International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Phytochemical Screening and Antimicrobial Activity on *Phyllanthus niruri*on Hydroethanolic Extract

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Abstract: ***The study is based on the antimicrobial effect on Phyllanthus niruri leaves using hydroethanolic extract at the concentrations 100µl and 200µl.8µl of chloramphenicol was used as control. Pure isolates of Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Shigella were employed as test organisms. Klebsiella pneumoniae was the most susceptible bacterium as it shows the maximum zone of inhibition whereasE.coli is the least susceptible bacterium. The present investigation also aimed to focus on the screening of phytochemical constituents. This phytochemical screening revealed the presence of phenol, saponin, and alkaloids in the extracts.***

Keywords: Antimicrobial, Phytochemicals, *Phyllanthus niruri*

1. Introduction

Phyllanthus niruri belongs to the family of Euphorbiaceae. Based on its uses and the arrangements of the flowers on the leaves the plant has several tribal names. “Chancapiedra” is the Spanish name meaning “stone breaker” or “chatter stone”, it is used as elimination agent for gall and kidney stones(Balle and William,1994).*Phyllanthus niruri* is known for protecting the liver. The plant has several protective properties such as antiviral, antifungal, antibacterial, pain relieving, diuretic, hypoglycamic(Barros *et al.,2003*). It acts as therapeutic agent against veneral disease, hepatic disease, mouth and throat infection, ulcer, syphilis, gastrointestinaldisorders, diarrhoea (Tona*et al.,2004*).

Phytochemical screening is the important steps to find out the chemical constituents which is used for the isolation of bioactive compounds. These compounds from plants inhibit bacterial growth by different mechanisms and they may have clinical value in the treatment of resistant microbial strains. In the present investigation hydroethanalic extract of plant leaves of *Phyllanthus niruri* has been studied for their antimicrobial efficiency.

Figure 1: *Phyllanthus niruri* plant and plant powder

2. Materials and Methods

Collection of Plant
The fresh leaves of *Phyllanthus niruri* were collected from outskirts of Dharapuram.

Preparation of plant powder
The leaves of *phyllanthus niruri* were cleaned and dried completely under shade and then ground into coarse powder with an electric grinder.

Solvent Extraction
The whole plant materials were air dried untill all the water molecules evaporated and plants become well dried for grinding. Using mechanical blender the plant material was well grinded into fine powder and then transferred into air tight containers with proper labeling for future use. Hydroethanol extract from the leaf sample was collected by using soxhlet apparatus by repeating for around 10 cycles.Further the extracts were cooled,weighed and tested for its antimicrobial activity at two different concentrations 100µl,200µl.

Phytochemical screening

1. Test for Alkaloids
In a clean dry test tube 2ml of extract was taken and added with 3-5 drops of Wagner’s reagent. The formation of reddish brown colour indicates the presence of alkaloids.

2. Test for Amino acids
A few drops of ninhydrin reagent were added in 2ml of the extract. A violet or purple colour was developed if amino acids were present. No colouration indicates the absence of amino acid

3. Test for Saponins
In a test tube 2ml of extract was added to 5ml of distilled water and shaken well. Theformation of persistent foam confirms the presence of saponin.(RajinikanthMarka*et al.,)*

4. Test for Phenols
Treated 1ml of extract with 3-4 drops of ferric chloride solution. The formationof bluish black colour indicates the presence of phenols.(Prashant Tiwari *et al.,2011)*

5. Test for Phytosterols
3ml of extract was dissolved in 3ml of chloroform. Then the solution was treated with few drops of acetic anhydride. Boiled and cooled. Added few drops of concentrated sulphuric acid. There will be a formation of a bluish green colour. No colour change in our solution confirmed the absence of phytosterol.

Tested microorganisms
Antimicrobial activity of the hydroethanolic extract of *P.niruri* leaf was tested against four different bacterial strains such as *E.coli, Stephylococcus aureus,Klebseilla pneumoniae,Shigella.*

Culture And Maintenance Of Bacteria
The pure cultures of *E.coli,S.aureus, K.pneumonia,Shigella* were used as indicater organisms.These bacterias were grown by inoculum prepared in conical flask containing nutrient agar(3.8g of muellar hinton agar and 0.5g of agar agar in 100ml distelled water)prepared by auto claving for 25-30 minutes and incubating at 37℃ for 18-24 hours.Each bacterial culture was maitained on the same medium after every 24 hours of sub-culturing.

Determination of Antimicrobial Assay
In vitro antimicrobial activity of the crude hydroethanolic extract was studied against the tested bacterial strains by agar well diffusion method. The Mueller Hinton in agar medium (MHA) was prepared by pouring 20ml of molten media into sterile petridishes to give a solid plate. Three wells were prepared in the agar plates. 8µl of chloramphenicol was used as a control in one well and the extracts were added in remaining two wells in two different concentrations as 100µl and 200µl and allowed to diffuse for one hour. Then incubated at 37°Cfor 24 hours and later observed to determine the diameter of zone of inhibition. The diameters of zones of inhibition were measured with metre rule and the zone formation of the plant extract was compared with the control antibiotic chloramphenicol.

