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Preliminary Phytochemical Screening and Antimicrobial Evaluation Of *Phyllanthus narayanaswamii* Gamble

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ABSTRACT

The present paper focus on the phytochemical screening and antimicrobial activity of *Phyllanthus narayanaswamii* (Euphorbiaceae), a critically endangered and endemic species to the East Godavari & Visakhapatnam hills of North Eastern Ghats. In the present study the alcoholic extracts of leaves, shoot and root of *P. narayanaswamii* were studied for their antibacterial activity by agar disc diffusion method against both Gram positive, Gram negative and one fungal strain. It was observed that, alcoholic extracts of leaf showed the highest antimicrobial activity against the pathogenic microorganisms followed by shoot and root extracts. The minimum inhibition and minimum bacterial concentrations (MIC and MBC) were determined by microdilution method using 96-well microtiter plate method. As the disc dosage level increases the inhibitory effect also increased.

Keywords: Phytochemical screening, Antimicrobial evaluation, *Phyllanthus narayanaswamii*, Endemic and Endangered species.

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INTRODUCTION

The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is found in "Rig-Veda", which is said to have been written between 4500 - 1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing[1]. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. India is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Herbal medicine is still the mainstay of about 75 - 80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents [2].

Phyllanthus is the largest genus in the family *Phyllanthaceae*, which has a remarkable diversity of growth forms including annual and perennial herbaceous, arborescent, climbing, floating aquatic and phyllocladus. *P. narayanaswamii* (*Euphorbiaceae*) is an annual shrub distributed in Andhra Pradesh that too restricted to the hills of Visakhapatnam and East Godavari districts only. The species is a critically endangered due to the mining activities especially bauxite from the area. The species is only seen with a countable population in the bauxite mining area in Anantagiri hills of Visakhapatnam district.

A number of the *Phyllanthus* species have been reported to have an extensive history in medicine systems. Substantial amount of secondary metabolites present in its members is used widely in traditional medicine for the treatment of flu, dropsy, diabetes, jaundice, gall and bladder calculus and liver diseases [3-5].

MATERIALS AND METHODS:

Plant material

The specimens were collected from the forests in Anantagiri hills of Visakhapatnam, Andhra Pradesh and identified with the help of regional and local floras [6, 7]. The voucher specimens were deposited at Sri Krishnadevaraya University Herbarium (SKU), Anantapur. The plant material was washed with water and shade dried, pulverized in mechanical grinder and stored in an airtight container till further successive extraction.

Preparation of extracts

The samples (leaf, shoot and root) were collected in bulk quantities for conducting preliminary phytochemical screening and antimicrobial activity. The samples were shade dried, powdered (100 g) and successively extracted with petroleum ether, methanol, alcohol (70%) and water using Soxhlet apparatus for 6 hours. The extracts were filtered and concentrated under reduced pressure to dryness.

Preliminary Phytochemical screening

Preliminary phytochemical screening was performed to identify phytochemicals in various solvent extracts of *P. narayanaswamii*. There are several sophisticated techniques used for identification of different groups of compounds in plant extract; however, in the present work, the phytochemicals were detected by color tests. The extracts were subjected to preliminary phytochemical screening using standard procedures [8-11].

Preparation of paper discs

Sterilized Whatmann No.1 filter paper discs of 5 mm diameter were saturated with 20 µl of the extract and allowed to dry at room temperature in laminar air flow bench, and used to test the inhibition activity on test microorganisms.

Microorganisms

The microbial strains viz., Gram positive *Bacillus cereus* MTCC 4079, *B. subtilis* MTCC 1133, *Micrococcus luteus*, MTCC 7256, *Staphylococcus aureus* MTCC 7443, Gram negative *Escherichia coli* MTCC 1668, *Klebsiella pneumoniae* MTCC 7028, *Pseudomonas aeruginosa* MTCC 7296, *Salmonella typhimurium* MTCC 98 and one fungal *Candida albicans* MTCC 7315, were used in the present study. The authentic strains were obtained from the Microbial Type Culture Collection Centre (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

Antimicrobial activity

The antimicrobial activity of the extracts were evaluated by disc diffusion method [12]. Standard antibiotics viz., ampicillin, kanamycin, tetracycline and vancomycin (30µg/disc) obtained from Hi-Media, Mumbai, were used as positive controls. The discs containing petroleum ether, ethyl acetate and methanol served as negative controls. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs.

The minimum inhibitory concentration (MIC) was determined by the broth –micro – dilutions [13] using 96 well microtiter plates. The lowest concentration of each extract showing growth was

taken as its minimum inhibitory concentration (MIC). The solution DMSO (100µl/ml) served as negative control. All samples were tested in triplicates to confirm the activity.

Minimum bacterial concentrations (MBC) were determined by adopting standard methods [14]. To determine MBC 10µl of broth medium from each well of MIC tested plate was taken and incubated in nutrient agar at 37° C for 24 h for bacteria or in Sabouraud's Dextrose agar at 30° C for 48 h for the yeasts. The least concentration showing no visible growth on agar subculture was taken as MBC value. This is the lowest concentration, expressed in mg/ml. Each test was performed in three replicates and repeated twice to confirm the activity and the results were tabulated.

RESULTS AND DISCUSSION:

The phytochemical screening of *P. narayaswamii* using Pet. ether, methanol and water (table-1) extracts from the leaf, shoot and root revealed the presence of alkaloids, coumarins, glycosides, anthocyanins as major constituents followed by catecholic compounds, flavones, dihydrochalcones, proteins, saponins and steroids while emodins and iridoids were obsent in all parts.

