



## ANTIBACTERIAL ACTIVITY OF BHUI AMLA (*PHYLLANTHUS NIRURI*)

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### ABSTRACT

Present study was conducted with the objective of evaluating the antibacterial activity of *Phyllanthus niruri* (Bhui amla) plant. Antibacterial effect was observed against Coagulase-positive *Staphylococcus aureus* (CoPS), *Escherichia coli*, *Pseudomonas* and *Listeria* species of bacteria. Hot infusion of fresh and dried plant and cold ethanolic extract from dried plant were observed for antibacterial activity by in vitro disc diffusion method using sterile discs of 6 mm diameter. The standard disc of Enrofloxacin (10 µg) were used as positive control. All the disc prior to the experiment were incubated at 37°C for 24 hours. Following incubation zone of inhibition was measured to the nearest millimeter. Results revealed that plant has antibacterial activity against the Coagulase-positive *Staphylococcus aureus* (CoPS) and *Escherichia coli*. Cold ethanolic extract of the plant showed antibacterial activity against Coagulase-positive *Staphylococcus aureus* with the zone of inhibition of 14 mm, 16 mm and 18 mm and with *E. coli* 11 mm and 13 mm zone of inhibition was revealed at concentration of 800 and 850 µg/disc respectively. The hot infusion of fresh plant showed mild antibacterial activity with 10, 13, 15 mm zone of inhibition with *Staphylococcus aureus* where as with *Escherichia coli* zone of inhibition of 12 mm was seen only at the concentration of 850 µg. Hot infusion of the dried plant did not show any antibacterial activity and also no appreciable zone of inhibition. None of the preparations of the plant showed antibacterial activity against, *Pseudomonas* and *Listeria*.

**KEYWORDS:** Antibacterial, *phyllanthus niruri*, Bhumi amla.

### INTRODUCTION

*Phyllanthus niruri* is an annual herb which usually grows in the humid areas. The *Phyllanthus* genus contains over 600 species of shrubs, trees, and annual or biennial herbs distributed throughout the tropical and subtropical regions of both hemispheres. The Spanish name of the plant, *chanca piedra*, means “stone breaker” or “shatter stone.” It was named for its effective use to generations of Amazonian indigenous peoples in eliminating gallstones and kidney stones. The natural remedy is usually just a standard infusion or weak decoction of the whole plant or its aerial parts. Its main uses are for many types of biliary and urinary conditions including kidney and gallbladder stones; for hepatitis, cold, flu, tuberculosis, and other viral infections; liver diseases and disorders including anemia, jaundice and liver cancer; and for bacterial infections such as cystitis, prostatitis, venereal diseases and urinary tract infections diuretic,

analgesic, stomachic, antispasmodic, febrifugal, and cell protective properties in many other conditions. The entire plant extracts of *Phyllanthus* was tested for its antibacterial effect on *E. coli*, *S. aureus* and *Salmonella typhi* [2]. Alkaloids, astragalin, brevifolin, carboxylic acids, corilagin, cymene, ellagic acid ellagitannins, gallocatechins, geraniin, hypophyllanthin, lignans, lintetralins, lupeols, methyl salicylate, niranthin, nirtetralin, niruretin, nirurin, nirurine, nirurisode, norsecurinines, phyllanthin, phyllanthine, phyllanthenol, phyllochrysin, phyltetralin, repandusinic acids, quercetin, quercetol, quercitrin, rutin, saponins, triacontanol, tricontanol are some of the phytochemicals found to be present in the plant [6]. Antihyperglycemic activity of methanol extract of *Phyllanthus niruri* (MEPN) in Streptozotocin (STZ) induced diabetic male Wistar rats study was undertaken for antioxidant potential of MEPN in STZ-induced diabetic rats. The decreased levels of thiobarbituric acid reactive substances (TBARS) and increased levels of reduced glutathione (GSH), and the activities of superoxide dismutase (SOD) and Catalase (CAT) resulted in reducing the free radical formation in various tissues such as liver, kidney, heart and brain of the diabetic rats. Results indicate that the methanol extract of *Phyllanthus niruri* (MEPN) exhibits significant antihyperglycemic and *in vivo* antioxidant activity in STZ-induced diabetic [17]. Its curative effect is also reported against disorders like anemia, jaundice and cystitis, prostatitis, venereal diseases and urinary tract infections caused by bacterial infections [3]. Uzoma reported diuretic, analgesic, stomachic, antispasmodic and hepatoprotective properties [18]. It was also found to be highly effective against intestinal problems [12]. One *in vitro* study and four *in vivo* studies (with rats and mice) document that extracts of chanca piedra effectively protect against liver damage from various chemical liver toxins [9,10,15,16]. *Phyllanthus* has also been used in the traditional system of Ayurveda for the treatment of wounds, jaundice, ulcers, diabetes, in the form of poultice in swelling and itching. The shoots of the plant is used as an infusion in chronic dysentery [1].

