PRELIMINARY PHYTOCHEMICAL AND ANTI FUNGAL ANALYSIS OF BARK, STEM AND LEAVES OF PREMNA PAUCINERVIS (C.B. CLARKE) GAMBLE (LAMIACEAE)

Steffy Francis[1], V. Anand Gideon[2], S. John Britto[3]

1. PhD Research scholar, Department Of Botany, Bishop Heber College, Trichy
2. Associate Professor, Department Of Botany, Bishop Heber College ,Trichy
3. The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College, Trichy

ABSTRACT: This study aims at validating the medicinal value of Premna paucinervis from the analysis of its antifungal properties and the underlying chemical profile of the above species, by using bark, stem and leaves of Premna paucinervis and three fungal strains Aspergillus niger, Mucor indicus and Penicillium vermiculatum. The preliminary phytochemical properties have been established that are responsible for the bioactivity. Well diffusion method was adopted to determine antifungal activity of plant extracts. 20ml of PDA, 100µl of each extract and for negative control 100µl of distilled water was used. Through standard chemical protocols, the group of phytochemicals present in the sample extracts were determined. The presence of Glycosides, flavonoids, terpenoids, steroids, carbohydrates, quinones and phenols became evident. Methanolic bark extract (sample 3) showed highest activity against A.niger and M.indicus strains than methanol stem and leaf extracts. Methanolic stem extract (sample 2) showed more activity against A.niger and methanolic leaf extract (sample 1) showed more activity against M.indicus strain. These three samples are showing poor activity against P.vermiculatum.

Keyword:Premna paucinervis,Antifungal,Phytochemical

INTRODUCTION

The effective use of the medicinal plants to control diseases is prevalent now globally. Above 35% of drugs have ingredients of natural products. Expensive and prolonged uses of synthetic drugs exhibit more side and toxic effects. As considered to be cost effective, the investigation of the efficacy of plant-based drugs used in the traditional medicine has received great attention because they are cheap and with little side effects (Rakh and Chaudhari, 2010). The species of *Premna* are well known for their medicinal properties and are in use in Indian traditional system of medicine especially for diarrhoea, stomach and hepatic disorders. Phytochemical research has isolated more than hundred secondary metabolites such as iridoid and their glycosides diterpenoids, sesquiterpenoids triterpenoids, flavonoids, isoflavones, lignans, xanthones and other classes of compounds. The various biological activities including antioxidant, antibacterial, anti-inflammatory, cytotoxic and heapatoprotective have been displayed both at extract and pure compound level. (Rekha *et al*., 2015). Medicinal plants are rich sources of antimicrobial agents and are a source of many potent and powerful drugs (Srivastava *et al.,* 1996). In recent years, antimicrobial properties of plant extracts have been reported with increasing frequency from different parts of the world (Cowan, 1999). The present study was intended to explore the antifungal properties of extraction of different parts of *Premna paucinervis.*

MATERIALS AND METHOD

Collection and authentication

The plant material was collected from Nelliyampathy forest Palaghat, Kerala, and authenticated by **Dr. S. John Britto S.J.**, Director, Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli. The voucher Specimen (RHT 68492) was deposited for future references.

Extraction

The bark, stem and leaves were shade dried and powdered using mechanical grinder. The powder sample was stored in an air tight container and the portion of the powder was taken in test tubes and solvents (Acetone, Ethanol, Methanol and Aqueous) were added to it, such that plant powder soaked in it and shaken well. The solution was then filtered with the help of muslin cloth and filtered extract were taken and used for antifungal studies and phytochemical analysis.

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Test's for Alkaloids

Mayer's Test: To a few ml of filtrate, one or two drops of Mayer's reagent were added by the side of the test tube. A white creamy precipitate indicated the test as positive.

Wagner's Test: To a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish brown precipitate confirmed the test as positive.

Hager's Test: To a few ml of filtrate 1 or 2 ml of Hager's reagent (Saturated aqueous Solution of picric acid) was added. A prominent yellow precipitate indicated the test as positive. (Kokate *et al.,* 2001).

Test for flavonoids

Pews Test: To 2-3ml extract, was added zinc powder in a test tube, followed by drop wise addition of conc. HCL. Formation of purple red or cherry colour indicated the presence of flavonoids (Peach *et al.,* 1956).

