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Research Article

Antimicrobial Effect of *Phyllanthus niruri* on Human Pathogenic Microorganisms

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Abstract

Seven microorganisms were used to study the antimicrobial effects of *Phyllanthus niruri* extracts. The bacterial pathogen used were *Escherichia coli, Pseudomonas aeruginosa, Enterococcus sp., Cogulase* (+) *Staphylococcus sp., Cogulase* (-) *Staphylococcus sp., Candida albicans* and *Candida parapsilosis.* Extracts of *Phyllanthus niruri* leaf, stem, root and fruit made in n-Butanol, Ethanol and Distilled water were tested for their antimicrobial activity. The extracts made in ethanol, water,n-Butanol were found to be effective against most of the human pathogenic microorganisms. Leaf, stem and fruit part of *Phyllanthus niruri* showed very effective results against pathogenic microorganisms.

Key Words : Antibacterial, Antimicrobial, *Phyllanthus niruri*

Introduction

The medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day to day practice. Medicinal plants are considerably useful and economically essential. They contain active constituents which are used in the treatment of many human diseases. Modern research has payed a way for the discovery of another plant of potential value to help in a wider range of ailments .Among the plants *Phyllanthus* has been used in ayurvedic medicine and has a wide number of traditional uses including internal use for Jaundice, Gonorrhea, Diabetes, Skin ulcers and Menstruation.

Phyllanthus niruri an annual herb is 50 cm tall which belongs to the *Euphorbiaceae* family. It has over 500 species and 300 genera. It usually grows in shade in the most tropical regions of the world. It is a wild herb in the Indian forest, but it can be cultivated easily. The generic name, *Phyllanthus* means “leaf and flower” because the flower as well as the fruit seems to become one with leaf. The specific name niruri may have come from a Hindu term. The most common species of the genus *Phyllanthus* is found in most of the countries including India. This investigations was aimed at finding out whether the plant *Phyllanthus niruri* could inhibit E.coli which is one of the agents of urinary tract infections, gastroenteritis and neonatal meningitis. Cogulase (+) *Staphylococcus sp.* is a major agent of food in toxication in humans. Cogulase (-) *Staphylococcus sp.* is a causative agent of endocarditis. *Pseudomonas aeruginosa* is a major agent of pneumonia, septicemia, urinary tract infection and gastrointestinal infection.

Enterococcus creates infection in urinary tract, bactermia, bacterial endocarditis, diverticulitis and meningitis. *Candida albicans* and *C.parapsilosis* is a causative agent of candidial infections in immunocompetent persons. Candidiasis is usually a very localized infection of the skin or mucosal membranes, including the oral cavity (thrush), the pharynx or esophagus, the gastrointestinal tract and the urinary bladder. Candidiasis is a very common cause of vaginal irritation, or vaginitis and can also occur on the male genitals.

Material and Methods

Method of extraction:- The fresh plant parts were collected, properly washed in tap water,

rinsed in sterile distilled water and then air dried in the hot air oven to remove moisture and

to dry them. They were then grounded soxhlet extracted using 70% ethanol. The extraction

lasted for 24 hours.

Antimicrobial susceptibility testing: -

The antimicrobial potential of the above plant extracts was seen against the test organisms using the agar-gel diffusion susceptibility test. Sterile Mueller – Hinton plates were taken one plate/organism tested. Three wells of about 3.0 mm diameter were aseptically punched on each agar plate using a sterile cork borer, with at least 30 mm distance between adjacent wells and the peripherary. According to the standard technique of Opara and Anasa (1993) - 2-4 colonies of the test organisms were inoculated in sterile water and these inoculums was swabbed using sterile swab on the surface of above punched Mueller - Hinton agar plates. A fixed volume (0.1 ml) of the plant extract was then introduced into the wells in the increasing concentration and then incubated at 37°C for 24 hours. The resulting zones of inhibition were measured.

