of *Phyllanthus niruri* shows significant antibacterial activity against cariogenic organisms. The anti microbial activity of aqueous extract is found to be more effective than the acetone extract of *Phyllanthus niruri* against pathogens responsible for common infections of skin, respiratory, urinary and gastro-intestinal tracts.

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1. Introduction

With the increase in antibiotic resistance rates and the need for novel antibiotics, which have optimal antimicrobial activity with minimal toxicity, there is renewed interest in exploring phytochemicals from everyday plants. A possible reason for the increased interest in extracting phytochemicals for the development of novel antibiotics is the threat of plant species extinction, hence inciting a need to explore the medicinal potential of these resources before they are lost.¹ These include rutin,^{2,3} gallic acid,⁴ prenylated flavanone glycosides⁵, quercetin⁶, quercitrin,⁷ p-Cymene,⁸ corilagin,⁹ diosgenin,¹⁰ securinine¹¹ and - glucogallin.¹²

P. niruri is rich in several compounds which have antioxidant, anti-protozoal, anti-viral and anti-microbial activity¹³. Aqueous, alcoholic, hydro-alcoholic and methanolic extracts of *P. niruri* has exhibited wide range of anti-microbial activity against *Bacillus pumillus*, *Bacillus cereus*, *E. coli*, *Vibrio cholera*, *Lactobacillus acidophilus* (*L. acidophilus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), *Coney lunata* and *Salmonella typhi*, *Listeria monocytogenes* *Helicobacter pylori* (*H. pylori*) and *Mycoplasma galisepticum*.

1.1. Botanical classification: *Phyllanthus niruri* L.

Kingdom – Plantae

Division – Magnoliophyta

Class – Magnoliopsida

Order – Euphorbiales

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Family – Euphorbiaceae
 Genus – Phyllanthus
 Species – Niruri

1.2. Vernacular names in India

Assamese: Holpholi; Poram-lokhi

Bengali: Noar

Hindi: Chalmeri, Harfarauri, Bhuiaonla.

Kannada: Kirunelli, Nela Nelli,

Konkani: Bhuin-avalae

Telugu: Ratsavusirike, Nela Usiri,

Tamil: Arunelli, Keela Nelli,

Malayalam: Arinelli, Kizhanelli, Nellipuli

Marathi: Rayavali, Bhuivali,

Oriya: Narakoli

Sanskrit: Amala, Bhumymlaki, Sukshmadala, Vitunika,

Bhoodatri

The extracts of *P. niruri* was tested against food borne & spoilage microorganisms. The growth of microorganisms was inhibited by the ethanolic extracts of *P. niruri*.¹⁴ This extract is strong against *Bacillus pumillus*, *Bacillus cereaus*, *E. coli* and *Vibrio cholera* at conc. of 750 µg/ml/disc. The potency was increased by 49 % when the extract was fermented with lactobacillus.¹⁵ It is also tested against the standard drug chloramphenicol at concentration of 10 µg/ml/disc shows potential source of antimicrobial agent.¹⁶

1.2.1. In vivo

The extract of alkaloids was tested on rabbits infected with *E.coli*. The results examined were found to have increased concentration of WBC, neutrophils and decreased hemoglobin and lymphocytes but no changes in enzyme concentration¹⁷.

1.2.2. In vitro

P. niruri can be used as a substitute of antibiotics in the treatment of Chronic Respiratory Disease (CRD) in broiler chickens caused by *Mycoplasma galisepticum*. A 30% plant extract caused up to 65% growth inhibition in *Mycoplasma galisepticum*.¹⁸

In a similar study conducted by Ramandeep and colleagues, they found that *P. niruri* extracts inhibits the growth of *Escherichia coli* (*E. coli*), *Lactobacillus acidophilus* (*L. acidophilus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*). Hydroalcoholic extracts of *P. niruri* L was found to inhibit urease activity. Quercetin is one of the major constituents which is thought to be the cause of noncompetitive urease inhibition.¹⁹

Another study used silver nanoparticles (AgNPs) obtained from a supercritical CO₂ extract of *P. niruri* to test the anti-bacterial potential against various strains like *Coney lunata* and *Salmonella typhi*.^{20,21}

These studies utilize different types of *P. niruri* extracts, with one study a comparison of methanolic, ethanolic and aqueous extracts, recognizing that different preparations yielded different compositions of pharmacophores.²² An agar well diffusion study conducted on the antimicrobial activity of aqueous and ethanolic extracts of the leaves and roots of 4 Indian herbs, including *P. niruri*, showed that the ethanolic extract was more effective against *Escherichia coli* and *Staphylococcus aureus*, whereas the aqueous preparation had greater activity against *Proteus vulgaris* and *Bacillus subtilis* but poor anticoliform activity.²³

The methanol extracts of *P. niruri* is twice strong as that of aqueous preparation. In addition, both aqueous and methanolic extracts of *P. niruri* demonstrated significant activity against *Listeria monocytogenes*, the bacteria responsible for listeriosis, suggesting the potential of *P. niruri* as a food preservative.²⁴ A subsequent disc diffusion study found that both ethanolic and aqueous extract of *P. niruri* failed to inhibit the growth of the Gram-negative bacilli but demonstrated statistically more significant inhibitory activity against Gram-positive bacteria.²⁵ The apparent difference in results between this study and that of Cheah and colleagues in 2011²⁴ could be due to the use of varying solvents.