Lead acetate test: 1ml extract was treated with 1ml 10% lead acetate ($Pb(OAc)_4$) solution. Formation of yellow colour precipitate indicated the presence of flavonoids.

Alkaline reagent test: To 2ml test solution, sodium hydroxide solution was added to give a yellow or red colour (Khandewal *et al.*, 2008).
Conc.H_2SO4 test: 5ml of dilute ammonia solution was added to the extract followed by conc.H_2SO4. Yellow colour indicated the presence of flavonoids.

Tests for Phenolic Compounds and Tannins

Ferric Chloride Test: The extract (50 mg) was dissolved in 5 ml of distilled water. To this few drops of neutral 5% Ferric Chloride solution was added. A dark green colour indicated the presence of phenolic compounds.
Potassium dichromate test: To the extract add 5% potassium dichromate solution. Positive result was confirmed by a formation of brown precipitate (for phenol).
Lead Acetate Test: The extract (50 mg) was dissolved in distilled water and to this 3 ml of 10% Lead Acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds (Treare *et al.*, 1985).
Braymer's Test: To 2 ml extract, added 2 ml H_2O and followed with 2-3 drops of $FeCl_3$ (5%). Green precipitate proved presence of tannins.

Tests for Saponins

Foam Test: To 1ml of extract, add 2ml of distilled water and shaken vigorously and allowed to stand for 10 min. There is the development of foam on the surface of the mixture. Then shake for 10 minutes, it indicates the presence of saponins(Khandewal *et al.*,2008).
$NaHCO_3$ Test: To extract a drop of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 min. A honey comb like froth was formed and it showed the presence of saponins.

Tests for Glycosides

Keller kiliani Test (*Test for cardiac glycoside*)**:** To 2 ml extract, was added 1 ml glacial acetic acid, one drop 5% $FeCl_3$ and 1 ml conc. H_2SO4. A brown ring of the interface indicated the presence of cardiac glycosides (Kokate *et al.*, 2001; Khandewal *et al.*, 2008).
Glycoside Test: To small amount of extract, was added 1 ml water and shake well. Then aqueous solution of NaOH was added. Yellow color appeared that indicated the presence of glycosides (Treare *et al.*, 1985).
Molisch's Test: To 1ml of extract, 2drops of Molisch's reagent was added in a test tube and 2ml of con. H_2SO_4 was added carefully keeping the test tube slightly curved. Formation of violet ring at the junction indicated the presence of glycosides (Khandewal *et al.*, 2008).

Tests for Carbohydrates

Molish's Test: To 2 ml of filtrate two drops of alcoholic solution of α- napthol was added, the mixture was shaken well and 1 ml of con. H_2SO_4 was added slowly along the sides of the test tube and allowed to stand. A violet ring indicated the presence of carbohydrates.
Benedict's test: To 0.5 ml of filtrate, 1 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 mins. A characteristic coloured precipitate indicated the presence of sugar.

Test for Terpenoids

Salkowski's Test: 2 ml of chloroform and 1 ml of conc. H_2SO_4 was added to 1 ml of extract and observed for reddish brown color that indicated the presence of terpenoids.
Tests for quinones: 1ml of extract was treated with alcoholic potassium hydroxide solution. Quinines give coloration ranging from red to blue.

Test for sterols

Salkowski's Test: To 2 ml of extract, was added 2 ml chloroform and 2 ml conc. H_2SO_4 from the side of the test tube. Chloroform layer appeared red and acid layer showed greenish yellow fluorescence indicated the presence of sterols (Khandewal *et al.*, 2008).

Tests for Proteins and Amino Acids

Biuret Test: An aliquot of 2 ml of filtrate was heated with 1 drop of 2 % $CuSO_4$ solution. To this 1 ml of ethanol (95%) was added, followed by excess of KOH Pellets. Pink colour in the ethanolic layers indicated the presence of proteins.
Conc. H_2SO_4 Test: 2 ml extract was treated with few drops of conc. H_2SO_4. Formation of white precipitate indicated the presence of proteins.
Xantho proteins Test: 2 ml extract was treated with few drops of conc. HNO_3 and NH_3 solution. Formation of reddish orange precipitate indicated the presence of xantho proteins.

