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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF PHYLLANTHUS NIRURI

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Abstract

Medicinal plants have been used as remedies for human diseases for centuries. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value. Knowledge about plants that were found to be most effective against particular ailments was passed down to succeeding generations.

The development of resistance in microorganisms due to the excessive use of conventional antibiotics and the increase in the emergence of infectious diseases has led to the search of new antimicrobial compounds with increased efficiency in terms of their mechanisms of action. Antioxidants are also of interest to biologists and clinicians, because they help to protect the human body against damages caused by reactive oxygen species (ROS). Compounds from natural sources capable of protecting against ROS mediated damage may have potential application in prevention and/or curing of diseases. In the present study, the antimicrobial and antioxidant potential of Phyllanthus niruri Linn belongs to family Euphorbiaceae, commonly known as Stonebreaker was studied in ethanol, methanol and aqueous extracts. Results revealed that the plant selected has good antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Salmonella typhi and high activity was observed in methanolic extract than ethanolic and aqueous extracts. Antioxidant activities of Phyllanthus niruri Linn in methanolic, ethanolic and aqueous extracts were 83.63%, 76.36 %, and 70.90 % respectively. Thus the selected plant has good antimicrobial and antioxidant property.

Keywords: Antioxidants, Reactive Oxygen Species, Antimicrobial

I Introduction

Many medicinal plants are used in modern medicine where they occupy a very significant place as raw materials for important drugs and plants used for traditional medicine contain a wide range of substances that can be used to treat

chronic as well as infectious diseases (1). Clinical microbiologists have great interest in screening of medicinal plants for new therapeutics (2). The development of resistance in microorganisms due to the excessive use of conventional antibiotics and the increase in the emergence of infectious diseases has led to the search of new antimicrobial compounds with increased efficiency in terms of their mechanisms of action (3). Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of phytomedicine to act against microbes (4).

Therefore, researchers are increasingly turning their attention to folk medicine to develop better drugs against microbial infections (5).

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centered free radicals and other reactive oxygen species (ROS), which are continuously, produced in vivo, result in cell death and tissue damage. The role of oxygen radicals has been implicated in several diseases, including cancer, diabetes and cardiovascular diseases, ageing, etc. (6, 7). Antioxidants are substances that may protect cells from the damage caused by unstable molecules known as free radicals. Antioxidants interact with and stabilize free radicals (8). Many synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are very effective and are used for industrial processing but they possess potential health risk due to toxic properties and should be replaced with natural antioxidants (9).

Phyllanthus genus contains over 600 species of shrubs, trees and annual or biennial herbs distributed throughout the tropical and subtropical regions of both hemispheres (10). Phyllanthus niruri Linn belongs to Family: Euphorbiaceae, commonly known as Stonebreaker (Eng.) due to its antilithic property. It has traditionally been used as a diuretic and cooling agent. It is extensively used to treat inflammation, diarrhea, eye sore, burns, suppurations and chafing of the skin (11). In the present study, the antimicrobial and antioxidant potential of Phyllanthus niruri were studied.

II Experimental Section

The aerial parts (leaves and fruits) of Phyllanthus niruri were collected from Chennai, Tamilnadu, India and were shade dried and powdered. 25 grams of powder was dipped into 250 ml of Ethanol, Methanol and water each into a conical flask closed with rubber corks and left for 7 days with occasional shaking. The extracts were filtered through Teflon cloth and WHATMAN No.1 filter paper. The resulting filtrates were taken and concentrated using a rotary evaporator. The crude extracts were subjected to anti-microbial and anti-oxidant assay.

Anti-microbial Assay

Two Gram-positive bacteria viz. Staphylococcus aureus, Bacillus subtillis and two Gram-negative bacteria viz. Escherichia coli, Salmonella typhi were included for anti-bacterial assay. Candida albicans, Aspergillus niger, Trichoderma viride, Rhizopus microsporus were included for anti-fungal assay. Anti-microbial activity was tested using the above microorganisms with Ampicillin and amphotericin (1000 µg/ml) as standard for bacteria and fungi respectively.

Disc diffusion method (12)

Antimicrobial assay of solvent extracts were performed by Disc diffusion method. Young cultures (8 hr old) of bacterial and fungal strains were swabbed separately on the Muller Hilton Agar and Potato dextrose agar plate respectively aseptically. The sterile disc, 5mm in diameter, is saturated with 20µl of different concentrations of solvent extracts viz., 1000 µg/ml, 750 µg/ml and 500 µg/ml separately. The sterile saturated discs were placed on the agar surface with flamed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. The bacterial plates were incubated at 37^{o}C for 24 hours and fungal plates were incubated at 28^{o}C for 24-48 hours. After incubation, antimicrobial activities were determined by measuring the diameter of the zone of inhibition.

II Analysis of Antioxidant Activity Assay

Free radical scavenging assay (DPPH) (13)

Aliquot 3.7 ml of absolute methanol in all the sample and standard test tubes and 3.8ml of absolute methanol to blank. Add 100µl of BHT to tube marked as standard and 100µl of respective samples to all other tubes marked as tests. 200µl of DPPH reagent was added to all the test tubes including blank. Incubate all test tubes at room temperature in dark condition for 30 minutes. The absorbance of all samples was read at 517nm.