This could suggest that the aqueous extracts contained a higher content of phenolic compounds compared with the ethanolic extract.²⁶ Agar diffusion assays in a study on *Helicobacter pylori* and three species of probiotic *Lactobacilli* revealed that *P. niruri* inhibited *H. pylori* in a dose-dependent manner while it did not affect the growth of lactic acid bacteria.²⁷

The anti- *H. pylori* property of *P. niruri* did not involve the inhibition of proline dehydrogenase, a membrane-associated protein linked with prokaryotic energy production.²⁸ This may be due to the presence of ellagitannins such as geraniin and corilagin contained in the aqueous extract, which have also been to act in a concentration-dependent manner against various antibiotic-resistant *H. pylori* strains²⁹ by rapidly precipitating agglutination of *H. pylori* cells.³⁰

In general, methanolic extracts are more potent against Gram-positive microbes, followed by aqueous extracts and ethanolic extracts. The antibacterial activity of *P. niruri* is also dose dependent. However, the compatibility of methanolic extracts to the mammalian subject may need to be investigated using animal models, as methanol, a polar organic solvent, may be disruptive of cellular phospholipid membranes. Although there is noticeably poor Gram-negative activity, there is still a need for large-scale molecular studies to investigate the relationship between the morphology of Gram-negative bacteria and the lack of Gram-negative activity in *P. niruri* extracts.

The in vitro anti-hepatitis B viral activity of *P. niruri* L. in Hep G2/C3A and SK-HEP-1 cells was studied by Li et

al.³¹ The ethanol fractions were analyzed and reported to be enriched with ellagic acid fractions which successfully inhibited the growth of HBV-infected HepG2 / C3A cells compared to the isolated active compound that showed a half-maximal inhibitory concentration (IC₅₀) of 120 µg/mL and had no effect on HBV DNA replication at the concentrations evaluated, hence failing to inhibit the reproduction of HBV.³¹ Hepatitis B virus claims around a million human lives annually. Sarma and colleagues attempted to explore a potent and efficient antiviral from *Phyllanthus* with a minimal risk of resistance for hepatitis B virus.³² Moreover, in this attempt the *Phyllanthus* active principles from among 93 phytochemicals were isolated to check the mechanism of action against hepatitis B virus reverse transcriptase (HBV RT), which is an active target for drugs used against HBV infections.³²

P. niruri is used as a home remedy for many diseases in Asia.³³ It is used to treat jaundice and to inhibit the hepatitis virus³⁴ and duck hepatitis B virus by inhibiting 50 % of DNA polymerase.³⁵ Wood chuck hepatitis virus (WHV) was tested against the extract in wood chucks (*Marmota monax*), it efficiently inhibited the wood chuck hepatitis virus (WHV) and it eliminated of both surface antigen and DNA polymerase activity.³⁶

Most prominent among the potential therapeutic effects of *P. niruri* is its antiviral activity. Studies conducted on sera obtained from chronic hepatitis B patients and woodchuck hepatitis (WHV)-infected woodchucks, which were treated with *P. niruri* extracts, showed decreased viral antigen levels.³⁷ Overall, aqueous extracts of *P. niruri* have been shown to possess significant antiviral potential and appear promising especially with regard to hepatitis B carriers.³⁶

Although not all the bioagents responsible for the antihepatitis B activity of *P. niruri* have been identified, molecular studies have determined the molecular structure of a novel lignin found in *P. niruri*, nirtetralin B and its two stereoisomers, nirtetralin and nirtetralin A.

1.3. Nirtetralin

Nirtetralin significantly inhibited HBsAg and HBeAg levels in vitro.³⁸ All three lignans had a dose-dependent inhibitory effect on the in-vitro titres of HBV antigens. Moreover, when compared with acyclovir, inhibition ratios for nirtetralin and nirtetralin B were significant suggesting that these compounds are promising novel anti-HBV antivirals. These lignans had low cytotoxicity on host cells, suggesting that these compounds could safely be given at non-toxic dosages without incurring undesirable adverse drug reactions.^{39,40} Although, no systematic reviews have been conducted on the anti-hepatitis B activity of *P. niruri*, there have been a number of reviews since 2000, on the utility of *Phyllanthus* genus as potential antiviral agents for chronic hepatitis B infection.^{41–43}

Despite the promising results with respect to the inhibition of HIV-1RT activity, repandusinic acid seemed to exert less significant inhibition of DNA polymerase alpha.⁴⁴ With regard to the resultant degree of cytopathogenicity, repandusinic acid reduced the number of pathogenic changes in HIV infected MT4 cells, and the results even suggested that repandusinic acid may be more potent than AZT in inhibiting HIV cytopathogenicity. Moreover, azidothymidine and repandusinic acid may work in synergy when administered as a combination. However, the action of repandusinic acid has only been studied at the cellular level, and no animal or human studies on the anti-HIV therapeutic effects of repandusinic acid have been carried out.