ANTI FUNGAL ACTIVITY

Fungal strains

Aspergillus niger, *Mucor indicus* and *Penicillium vermiculatum a*re the fungal strains were used for the antifungal analysis.

Determination of antifungal activity

Petri plates containing 20ml PDA were seeded with mature culture of fungal strains. Wells were cut using a sterile Cork Borer and 100µl (200µg/well) of extracts were added into the well. For the negative control, 100µl of the distilled water was added into the wells. The plates were then incubated at room temperature for about a week. The antifungal activity was
assayed by measuring the diameter of the inhibition zone formed around the well.

RESULTS AND DISCUSSION

Table: 1.1 Preliminary phytochemical analysis in *Premna paucinervis* (leaf)

	TEST	ACETONE	ETHANOL	METHANOL	AQUEOUS
Alkaloids	Wager's	+++	+++	+++	+++
	Hager's	+++	+++	+++	+++
	Mayer's	++	+++	+++	+
Flavonoids	Pew's	-	-	-	-
	Lead Acetate	+++	+++	+++	+++
	NaOH	+++	+++	+++	+++
	Con.H_2SO_4	-	-	-	-
Phenol & tannin	$FeCl_3$	+++	+++	+++	+++
	$K_2Cr_2O_7$	+	++	+	+
	Lead Acetate	-	-	-	-
	Braymers	+	+	+	+

Saponins	Foam	+	+	+	+
	$NaHCO_3$	++	++	++	++
Glycosides	Keller kiiani	+++	+++	+++	+++
	Glycosides	+++	+++	+++	+++
	Molish	++	+++	+++	++
Carbohydrates	Molish	++	++	++	++
	Benedicts	-	+	+	-
Terpenoids	Salkowskis	-	-	-	-
Quinones	Quinones	-	-	++	+++
Sterols	Salkowskis	-	-	-	-
	Keller kiiani	+++	+++	+++	+++
Protein	Biuret	+	+	+	+
	Con. H_2SO_4	++	+	++	+++
	Xanthoprotein	-	-	-	-

Table: 1.2 Preliminary phytochemical analysis in *Premna paucinervis* (stem)

	TEST	ACETONE	ETHANOL	METHANOL	AQUEOUS
Alkaloids	Wager's	+++	+++	+++	+++
	Hager's	+++	+++	+++	+++
	Mayer's	+++	++	+++	++
Flavonoids	Pew's	-	-	-	-
	Lead Acetate	+++	+++	+++	+++
	NaOH	+++	+++	+++	+++
	Con.H_2SO_4	++	++	++	++
Phenol & tannin	$FeCl_3$	-	-	-	-
	$K_2Cr_2O_7$	++	++	++	++
	Lead Acetate	-	-	-	-
	Braymers	-	-	-	-
Saponins	Foam	+	+	+	+
	$NaHCO_3$	-	+	-	++
Glycosides	Keller kiiani	+++	+++	+++	+++
	Glycosides	+++	+++	+++	++
	Molish	+++	+++	+++	+++
Carbohydrates	Molish	+++	+++	+++	+++
	Benedicts	+	+	+	+
Terpenoids	Salkowskis	+	+	+	-
Quinones	Quinones	-	-	-	-
Sterols	Salkowskis	+	+	+	-
	Keller kiiani	+++	+++	+++	+++
Protein	Biuret	+	+	+	+
	Con. H_2SO_4	++	+	++	+++
	Xanthoprotein	++	++	++	++

Table: 1.3 Preliminary phytochemical analysis in *Premna paucinervis* (bark)

	TEST	ACETONE	ETHANOL	METHANOL	AQUEOUS
Alkaloids	Wager's	+++	+++	+++	+++
	Hager's	+++	+++	+++	+++
	Mayer's	+++	+++	+++	+++
Flavonoids	Pew's	-	-	-	-
	Lead Acetate	+++	+++	+++	+++
	NaOH	+++	+++	+++	+++
	Con.H_2SO_4	+++	+++	+++	+++
Phenol & tannin	FeCl3	+++	+++	+++	+++
	$K_2Cr_2O_7$	+	+	+	+