Calculation:

$$\% \text{ Antioxidant activity} = \frac{(\text{Absorbance at blank}) - (\text{Absorbance at test})}{(\text{Absorbance at blank})} \times 100$$

III Results and Discussion

The zone of inhibition in millimeters for the methanolic extracts of Phyllanthus ninuri against Staphylococcus aureus, Bacillus subtilis, E.coli and Salmonella were 26, 26, 17 and 26 respectively. Whereas in the case of ethanolic extracts, the values were 15, 20, 9 and 11 respectively. The zone of inhibition of the aqueous extract against the bacteria in the same order as mentioned was 20, 25, 8 and 8 respectively.

The zone of inhibition for the methanolic extracts against Candida albicans, Aspergillus niger, Trichoderma viridae and Rhizopus microsporus were 9, 12, 33 and 44 respectively. The ethanolic extracts showed 10, 9, 30 and 12 and with aqueous extract were 8, 10, 35 and 45 respectively.

The antibacterial activity results clearly showed that the methanolic extracts had greater potential when compared to the ethanolic and aqueous extracts. This may be reasoned that methanol stands as the better solvent solubilizing more amounts of phytochemicals compared to other solvents. But in the case of antifungal activity, all the three extracts showed comparative inhibition. The present study was compared to the antimicrobial activity of Phyllanthus ninuri by (14), in which it was found that the results were similar, compatible.

The antioxidant activities of methanolic, ethanolic and aqueous extract of Phyllanthus niruri were evaluated using DPPH assay. The results indicate that when compared, the antioxidant activity of the three extracts of Phyllanthus ninuri decreased in the order methanolic > aqueous > ethanolic extract with 83.63%, 76.36 %, and 70.90 % respectively. Overall it can be observed from the table (7) that the antioxidant activity was consistently higher in the methanolic extract when compared to other extracts. This research finding is supported by investigation carried out by (15).

Table 1: **Antibacterial activity of ethanolic extract of Phyllanthus niruri.**

Organisms	Zone of Inhibition (mm)			Antibiotic (1mg/ml)
	Concentration (µg/ml)			
	1000	750	500	
Staphylococcus aureus	15	15	15	28
E. coli	9	8	8	16
Salmonella spp.	11	10	10	1
Bacillus spp.	20	20	20	40

Table 2: **Antibacterial activity of methanolic extract of Phyllanthus niruri.**

Organisms	Zone of Inhibition (mm)			Antibiotic (1mg/ml)
	Concentration (µg/ml)			
	1000	750	500	
Staphylococcus aureus	26	14	12	32
E. coli	26	4	24	42
Salmonella spp.	17	15	14	48
Bacillus spp.	26	24	23	33

Table 3: **Antibacterial activity of aqueous extract of Phyllanthus niruri.**

Organisms	Zone of Inhibition (mm)			Antibiotic (1mg/ml)
	Concentration (µg/ml)			
	1000	750	500	
Staphylococcus aureus	20	15	13	25
E. coli	8	8	7	12
Salmonella spp.	8	8	7	9
Bacillus spp.	25	18	15	34

Table 4: **Antifungal activity of ethanolic extract of Phyllanthus niruri.**

Organisms	Zone of Inhibition (mm)			Antibiotic (1mg/ml)
	Concentration (µg/ml)			
	1000	750	500	
Candida albicans	10	7	7	10
Aspergillus niger	9	8	7	10
Trichoderma viridae	30	10	10	-
Rhizopus spp.	12	8	8	35

Table 5: **Antifungal activity of methanolic extract of Phyllanthus niruri.**

Organisms	Zone of Inhibition (mm)			Antibiotic (1mg/ml)
	Concentration (µg/ml)			
	1000	750	500	
Candida albicans	9	8	7	10
Aspergillus niger	12	11	10	15
Trichoderma viridae	33	31	30	35
Rhizopus spp.	44	41	37	45

Table 6: **Antifungal activity of aqueous extract of Phyllanthus niruri.**

Organisms	Zone of Inhibition (mm)			Antibiotic (1mg/ml)
	Concentration (µg/ml)			
	1000	750	500	
Candida albicans	8	8	8	10
Aspergillus niger	10	10	9	15
Trichoderma viridae	35	33	5	35
Rhizopus spp.	45	40	35	45

IV Antioxidant Activity

Table 7: **Antioxidant activity of Phyllanthus niruri.**

S.No.	SAMPLE	DPPH activity (%)
1	Ethanol extract	70.90
2	Methanol extract	83.63
3	Aqueous extract	76.36
4	BHT	99.9

V Conclusion

In the present study, Phyllanthus niruri Linn in methanolic, ethanolic and aqueous extracts were studied. Results revealed that Phyllanthus niruri showed good antimicrobial activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Salmonella typhi. High activity was observed in methanolic extract than ethanolic and aqueous extracts. Antioxidant activities in methanolic, ethanolic and aqueous extracts were 83.63%, 76.36 %, and 70.90 % respectively. Thus the selected plant has good antimicrobial and antioxidant property.

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