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ANTIMICROBIAL POTENCY OF *PHYLLANTHUS NIRURI* L. ON SOME CLINICAL ISOLATES

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ABSTRACT

The present work was designed to evaluate the antimicrobial activities of the ethanol extract of different parts of *Phyllanthus niruri* (EPN) (Family: Euphorbiaceae). The antimicrobial activity of EPN was determined by the well diffusion method with various gram-positive, gram-negative microorganisms and pathogenic fungus. We have collected medicinally important medicinal plant *Phyllanthus niruri* for antimicrobial and antifungal studies. The experiment carried out in the medicinal plant leaves, roots, stem, seed and callus. The results are discussed with the available literature. The results obtained in the present investigation clearly suggest that EPN can be a potential source of antimicrobial agent.

KEY WORDS: **Anti microbial,Well diffusion, Clinical isolates, *Phyllanthus Niruri***

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INTRODUCTION

There has been growing interest in the investigation of new antimicrobial agents which serves as an alternative route for the substitution of synthetic chemicals, side effects of which are always in question. The advent of orthodox medicine has not wiped the use of medicinal plants[1,2]. W.H.O reported that more than 80% of the world's population relies on traditional medicine for their primary health care needs[3]. In the last few years, a number of studies have been conducted in different countries to prove antimicrobial efficacy of botanicals[4]. This worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care and the development of microbial resistance to the available antibiotics has led the authors to investigate the antimicrobial activity of medicinal plants. For this, the essential oils and the extracts of many plants have been prepared and screened for their antimicrobial leading to the accumulation of large number of reports in the literature concerning the above-mentioned properties of plants. Much attention has been paid to the plant extracts and the isolated compounds because of their less side effects and the strong resistance towards various microorganisms. Plant-based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials is needed as antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The phytochemical research based on ethnopharmacological informations is generally considered an effective approach in the discovery of new anti-infective agents from higher plants. *Phyllanthus niruri* has demonstrated *in vitro* antibacterial actions against *Staphylococcus, Micrococcus* and *Pasteurella* bacteria as well as *in vivo* and *in vitro* anti-malaria properties, which validates other traditional uses of the genus[3]. Extracts of Phyllanthus had been used as antiviral source to treat hepatatis B[5,6,7,8]. Powis and Moore[9] studied the aqueous extracts of *Phyllanthus amarus* and found it to inhibit viral DNA in vitro. In addition, they eliminated detectable virus from the sera of woodchucks (Marmota monax) acutely or chronically infected with the woodchuck hepatitis virus (WHV). The methanol extracts of five Phyllanthus species from India was reported to have strong antioxidant activity[10]. *P. niruri* has antifungal activity on ringworm, ulcers, cabies and jaundice.

MATERIALS AND METHODS

(i) Microorganisms

The following strains of bacteria were used: *Miromonospora purpurea, Acinetobacter calcoaceticus, Staphylococcus epidermidis, Zymomonas mobilis, Alternaria solani, Fusarium culmorum, Penicillium chrysogenum, phanerochaete chrysosporium.*

(ii) *Plant Collection and Identification*

The plant used in this study, *Phyllanthus niruri* was collected from the Department of Botany, University of Rajasthan, Jaipur. The plants were identified and confirmed at Department of Botany, University of Rajasthan. are collected and shade dried under room temperature The dried *in vivo* and *in vitro* material are grained into a coarse powder and used for further investigations.

(iii) Extraction

Plant materials were harvested and fresh leaves, stem, seeds and roots along with the *invitro* grown callus cultures. A soxhlet apparatus were used for the extracting ethanol fraction from the plant leaves, stem, seed and roots as well as callus. The collected plant leaves and roots were shade dried and

powdered separately. 20gm of dried powder was packed with thimble and then subjected to extraction with the ethanol .The collected extracts were concentrated by evaporation under room temperature. The collected extracts were then investigated for antimicrobial activity.

(iv) Standardization of microbial cell suspension

Five colonies of each test organisms were picked into sterile test tubes containing sterile Mueller-Hinton broth for the bacteria and Potato dextrose broth for fungi and incubated at 37^{o}C for 18h. The turbidity produced by this was adjusted and used to match the turbidity (opacity) standard prepared as described by Cheesbrough[11]. The test organisms are: *Miromonospora purpurea, Acinetobacter calcoaceticus, Staphylococcus epidermidis, Zymomonas mobilis,Alternaria solani, Fusarium culmorum, Penicillium chrysogenum, phanerochaete chrysosporium.* The pure clinical isolates were obtained from the SMS Medical College, Department of microbiology, Jaipur. All clinical isolates were checked for purity and maintained on Nutrient and Potato dextrose agar (PDA) slants at 4 ^{o}C in a refrigerator till required for use.

ANTIMICROBIAL SENSITIVITY TESTING

(v) Agar Well Diffusion Method

The Agar well diffusion method described by Hugo and Russel[12] was employed. The antibacterial activity was tested against leaves, roots, stem, seeds and callus ethanolic extracts of *Phyllanthus niruri.*. The inoculation of microorganism was prepared from bacterial culture. About 15-20 ml of Muller-Hilton agar medium and potato dextrose agar was poured into the sterilized petridish and allows solidifying. One drop of bacterial strains was spread over the medium by a rod. Wells of 6nm in diameter and about 2 cm apart punctured in the culture medium using sterile Cork borers. About 100 ml of plant extracts was added to the wells. Plates were incubated in air at 37°C for 24 hours. Antimicrobial studies were done in triplicates and inhibition zones were measured in mm. Blanks solvents of acetone, water and methanol were used as control. Antibacterial activities and antifungal activities were evaluated by measuring inhibition zone against standard ampicillin and flucanzole.