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Results

All the bacterial and fungal pathogens used in this work demonstrated susceptibility to the Ethanol, n-Butanol, Chloroform, Distilled water and Petroleum ether using extracts of *Phyllanthus niruri.* Ethanol extract gave the highest zone of inhibition (28.9mm) on *Escherichia coli*. With Cogulase (+) *Staphylococci* showed the highest zone of inhibition i.e. 22.0 mm in diameter. Cogulase (-) *Staphylococci* showed the highest zone of inhibition i.e. 24.2 mm in diameter. With *Pseudomonas aeruginosa* the highest zone of inhibition was 20.3 mm in diameter. With *Enterococcus sp.* highest zone of inhibition recorded was 25.0 mm in diameter. With *Candida albicans and Candida parapsilosis* it showed the highest zone of inhibition i.e. 23.0 mm and 19.0 mm in diameter respectively. The observation that *Phyllanthus niruri* has good inhibition against *Escherichia coli, Pseudomonas aeruginosa, Enterococcus sp.,* Cogulase *(+) Staphylococcus sp., Cogulase (-) Staphylococcus sp., Candida albicans and Candida parapsilosis* tends to prove worthy remedy to the problem of drug resistance against these pathogens which are already known to be resistant to the most of the standard antibiotics (Penicillin, Tetracycline, Erythromycin, Streptomycin, Fluconazole and Amphotericin B) Tetracycline showed no zone of inhibition against all tested microorganisms. *E.coli* developed resistance against most of the antibiotics except streptomycin. *Pseudomonas aeruginosa* and *Enterococci* showed resistance against all tested antibiotics. Table showed the commercially available antibiotics susceptibility against tested pathogenic microorganisms. Graph showed comparison of commercially available antibiotics with maximum zone found in *Phyllanthus niruri*. But *Phyllanthus niruri* showed effective results against most of the resistant organisms. Bacteria developed resistance against most of the tested antibiotics. Fruit and Leaves showed very effective results as compared to root and stem of *Phyllanthus niruri*. The results of these investigation showed that plant extracts of *Phyllanthus niruri* possess appreciable and potential antimicrobial activity against commonly encountered microorganisms in humans. It is interesting to note that the action of the extracts of *Phyllanthus niruri* is non toxic. Studies have shown that the alcohol and water based extracts of the plant have very low toxicity in mammals.

Table : A Comparison of commercially available antibiotics with maximum zone found in *Phyllanthus niruri*

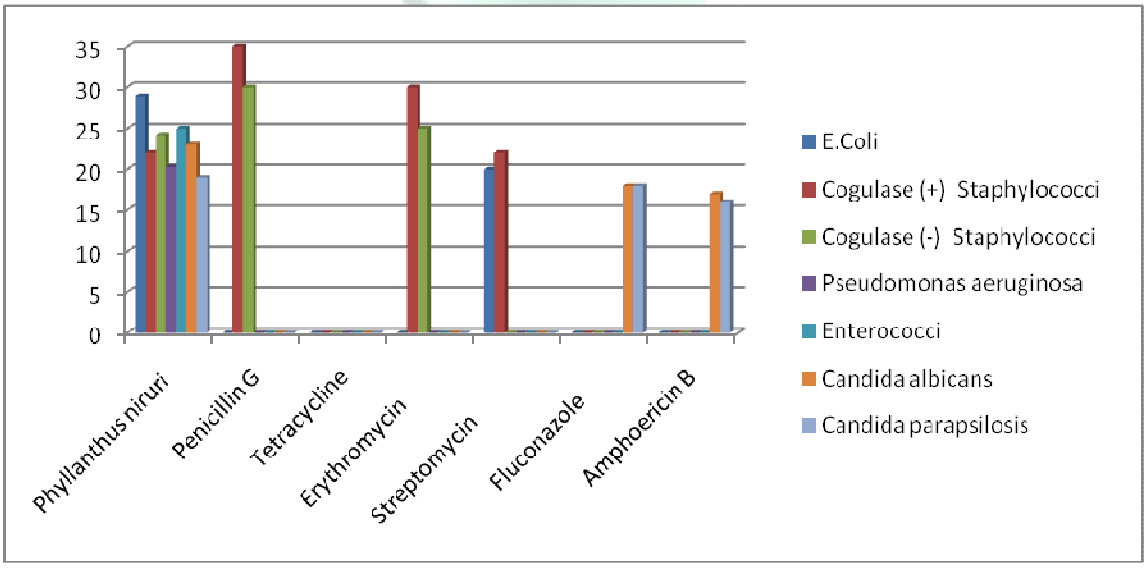
Name of Tested Organisms	*Phyllanthus niruri*	Penicillin G 10 mcg/.1 ml	Tetracycline 30mcg/.1 ml	Erythromycin 15 mcg/.1 ml	Streptomycin 10 mcg/.1ml	Fluconazole 25mcg/.1ml	Amphoericin B mcg/.1ml
E.Coli	28.9mm	Nil	Nil	Nil	20	Nil	Nil
Cogulase (+) Staphylococci	22.0mm	35	Nil	30	22	Nil	Nil
Cogulase (-) Staphylococci	24.2mm	30	Nil	25	Nil	Nil	Nil
Pseudomonas aeruginosa	20.3mm	Nil	Nil	Nil	Nil	Nil	Nil
Enterococci	25.0mm	Nil	Nil	Nil	Nil	Nil	Nil
Candida albicans	23.0mm	Nil	Nil	Nil	Nil	18	17
Candida parapsilosis	19.0mm	Nil	Nil	Nil	Nil	18	16