	Lead Acetate	+++	+++	+++	+++
	Braymers	-	-	-	-
Saponins	Foam	+	-	+	+
	$NaHCO_3$	-	-	-	+
Glycosides	Keller kiiani	+++	+++	+++	+++
	Glycosides	+++	+++	+++	+++
	Molish	+++	+++	+++	+++
Carbohydrates	Molish	+++	+++	+++	+++
	Benedicts	+++	+++	+++	+++
Terpenoids	Salkowskis	++	++	++	++
Quinones	Quinones	++	++	+++	+++
Sterols	Salkowskis	++	++	++	++
	Keller kiiani	+++	+++	+++	+++
Protein	Biuret	+	+	+	+
	Con. H_2SO_4	++	+	++	+++
	Xanthoprotein	+++	+++	+++	+++

- Absent, + Present, ++ good, +++ very good

Table 2. Antifungal assays of *Premna paucinervis*

Column1	*A.Niger*	*M.Indicus*	*P.Vermiculatum*
Sample1	9.7±0.5	13.2±1.2	0
Sample2	15.3±0.5	12.5±0.5	6.3±0.5
Sample3	15.5±0.5	14.5±0.5	6.3±0.5

Sample 1-leaf methanol extract, sample 2 –stem methanol extract and sample 3 –bark methanol extract.

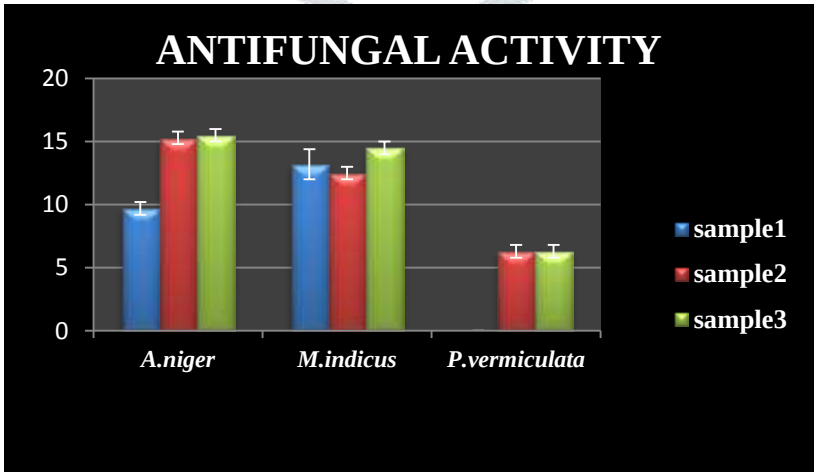

Figure.1. Antifungal activity of *P. paucinervis* extracts (leaf, stem and bark)

Figure.2. Antifungal activity of *P. paucinervis* extracts (leaf, stem and bark)

The results obtained for qualitative screening of phytochemicals in different plant parts (leaf,stem,bark) of *P.paucinervis* is presented in Table 1.1,1.2,1.3. Of the ten phytochemicals screened, all were found present they are Alkaloids, flavonoids, phenol & tannins, saponins, glycosides, carbohydrates, terpenoids, quinones ,sterols and proteins. On the other hand *P.paucinervis* showed strong properties against fungi. The antifungal activities of methanolic extracts of *P.paucinervis* leaf, stem and bark were carried out against three pathogenic fungi, namely *Aspergillus niger, Mucor indicus* and *Penicillium vermiculatum*. Sample 3 exhibited maximum inhibitions against *A.niger* 15.5±0.5 (mm) and *M.indicus* 14.5±0.5 (mm) than sample1 and sample 2 followed by *P.vermiculatum* 6.3±0.5 (mm). Sample 2 showed more activity against *A.niger* 15.3±0.5(mm) strain followed by M.*Indicus*12.5±0.5*(mm),P.vermiculatum* 6.3±0.5(mm). Sample 1 showed more activity against *M. indicus*13.2±1.2 (mm), followed by *A.niger* 9.7±0.5 (mm). For *P.vermiculatum* sample 1 did not show any activity but showed similar activity against sample 2 and sample 3 (Table 2, Fig. 1 and Fig. 2).

CONCLUSION

The study on the leaf, stem and bark of *P. paucinervis* for its phytochemical constituents and antifungal activity has proved the presence of secondary metabolites along with activity against various fungal strains. More purification needs to be done and checked for more resistant type of micro-organisms. Further research on *P. paucinervis* is necessary for elucidating the active principles and their mode of action.

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