## MATERIAL AND METHODS

1. Collection of plant material - *Phyllanthus niruri* plants were collected from the campus of college of veterinary science and animal husbandry Rewa (M.P.) and were authenticated for their micro and macro characteristics as described in the literature.
2. Preparation of Extract - The whole plant collected were thoroughly washed in running tap water and distilled water and were air dried in shade at room temperature for 3-4 days. The dried plant were then churned in the sterile mixture to prepare dry powder and stored in the air tight container.

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Hot infusion was prepared from fresh plant as well as dry powder as per the conventional methods. Prepared infusion was filtered with the sieve and later with the sterile filter of 0.22 micron to remove residues. It was dried and crude extract obtained was kept in the refrigerator till further use to avoid loss of any active principle.

In the same way cold extract was prepared with dried powder with the conventional methods later filtered with a sieve and 0.22 $\mu$  filter to remove residues completely later dried. The crude extract was obtained and then transferred in an autoclaved air tight container. Both the hot infusion and cold extract was stored in the refrigerator.

3. Evaluation of Antibacterial activity – Activity of plant extract was evaluated by the Kirby-Bauer disc diffusion method<sup>[5]</sup> using sterile blank disc of 6 mm diameter. Disc were sterilized in the autoclave at 121<sup>0</sup>c for 60 minutes, dipped in the plant extracts (infusion and ethanolic extract) allowed to dry and then used as extract loaded antimicrobial discs. Disc containing 700, 800 and 850  $\mu$ g of different extract were used to study the antimicrobial activity against Coagulase-positive *Staphylococcus aureus* (CoPS) and *Escherichia coli* obtained from the department of microbiology of the college. Disc containing three different concentration of the extract were placed at the even distance on nutrient agar plates. Standard disc of Enrofloxacin 10 $\mu$ g/disc were used as the positive control. All the loaded petri plates were incubated at 37<sup>o</sup>c for 24 hours in an incubator. Following incubation the zone of inhibition was measured to the nearest millimeter using a ruler including the diameter of disks from the back of petri plates and expressed in mm.

## RESULTS AND DISCUSSION

In vitro antibacterial assay of the cold ethanolic extract of Bhui amla plant showed concentration dependent antibacterial activity against Coagulase-positive *Staphylococcus aureus*. The maximum zone of inhibition was observed at 850 $\mu$ g per disc against Coagulase-positive *Staphylococcus aureus*. Hot infusion showed mild antibacterial activity against Coagulase-positive *Staphylococcus aureus* and *E.coli* at 850  $\mu$ g concentration where as none of the form of extract revealed antibacterial activity against *Pseudomonas* and *Listeria* organism. However hot infusion at the concentration of 850  $\mu$ g revealed 15 mm zone of inhibition depicting good antibacterial activity against *Staphylococcus aureus*. Cold ethanolic extract of Bhui amla plant exhibited good antibacterial activity against *Staphylococcus aureus* as well as *E.coli* bacteria. The active phytochemicals, flavonoids, alkaloids, terpenoids, lignans, polyphenols, tannins, coumarins and saponins, have been identified from various parts of *P. niruri* which may be responsible for the antibacterial and various other activities of the plant.