Discussion and Conclusion:

The effect of *Phyllanthus niruri* extracts agrees with the work of Contreras and Gamarra (1993) that showed antibacterial effect of *Phyllanthus niruri* over *E.coli*. The present investigation agrees with the results of Uchechi and Njoku (2006) that showed antibacterial effect of *Phyllanthus niruri* over *E.coli, S.aureus and S.typhi.* Harisaranraj *et al*., (2010) worked on antimicrobial properties of selected Indian medicinal plants against acne-inducing bacteria. They worked on Ethanolic extracts of *Rauwolfia serpentina* (roots), *Piper nigrum* (seeds), *Azadirachta indica* (leaves), *Cardiospermum halicacabum* (leaves), *Momordica charantia* (fruits), *Casuarina equisetifolia* (fruits), *Cynodon dactylon* (leaves), *Ficus religiosa* (leaves), *Euphorbia hirta* (roots), *Ocimum sanctum* (leaves), *Phyllanthus niruri* (Whole plant), and *Jasminum sambac* (flowers) In case of Phyllanthus niruri they concluded that whole plant of *Phyllanthus niruri* have a good antimicrobial properties. Present investigation agrees with their statement. Kiran *et al.* (2011) evaluated the pleiotropic multifaceted therapeutic potential of *Phyllanthus amarus.* They concluded that anti-inflammatory, antidiabetic, antimicrobial, antihyperlipidemic, antioxidant, anticancer, hepatoprotective, antifertility, antidiarrhoeal, antiallodynic, antioedematogenic, antispasmodial, chemoprotective, antihypercalciuric, antiviral, antispasmodic, antinociceptive and diuretic properties associated with *Phyllanthus amarus.* Venugopalan *et al*., (2010) worked on the enhancement of antimicrobial potential of *Phyllanthus niruri* by fermentation. According to their findings antimicrobial capabilities of the fermented product increases along the fermentation period by about 65%-95% irrespective of the source of lactic acid bacteria used. Another salient feature of the study is that *E.coli* is the most sensitive while *Klebsiella species* is the least sensitive to both the crude as well as the fermented extracts. Our results agrees with their finding regarding the crude extracts effects against *E.coli* .The antimicrobial effect of *Phyllanthus niruri* disagree with the results of Mohana, *et al.,* (2008) They worked on antibacterial evaluation of some plant extracts viz., *Argemone mexicana*, *Caesalpinia coriaria*, *Decalepis hamiltonii*, *Euphorbia tirucalli*, *Leucas aspera,Phyllanthus amarus*, *Phyllanthus niruri*, *Tinospora cordifolia* and *Tribulus terrestris* against some human pathogenic bacteria. Whereas our work was conducted on *Phyllanthus niruri*. Raghu *et al*., (2010) determine the antimicrobial activity and phytochemical study of *Phyllanthus emblica*. The results of the investigation showed that the chloroform, methanol, diethyl ether extracts from the fruits of *Phyllanthus emblica* possess antimicrobial activity against *Klebsiella pneumoniae* and *Staphylococcus aureus.* Doughari *et al*., (2008**)** showed the antibacterial activity of *Phyllanthus muellerianus*. They concluded that both the aqueous and methanol extracts of the leaves and stem bark showed high antibacterial activity against the test organisms. Our results agree with the statement of Doughari *et al*., (2008**)**.