RESULTS

Medicinally important plant species *Phyllanthus niruri* was selected for screening of antimicrobial activity. During this investigation, an attempt has been made to decipher the effects of *invivo* and *invitro* plant parts and callus for its antimicrobial activity. Ethanolic extracts of leaf, seed, stem, roots and callus exhibit antibacterial activity against *Miromonospora purpurea, Acinetobacter calcoaceticus, Staphylococcus epidermidis, Zymomonas mobilis,Alternaria solani, Fusarium culmorum, Penicillium chrysogenum, phanerochaete chrysosporium*

Antibacterial Activity

The effect of ethanolic extracts against gram positive micromonospora and staphylococcus and gram negative *Acinetobacter* and *Zymomonas* is shown in table 1. The results clearly showed that plant extracts were specific in action against the growth of bacteria. *M.* purpurea was found sensitive against leaf extract showed 18mm inhibition zone which was comparable to standard antibiotic ampicillin 20mm and seed and leaf extract showed higher sensitivity against *A. calcoaceticus. Z. mobilis* was strongly inhibited by root extract 18mm and *S. epidermidis* by seed extract 17mm. other parts of plant were also equally potent in inhibiting bacterial growth. The results of the antimicrobial activity presented in Table 1 show that all extracts exhibited appreciable antibacterial properties inhibiting the growth of all bacteria**.**

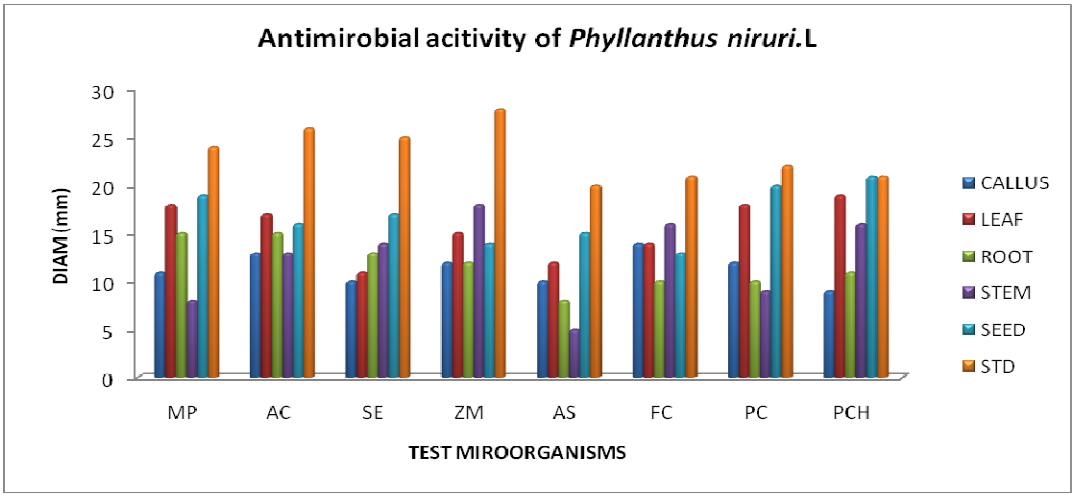
Antifungal activity

The results were found promising against fungal strains too. Leaf and seed extract

showed appreciable inhibition zone 20mm and 18 mm showed in table 1 compared to other parts which proves *phyllanthus* to be a potent antimicrobial agent having no serious side effect when compared to antibiotics and overcomes the problem of drug resistance increasing reliance over herbal remedies to cure serious disease caused by above mentioned microbes.

Table 1
Antimirobial acitivity of *Phyllanthus niruri*.L

Test organisms	Plant parts Zones of growth inhibition (mm)					
	Standard	Callus	Leaf	Stem	Root	Seed
M. purpurea	20	11	18	15	8	19
A. calcoaceticus	16	13	17	15	13	16
S. epidermidis	17	10	11	13	14	17
Z. mobilis	28	12	15	12	18	14
A. solani	20	10	12	8	7	15
F. culmorum	21	14	14	10	16	13
P. chrysogenum	19	12	18	10	9	20
P. chrysosporium	21	9	19	11	16	21



MP: M. purpurea, AC: A. calcoaceticus, SE: S. epidermidis, ZM: Z. mobilis, AS: A. solani, FC: F. culmorum , PC: P. chrysogenum , PCH: P. chrysosporium

DISCUSSION

Several investigations had reported that plants contain antimicrobial substances[13,14,15,16,17]. The results of the present study agrees essentially with the reports of these previous workers. The antimicrobial properties exhibited by the extracts may be associated with the presence of tannins, sapoins, cardiac glycosides and alkaloids found in the plant extracts. A large number of flavonoids have been reported to possess antimicrobial properties[18,19,20,21,22]. Tsuchiya *et al*[18] attributed the antimicrobial activities of flavonoids to their ability to complex with extracellular and soluble proteins as well as their ability to complex with bacterial cell walls. They suggested that more lipophylic flavonoids exert antimicrobial activity by disruptiog microbial cells membranes. The results of this study show that *Phyllanthus niruri* appreciable antimicrobial properties thus justifying its use as antimicrobial agent in Nigerian ethnomedicine.

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