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Phytochemical and Antimicrobial Studies of *Phyllanthus Wightianus* Leaf Extract

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Abstract

The genus *Phyllanthus* contains highly valued medicinal plants which produce a variety of secondary metabolites. The present paper was designed to carry out preliminary phytochemical screening and antimicrobial activity of leaves of *Phyllanthus wightianus*. The dried leaves of the plant were subjected to phytochemical analysis by using of systematic extraction method. The ethanolic extract of leaves of *P. wightianus* was screened for antimicrobial activity on 20 types of pathogenic bacteria. The investigation reveals that the leaves of *P. wightianus* contain phytoconstituents-alkaloids, terpenoids, flavanoids, tannins and carbohydrates. The ethanolic extract of the plant showed good antimicrobial resistance.

Keywords: *Phyllanthus wightianus,* phytochemicals, antimicrobial.

Introduction

Nature has provided a complete store-house of remedies to cure all ailments of mankind. The medicinal properties of several herbal plants have been documented in ancient Indian literature. The genus *Phyllanthus* (Euphorbiaceae) have long been used to cure disturbances of the kidney and urinary bladder and against intestinal infections. The literature shows that a number of studies seemed to be done with respect to *phyllanthus* species. The role on free radical scavenging activity and chemical constituents were studied for the plant *Phyllanthus wightianus*.The whole plant of *Phyllanthus wightianus* has long been used as a constituent of an ethno medicine for bone setting, as an antidiarrhoeal, against jaundice and for treating dieresis[1].The leaves of the research plant are dark green above, glaucous below. Flowers reddish, and fruits pendulous. The present work investigates the preliminary phytochemical screening and antimicrobial activity of leaves of *Phyllanthus wightianus*.

Material and Methods

Chemical and reagents

The solvents and the reagents were purchased from Merck. They were used without further purification.

Plant Material

The leaves of *Phyllanthus wightianus* [2] were collected Javadi Hills, Vellore district, Tamilnadu, India, during December 2010. The species was verified with authentic specimen at Rapinat herbarium, St.Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India.

Preparation of extracts

The shade-dried leaves of *Phyllanthus wightianus* (1kg) were extracted, with 80% ethanol (4x500) for 3-5 days subjected to cold percolation method. The alcoholic extract was concentrated in a flash evaporator. The systematic extraction procedure [3] is given in Scheme (Fig.1).

Phytochemical screening

Phytochemical analysis of different ether extracts [4-6] and the aqueous extracts were subjected for preliminary phytochemical screening, the details of the analysis, given in Table 1.

Antimicrobial activity

Test organisms

The following organisims were employed for this study as test organisims:

Bacteria

Bacteroides fragilis, Bacteroides melaninogenicus, Bacteroides oralis, Shigella sp, Clostridium septicum, Clostridium tetani, Bifidobacterium bifidum, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Salmonella enteritidis, Klebsiella pneumonia, Enterobacter sp., Proteus mirabilis, Pseudomonas mutant, streptococcus sp., Proteus vulgaris, Bacillus substilis mutant, Yersinia.

The bacterial pathogenic strains were obtained from the Amphigene research laboratories, Thanjavur, Tamilnadu, South India.

Preperation of inoculum

Using sterile inoculation loop 20 pure colonies of the test organism are transferred to 5ml of sterile nutrient broth and incubated at 37 ^{0}C overnight for 18 hrs. Then this bacterial culture were suspended in saline solution (0.85%NaCl) and adjusted to a turbidity of 0.5 Mac Farland standards (10^8cfu/ml). This suspension was used for preliminary screening of anti bacterial activity.

Agar well diffusion assay

The modified agar well diffusion method of Perez et. al.[7] was employed. Each selective medium was inoculated with the microorganism suspended in sterile water. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 25 µL of the plants extracts and blanks (ethanol, distilled water, and n-hexane). The concentration of the leaves extracts of *P. wightianus* employed was 25 µg/ml. The test was carried out by triplicate. The plaques were incubated at 35 ± 2 C for 24 h. The antimicrobial activity was calculated by applying the expression in mm as shown in Table 2. The graphical representation of the zone of inhibition has shown in Fig. 2.

