



“To study the Antimicrobial activity of *Phyllanthus amarus* extract for the treatment of bacterial infections”

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Abstract

In India it is present in the coastal areas. It is an annual herb and field weed having very short life. *Phyllanthus amarus* 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. *Phyllanthus* comprises of 600-700 species with minor distinguishing features among them. In Indian ayurvedic system, plant extract of *P. amarus* is used as a medicine for asthma, bronchitis, anemia, leprosy, etc., As mentioned in the book Charaka Samhita *P. amarus* is used as an effective treatment for stimulating liver, improving digestion, to increase appetite and produce laxative effects. Methanolic extract of *P. amarus* is an effective antibacterial agent to treat bacterial infections since the extract exhibited significant antimicrobial potency. The alcoholic extract of leaves of *Phyllanthus amarus* 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 amarus* against pathogens responsible for common infections of skin, respiratory, urinary and gastro-intestinal tracts.

Keywords: *Phyllanthus amarus* , Antimicrobial Antiviral, Antibacterial, Herb.

1. Introduction

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 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. 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. amarus* 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. amarus* 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*.

Botanical classification: *Phyllanthus amarus* L.

Kingdom – Plantae

Division – Magnoliophyta

Class – Magnoliopsida

Order – Euphorbiales

Family – Euphorbiaceae

Genus – *Phyllanthus*

Species – *Amarus*

The extracts of *P. amarus* was tested against food borne & spoilage microorganisms. The growth of microorganisms was inhibited by the ethanolic extracts of *P. amarus*.¹⁴ This extract is strong against *Bacillus pumillus*, *Bacillus cereus*, *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

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.

In vitro

A 30% plant extract caused up to 65% growth inhibition in *Mycoplasma galisepticum*.¹⁸ *P. amarus* can be used as a substitute of antibiotics in the treatment of Chronic Respiratory Disease (CRD) in broiler chickens caused by *Mycoplasma galisepticum*. In a similar study conducted by Ramandeep and colleagues, they found that *P. amarus* extracts inhibit the growth of *Escherichia coli* (*E. coli*), *Lactobacillus acidophilus* (*L. acidophilus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*). Hydroalcoholic extracts of *P. amarus* 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. amarus* to test the anti-bacterial potential against various strains like *Coney lunata* and *Salmonella typhi*.^{20,21} These studies utilize different types of *P. amarus* 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. amarus*, 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. amarus* is twice as strong as that of aqueous preparation. In addition, both aqueous and methanolic extracts of *P. amarus* demonstrated significant activity against *Listeria monocytogenes*, the bacteria responsible for listeriosis, suggesting the potential of *P. amarus* as a food preservative.²⁴ A subsequent disc diffusion study found that both ethanolic and aqueous extract of *P. amarus* 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. amarus* 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. amarus* 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. amarus* 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. amarus* extracts.

The in vitro anti-hepatitis B viral activity of *P. amarus* 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. amarus* is used as a home remedy for many diseases in Asia.³³ It is used to treat jaundice and to inhibit the hepatitis B 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 both surface antigen and DNA polymerase activity.³⁶

Most prominent among the potential therapeutic effects of *P. amarus* 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. amarus* extracts, showed decreased viral antigen levels.³⁷

Overall, aqueous extracts of *P. amarus* 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 anti-hepatitis B activity of *P. amarus* have been identified, molecular studies have determined the molecular structure of a novel lignin found in *P. amarus*, nirtetralin B and its two stereoisomers, nirtetralin and nirtetralin A.

Despite the promising results with respect to the inhibition of HIV-1 RT 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. amarus* 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. amarus*⁴⁵ with a greater preference for HIV-2-infected cell lines. Alkaloid extract of *P. amarus* also has an inhibitory effect on HIV infection.

Qian-Cutrone and colleagues isolated a glucopyranoside, *amarusside*, 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, *amarusside* 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. amarus*-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. amarus* and *Mimosa pudica* showed antiplasmodial activity, when feed with ethanol extracts in albino mice.⁴⁹ *P. amarus*'s ethanolic extract of one month old in vitro grown callus showed higher antiplasmodial activity than extract prepared from fresh apical stem extract.⁵⁰

Conclusion

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 amarus* 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 amarus*. Clinical studies on hepatitis B patients showed that 50–60% of patients who were administered *P. amarus* extract experienced HBsAg seroconversion. The reduction in HBsAg antigen may have been due to the inhibitory effect of *P. amarus* 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. amarus* were more likely to induce reductions in HBeAg titers. Although not all the bioagents responsible for the antihepatitis B activity of *P. amarus* have been identified, molecular studies have determined the molecular structure of a novel lignin found in *P. amarus*, nirtetralin B and its two stereoisomers, nirtetralin and nirtetralin A are responsible.

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