A study conducted by Ogata et al., noted that tannins of *P. niruri* such as repandusinic acid, as another novel HIV-1-RT inhibitor were important antiviral agents in HIV therapy.⁴⁴

This significant toxic selectivity for virus infected cells was replicated in a subsequent study on alkaloidal extracts of *P. niruri*⁴⁵ with a greater preference for HIV-2-infected cell lines. Alkaloid extract of *P. niruri* also has an inhibitory effect on HIV infection.

Qian-Cutrone and colleagues isolated a glucopyranoside, niruriside, which was found to inhibit REV/RRE binding during the movement of viral RNA from the cell nucleus to the cytoplasm. However, despite being found to be a specific REV/RRE inhibitor, niruriside did not display satisfactory levels of cellular protection in cases of acute HIV-1 infection.⁴⁶

A study exploring the antidengue activity of members of the genus *Phyllanthus* showed that *Phyllanthus* extracts worked best when administered simultaneously with DENV-2 inoculum implying that the *Phyllanthus* extract most probably affected the early phases of viral infection such as the viral attachment and entry.⁴⁷ Proteome analysis showed that the expression of 13 host and viral proteins involved in viral entry and replication, molecular chaperoning, cytoskeletal assembly and cellular metabolisms was altered, including calreticulin, Trim 1, heat-shock 70-kDA protein, beta-actin, DNA topoisomerase I, NS3, G3PD (glyceraldehyde-3-phosphate dehydrogenase), RBM1 (RNA-binding motif 1), DNA mismatch repair protein Msh2, dengue virus NS2bNS3 and polysialyl transferase.⁴⁷

Apart from that, *P. niruri*-synthesized silver nanoparticles demonstrated significant larvicidal, pupicidal and adulticidal activity against *Aedes aegypti* both in laboratory and field settings.⁴⁸ Malaria is one of the most prominent health problems in the tropical and subtropical countries. The herbal plants show antagonistic properties against malaria. *P. niruri* and *Mimosa pudica* showed antiplasmodial activity, when feed with ethanol extracts in albino mice.⁴⁹ *P. niruri*'s ethanolic extract of one month old in vitro grown callus showed higher antiplasmodial activity than extract prepared from fresh apical stem extract.⁵⁰

Table 1: Antimicrobial activity of *Phyllanthus niruri*

Extract	Effect	Part used	References
Ethanolic	Inhibit the growth of microorganism	Plant	14
Alkaloids	Increase concentration of WBC, neutrophils and decreased hemoglobin, lymphocytes	Plant	17
Alkaloids	65% growth inhibition in mycoplasma galisepticum	30% plant	18
Hydro alcoholic	Noncompetitive urease inhibition	Plant	19
Ethanolic	Effective against E.coli, staphylococcus	Root, leaves	23
Ethanolic and aqueous	Inhibitory activity against gram positive	Plant	25
Ethanolic	Inhibited the growth of HBV – infected HepG2/C3A cells	Plant	31
Alkaloid	Inhibitory effect on HIV -1 replication	Plant	46

**Fig. 1:** Antimicrobial activity of *Phyllanthus niruri*

2. Conclusion

Phyllanthus niruri belongs to a family Euphorbiaceae and it has many health benefits, one of the most important health benefits is antimicrobial activity. The anti-microbial activity has been studied in in vitro, in vivo and humans. In in vivo experiments on the rabbit it was found increased concentration of WBC, neutrophils and decreased hemoglobin, lymphocytes infected with an E.coli but there are no changes in enzyme concentration while given *Phyllanthus niruri* as a dose to overcome the antimicrobial activity in rabbit. In in vitro condition lactobacillus bacteria of curd as taken and studied even there, they have found the microbial activity has been reduced by incorporating the *Phyllanthus niruri*. Clinical studies on hepatitis B patients showed that 50–60% of patients who were administered *P. niruri* extract experienced HBsAg seroconversion. The reduction in HBsAg antigen may have been due to the inhibitory effect of *P. niruri* on hepatitis B viral genetic

replication of note, in a study where patients were treated with extracts of three different members of the genus *Phyllanthus*, it was observed that extracts of *P. niruri* were more likely to induce reductions in HBeAg titers. Although not all the bioagents responsible for the antihepatitis B activity of *P. niruri* have been identified, molecular studies have determined the molecular structure of a novel lignin found in *P. niruri*, nirtetralin B and its two stereoisomers, nirtetralin and nirtetralin A are responsible.

3. Conflicts of Interest

All contributing authors declare no conflicts of interest.

4. Source of Funding

None.

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Cite this article: Chandana G, Manasa R, Vishwanath S, Naik R S, Mahesh MS. Antimicrobial activity of *Phyllanthus niruri* (Chanka piedra). *IP J Nutr Metab Health Sci* 2020;3(4):103-108.