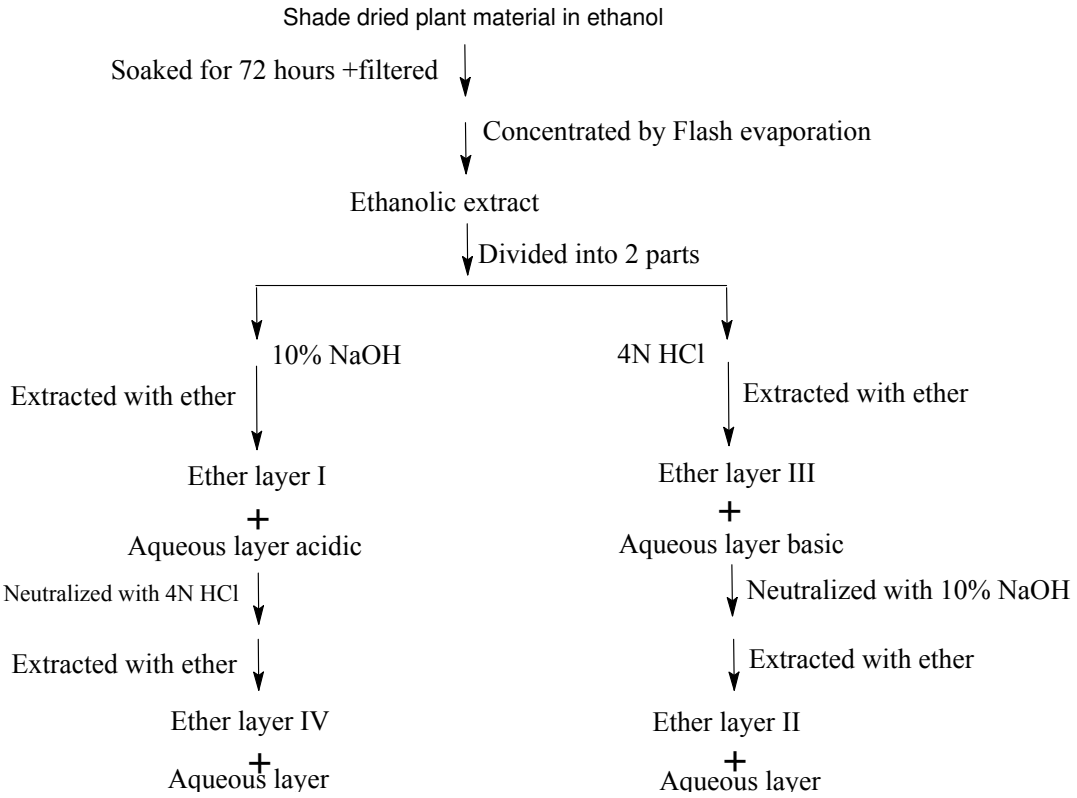

Fig.1: Scheme of extraction using ethanol, alkali, acid and ether solvents.

Table 1: Phytochemical analysis of different ether layers from ethanolic leaves extract of *P. wightianus*

S. NO	METABOLITES	TEST (S)	OBSERVATION	EI	EII	EIII	EIV	Aq.
1	Alkaloids	Dragondroffs reagent	Orange colour	-	-	-	+	-
		Mayer's reagent	White ppt	-	-	-	+	-
		Hager's reagent	Yellow ppt	-	-	-	+	-
2	Glycosides	Anthrone + H_2SO_4+Heat	Purple or green	-	-	-	-	+
3	Carbohydrates	Molish's reagent+ conc. H_2SO_4.	Purple colour	-	-	-	-	+
		Fehling's solution A&B	Brick red colour	-	-	-	-	+
4	Phytosterols /triterpenoids	LiebermannTest	Bluish green	+	-	+	-	-
		Salkowski Test	Red fluorescent	+	-	+	-	-
		Noller's test	Pink colour	+	-	+	-	-
5	Proteins & Amino acids	Biuret test	Violet colour	-	-	-	-	-
		Xanthoprotein test	Orange colour	-	-	-	-	-
		Millon's reagent test	White ppt	-	-	-	-	-
		Ninhydrin test	White ppt	-	-	-	-	-
6	Saponins	Water + shaking	Formation of honey comb like froth.	-	-	-	-	+
7	Flavonoids	Shinoda's test	Red colour	-	+	-	-	+
		Zn-HCl acid reduction test	Magenta colour	-	+	-	-	+
8	Fixed oils & Fats	Spot test	Stains appear after drying	+	-	+	-	-
9	Gums/Mucilage	water	No thickening of the substance	+	-	+	-	-
10	Phenolics/ Tannins	Extract+$FeCl_3$	Intense colour Formation of White ppt	-	+	-	-	-
		Extract + lead acetate+ water		-	-	-	-	+