The present investigation agrees with the findings of Chitravadivu *et al*., (2009) who analyzed the antimicrobial studies on selected medicinal plants such as *Acalypha indica, Cassia auriculata, Eclipta alba and Phyllanthus niruri*. Their experiment was carried out on the selected medicinal plants, leaves and roots parts. The extracts of leaves & roots of *Phyllanthus niruri* exhibit relatively higher zone of inhibition followed by *Cassia auriculata, Eclipta alba* and *Acalypha indica* . Other

Graph : A Comparison of commercially available antibiotics with maximum zone found in *Phyllanthus niruri*



relevant work was performed on the other species of *Phyllanthus*. Oluwafemi (2008) showed that *Phyllanthus amarus* possesses significant and appreciable antimicrobial activity. They concluded that cold and hot water extract was not as effective as ethanolic extracts because ethanol is generally able to dissolve multivariable types of compounds. Similar conclusions were obtained using *Phyllanthus niruri*. Rajeshwar *et al*., (2008) worked on in vitro lipid peroxidation inhibitory and antimicrobial activity of *Phyllanthus niruri* extract. Similar conclusion on antimicrobial activity was drawn with the present work. Naik *et al*., (2003) showed the effects of alkaloidal extract of *Phyllanthus niruri* on HIV replication. Phytochemical estimation of *Phyllanthus niruri* agrees with the results of Sule *et al.,* (2008) worked on antibacterial effect of some plant extracts on selected Enterobacteriaceae. In this study *Vernonia amygdalina, Eucalyptus citriodora* and *Phyllanthus amarus* were investigated for their antibacterial properties against pure cultures of clinical isolates of *Escherichia coli, Klebsiella sp., Salmonella sp.* and *Shigella sp.* water extracts of *Vernonia amygdalina* and *Phyllanthus amarus* were not effective on majority of the test organisms.

The Ethanolic extracts of *Eucalyptus citriodora* were most effective on all the test organisms. They concluded the possibility of using the ethanolic extracts of these plants in treating diseases caused by the test organisms. Similar conclusion was drawn with the present work. Sumathi *et al.,* (2010) worked on antimicrobial activity of some traditional medicinal plants. The results of their studies revealed that the dimethyl sulphoxide extracts of leaves of *P. niruri* possess appreciable potentiality of inhibiting the growth of all the strains of *Salmonella typhi* and *Staphylococcus aureus.* Similar results against *Staphylococcus aureus* were obtained using different solvents. Akinjogunla *et al*., (2010) analyzed the antibacterial activity of ethanolic extracts *of Phyllanthus amarus* against extended spectrum lactamase producing *Escherichia coli* isolated from stool samples of HIV sero-positive patients with or without diarrhea. In the end it can be concluded that the plant possesses appreciable medicinal properties that make the plant to be investigated with more doors open. Together with the vast improvements in the approaches for natural-product isolation, characterization and synthesis, this could be opening to a new epoch in the investigation of natural products in academia and industry. This would clearly indicate large share of natural product in new drug discovery and it is strongly advocated to expand the exploration of nature as novel active agents that may serve as scaffolds to develop more efficacious drugs.

Escherichia coli, Pseudomonas aeruginosa, Enterococcus sp., Cogulase *(+) Staphylococcus sp., Cogulase (-) Staphylococcus sp., Candida albicans and Candida parapsilosis* produced appreciable susceptibility with the plant extracts. The result of this research has revealed that many active bioconstituents of *Phyllanthus niruri* constitute potential qualities in its curative action. Thus it must be exploited upon by scientists in the development of human medicines and drugs. *Phyllanthus niruri* research for human gastroenteritis, neonatal meningitis ,pneumonia, septicemia, urinary tract infection ,gastrointestinal infection, bactermia, bacterial endocarditis, diverticulitis , meningitis and candidiasis has thus opened a way for the possibility of finding cures for many strains of enteric microorganisms which are now resistant to many usable and common antibiotics.

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