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

Steffy Francis¹, V. Anand Gideon², S. John Britto³
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, sesqui terpenoids triterpenoids, flavonoids, isoflavones, lignans, xanthenes and other classes of compounds. The various biological activities including antioxidant, antibacterial, anti-inflammatory, cytotoxic and hepatoprotective have been displayed both at extract and pure compound level. (Rokha et al., 2015). Medicinal plants are rich sources of antimicrobial agents and are a source of many potent and powerful drugs (Srivasatava et al., 1996). In recent years, antimicrobial properties of plant extracts have been explored 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 forestPalaghat, 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 such that 1ml extract was treated with 1ml 10% lead acetate (Pb(OAc)₂) solution. Formation of purple red precipitate indicated the presence of flavonoids (Peach et al., 1956).

Test for flavonoids

Pew's 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)₂) 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. H₂SO₄ test: 5ml of dilute ammonia solution was added to the extract followed by conc. H₂SO₄. 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 H₂O and followed with 2-3 drops of FeCl₃ (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).

**NaHCO₃ 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 glycosides):* To 2 ml extract, was added 1 ml glacial acetic acid, one drop 5% FeCl₃ and 1 ml conc. H₂SO₄. 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).

**Molish’s Test:** To 1ml of extract, 2drops of Molish’s reagent was added in a test tube and 2ml of con. H₂SO₄ 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**

**Salkowski’s Test:** 2 ml of chloroform and 1 ml of conc. H₂SO₄ was added to 1 ml of extract and observed for reddish brown color that indicated the presence of terpenoids.

**Tests for quinones:**

**Acetoacetate Test:**

**Biuret Test:** An aliquot of 2 ml of filtrate was heated with 1 drop of 2% CuSO₄ 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 quinones.

**Conc. H₂SO₄ Test:** 2 ml extract was treated with few drops of conc. H₂SO₄. Formation of white precipitate indicated the presence of proteins.

**Flavonoids Test:** 2 ml extract was treated with few drops of conc. HNO₃ and NH₄ solution. Formation of reddish orange precipitate indicated the presence of xantho proteins.

**ANTI FUNGAL ACTIVITY**

**Fungal strains**

*Aspergillus niger, Macor indicus* and *Penicillium vermiculatum* are 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)

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

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

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Table 2. Antifungal assays of *Premna paucinervis*

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<td>Sample 3</td>
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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)
The results obtained for qualitative screening of phytochemicals in different plant parts (leaf, stem, bark) of *P. paucinervis* is presented in Table 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 sample 1 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.

ACKNOWLEDGEMENT

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Reference