The antimicrobial activity of alcoholic extracts of different parts of *P. narayanaswamii* was observed using agar disc diffusion method by measuring the diameter of the growth inhibition zone (Table-2).

The alcoholic extracts of leaf, shoot and root showed antimicrobial activity against gram positive as well as gram negative bacteria and one fungal strain respectively. In case of leaf alcoholic extract showed significant and highest antibacterial activity against *Escherichia coli* (19mm), *Candida albicans* (17mm) whereas moderate degree of activity was noticed on *Bacillus subtilis*, *Staphylococcus aureus* (15mm each), *Klebsiella pneumoniae*, *Micrococcus luteus* (14mm each) at higher concentration (50mg/ml). The MIC values, 156-625 were obtained from leaf extract while the MBC values revealed that *Candida albicans* and *E. coli* were completely inhibited at 4 mg/ml concentration while *Pseudomonas aeruginosa* was the least sensitive organism to leaf extract.

The root extract of alcohol showed a positive significant antimicrobial activity against *B. subtilis, E. coli, Pseudomonas aeruginosa* (7mm each) and moderate degree of activity against *Klebsiella pneumoniae, Micrococcus luteus* (6mm each) no inhibitory activity was found against *Candida albicans* and *Salmonella typhimurium*. Whereas shoot alcoholic extract showed highest inhibition activity against *B. subtilis, Candida albicans* (7 & 8mm) no activity was found against

Table 1: Phytochemical constituents of *Phyllanthus narayanaswamii* Gamble

Sr.	Compound	Leaf			Shoot			Root		
		Pet.ether	Methonol	Water	Pet.ether	Methonol	Water	Pet.ether	Methonol	Water
1	Alkaloids		+	+	+	+	+		+	T
2	Anthocyanins		+			+			+	
3	Anthocyanidins		+							
4	Anthracene glycosides					T				
5	Anthraquinones									
6	Aucubins									
7	Carbohydrates									
8	Carotenoids	+								
9	Catecholic compounds					+			+	
10	Coumarins	+	+	+		+		T	+	+
11	Dihydrochalcones		+	+					T	
12	Emodins									
13	Flavonoids		++	+		+			+	
14	Flavonols								+	
15	Flavonones								+	
16	Flavones		++	+						+
17	Fatty acids									
18	Gallic tannins		+							
19	Glycosides	T	+	+	T		+		+	
20	Iridoids									
21	Lignans		+							
22	Phenols		+	T		+			+	
23	Proteins	+						+	+	
24	Polyoses			T						
25	Reducing compounds		+							
26	Saponins			+			+			
27	Steroids	+			+					

28	Tannins					
29	Triterpenoids	++	+	+	+	+
30	Volatile oils		T			

++: Strong reaction, +: Present, T: Trace.

Table 2: Antimicrobial activity of alcohol extracts of *Phyllanthus narayanaswamii* Gamble

Organism	**Inhibition Zone (mm^{-1})**																		
	Leaf						**Shoot**						**Root**						**Standard µg/disc**
	mg/ml																		
	5	**10**	**25**	**50**	**MIC**	**MBC**	**5**	**10**	**25**	**50**	**MIC**	**MBC**	**5**	**10**	**25**	**50**	**MIC**	**MBC**	
Bacillus cereus (MTCC - 4079)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22^{A}
Bacillus subtilis (MTCC-1133)	7	12	13	15	312	8	-	-	-	7	625	10	-	6	6	7	625	10	25^{V}
Candida albicans (MTCC – 7315)	7	11	13	17	156	4	-	7	-	8	625	10	-	-	-	-	-	-	25^{V}
Escherichia coli (MTCC – 1668)	13	18	19	19	156	4	-	8	-	-	625	10	6	-	-	7	625	10	22^{T}
Klebsiella pneumoniae (MTCC – 7028)	6	7	13	14	312	8	-	-	-	-	-	-	-	7	-	6	625	10	23^{T}
Micrococcus luteus (MTCC – 7256)	6	7	12	14	312	8	-	-	-	10	625	10	6	6	7	6	625	10	23^{K}
Pseudomonas aeruginosa (MTCC – 7296)	-	6	7	7	625	10	-	-	-	-	-	-	-	6	7	7	625	10	28^{T}
Salmonella typhimurium (MTCC - 98)	-	-	-	-	-	-	7	8	-	-	625	10	-	-	-	-	-	-	22^{A}
Staphylococcus aureus (MTCC – 7443)	9	9	12	15	312	8	-	-	-	-	-	-	-	-	-	-	-	-	23^{K}

MIC: Minimum inhibitory concentration; **MBC:** Minimum bacterial concentrations**; A – *Ampicillin,* K – *Kanamycin,* T – *Tetracycline,* V – *Vancomycin.***

B. cereus (leaf, shoot and root), *Candida albicans* (root), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* (shoot), *Salmonella typhimurium* (leaf and root) and *Staphylococcus aureus* (shoot and root). The MIC values, 625 µg/ml were obtained in both shoot and root extracts while the MBC values revealed that 10 mg/ml in both extracts.

CONCLUSION

From the above results it can be concluded that leaf extract has great potential with antibacterial compounds against pathogenic microorganisms and that they can be used in the treatment of infectious diseases caused by such microorganisms. *P. narayanaswamii* showed maximum antibacterial activity, hence, can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hitherto unmet therapeutic needs. However, further investigation on isolation and characterization of the active principles, responsible for the antibacterial activity is necessary and it would provide a comprehensive evidence of bioactive potential of medicinal plants.

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