3. Results

Phytochemical test
Phytochemical screening of *P.niruri* extract revealed the presence of Alkaloids,Saponins,Phenols and absence of proteins,amino acids and phytosterol.

Table 1: Phytochemical analysis of *Phyllanthus niruri*

Phytochemical test	*Presence/Absence*
Saponin	++
Protein	-
Amino acids	-
Alkaloids	+
Phytosterols	-
Phenols	+++

Antimicrobial activity of hydroethanol extract on *Phyllanthus niruri* leaf
The study is based on the antimicrobial effect of *Phyllanthus niruri* leaves using hydro ethanol extracts of leaves at concentration 100µl and 200µl.Chloramphenicol was used as a positive control at concentration of 8µl. Pureisolates of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Shigella* were employed as test organisms.

The diameter of the zone of inhibition of the hydroethanolic extract of *Phyllanthus niruri* was found in different concentration. Against *E.coli* the zone of inhibition for the control was measured to be 3.2cm and for extract the growth was inhibited at 2.5cm (100µl) and 2.8cm (200µl).Against *S.aureus* the zone of inhibition of the control was measured to be 3.1cm and for extract the growth was inhibited at 2.6cm (100µl) and 3.0cm (200µl).Against Shigella the zone of inhibition of the control was measured to be 3.2cm and for extract the growth was inhibited at 2.8cm (100µl) and 2.9cm (100µl).Against *Klebsiella pneumoniae* the zone of inhibition of the control was measured at 3.4cm and for extract the growth was inhibited at 3.0cm (100µl) and 3.7cm (200µl).

According to the result the antimicrobial activity of *Phyllanthus niruri* extract was more effective against the bacterium

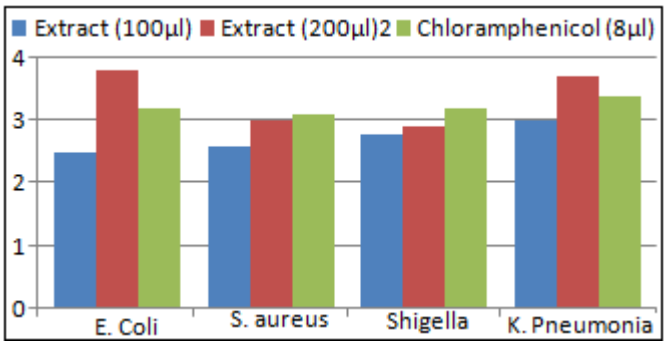

Figure 2: Antimicrobial activity of *Phyllanthus niruri*

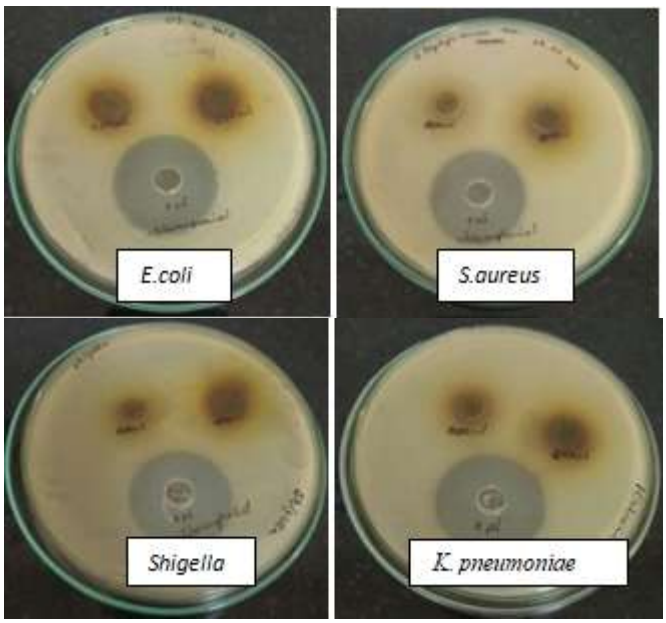

Figure 3: Antimicrobial activity

4. Summary and Conclusion

The phytochemical screening of the *phyllanthus niruri* leaves shows the presence of alkaloids,saponins and phenols.For antimicrobial activity on the *Phyllanthus niruri* were evaluated by collecting the fresh leaves.The leaves are allowed to shadow dried and powdered well.then the

powdered leaves were extracted using hydroethanol. Pure culteres of E.coli,Klebseilla pneumoniea,Sheigella and Staphyllococcus aureus were used as test organisms.8µl of Chloramphenicol is used as control.The extracts were added in two different concentrations as 100µl and 200µl. The activity were studied by agar well diffusion method. Then incubated at 37ºCfor 24 hours and later observed to determine the diameter of zone of inhibition to compare the extract against the control chloramphenicol.

Results showed that the hydro ethanol extracts have antimicrobial activity against these test bacteria's. *Klebsiella pneumoniae* was the most susceptible bacterium and *E.coli* is the least susceptible bacterium. Phytochemical screening revealed the presence of phenol, saponin, and alkaloids in the extracts. The ability of the crude extracts of *Phyllanthus niruri* leaves inhibit the growth of these bacteria's showed its broad spectrum antimicrobial potential, which may be employed in the microbial infections and to treat some diseases.

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