In most West African nations the leaves of *Phyllanthus amarus* are made into a paste and licked to expel intestinal worms. The decoction of the plant is also used as a purgative. Topically, this plant has soothing effect on the skin and is used for genital and anal infections. Poultice is used for the treatment of certain types of ulcers or sores. The infusion of the root and leaf is a very good tonic and causes diuresis when taken in repeated doses. According to <sup>[14]</sup> administration of *P. amarus* extract is non-toxic with no signs of toxicity or mortality in experimental rats.

**Table -1**

Type of Extract	Microorganism tested	Zone of Inhibition	Result
<b>Tincture</b>	<i>Staphylococcus aureus</i>	<b>14 mm</b>	<b>Active</b>
	<i>Escherichia coli,</i> <i>Pseudomonas spp</i>	-	<b>No activity</b>
	<i>Listeria spp</i>	<b>10 mm</b>	<b>Weakly active</b>
		-	<b>No activity</b>
<b>Hot Infusion Fresh plant</b>	<i>Staphylococcus aureus</i>	<b>9 mm</b>	<b>Mild active</b>
	<i>Escherichia coli,</i> <i>Pseudomonas spp</i>	-	<b>No activity</b>
	<i>Listeria spp</i>	-	<b>No activity</b>
		-	<b>No activity</b>
<b>Hot Infusion Dried plant</b>	<i>Staphylococcus aureus</i>	-	<b>No activity</b>
	<i>Escherichia coli,</i> <i>Pseudomonas spp</i>	-	<b>No activity</b>
	<i>Listeria spp</i>	-	<b>No activity</b>
		-	<b>No activity</b>

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Reported that *Phyllanthus niruri* plant showed antibacterial activity against common pathogens of man like *E.coli*, *Salmonella* species. *P. niruri* 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), whereas, *P. niruri* has no inhibitory effect on the other 3 bacterial strains of *E. coli*, *K. pneumoniae* and *S. paratyphi A* even at 400 µg/ml [11,19]

Tannin can be toxic to bacteria, filamentous fungi and yeast [4] A poultice of the leaves with salt cures scabies infection and without salt is applied on bruise and wounds [8] In the study conducted by Oliver and it was found that, the crude extract inhibit the growth of *E. coli*, *Streptococcus* and *S. aureus*. However its effects were low when compared with standard antimicrobial agents such as ofloxacin and ciprofloxacin which was used as positive controls and the results revealed that the alcoholic extracts were more effective than the aqueous extract in inhibiting the growth of the test microbes Results obtained from the present study also revealed the antibacterial activity of *Phyllanthus niruri* against coagulase positive *Staphylococcus aureus* (CoPS) and *Escherichia coli* Higher sensitivity to gram positive organism could be attributed to their outer peptidoglycan layer which is not an effective permeability barrier [7] while the gram negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharides components make the cell wall impermeable to lipids. Antibacterial activity of ethanolic extract was found to be better than hot infusion .Activity against *E coli* was not so prominent at low concentration. This difference in the antibacterial activity may be due to some active principles lost in heating. Therefore it is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [13]. In one of the study conducted for the antibacterial activity the crude extract was observed to inhibit the growth of *E. coli*, *Streptococcus* and *S. aureus*. However its effects were low when compared with standard antimicrobial agents such as ofloxacin and ciprofloxacin which was used in the study as positive controls, However, the results revealed that the alcoholic extracts were more effective than the aqueous extract in inhibiting the growth of the test microbes. Correlating with the various work undertaken previously still further studies is to be conducted using different fraction of these extract including isolation and chemical characterization of the active principles of the plant.

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