EI =Ether layer 1, EII =Ether layer 2, EIII = Ether layer 3, EIV= Ether layer IV, Aq=Aqueous - = absent, + = present

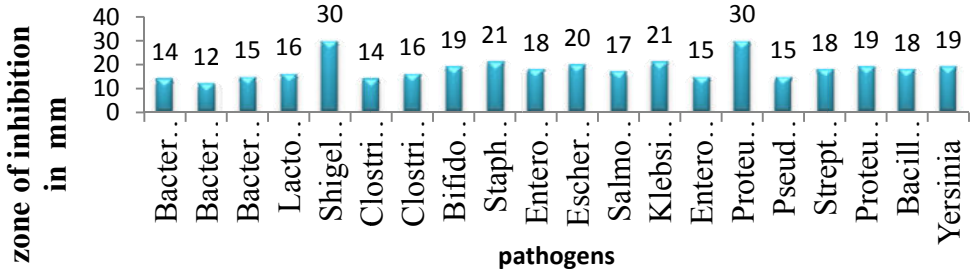

Figure 2: Zone Of Inhibition Vs Different Bacteria

Table: 2. Zone of inhibition of *P. wightianus* leaf extract against selected Microorganisms.

S.No	Name of Pathogens	Control	Zone of inhibition (mm)	S. No	Name of Pathogens	Control	Zone of inhibition (mm)
1	*Bacteroides fragilis*	0	14	11	*Escherichia coli*	0	20
2	*Bacteroides melaninogenicus*	0	12	12	*Salmonella enteritidis*	0	17
3	*Bacteroides oralis*	0	15	13	*Klebsiella pneumoniae*	0	21
4	*Lactobacillus*	0	16	14	*Enterobacter sp.*	0	15
5	*Shigella sp*	0	30	15	*Proteus mirabilis*	0	30
6	*Clostridium septicum*	0	14	16	*Pseudomonas mutant*	0	15
7	*Clostridium tetani*	0	16	17	*Streptococcus sp.*	0	18
8	*Bifidobacterium bifidum*	0	19	18	*Proteus vulgaris*	0	19
9	*Staphylococcus aureus*	0	21	19	*Bacillus substilis mutant*	0	18
10	*Enterococcus faecalis*	0	18	20	*Yersinia*	0	19

Results and Discussion

The phytochemical investigation of *Phyllanthus wightianus* [Table 1], showed the presence of basics compounds alkaloids in the Ether layer IV. The acidic compounds like flavonoids, phenolics and other acidic non-glycoside moiety (aglycone) were present in the ether layer II. The compound like steroids, terpenoids, volatile oil, gums/mucilage was present in the Ether layer I and Ether layer III. Highly polar compounds like saponins, glycosides and tannins were present in the aqueous. Amino acids and proteins were absent in the extract.

The concentrated ethanolic leaf extract of *P.wightianus* was subjected to further evolution of antimicrobial activities against different pathogens of 20 bacteria was determined by Agar Well diffusion method. The concentration of the leaves extracts of *P. wightianus* employed was 25 µg/ml.The zone of inhibition of *Shigella sp* and *Proteus mirabilis* (30mm) was maximum whereas *Bacteroides melaninogenicus showed* minimum inhibition activity.

Conclusion

From the above studies, it is concluded that the selected traditional plant *Phyllanthus wightianus* may represent new sources of anti-microbials with stable, biologically active components present in that plant and it can establish a scientific base for the use of plants in modern medicine. These local ethnomedical preparations and

prescriptions of plant sources can be scientifically evaluated and then disseminated properly.

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