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ANTIFUNGAL ANALYSIS OF STEM AND LEAF OF *PREMNA RAJENDRANII* (LAMIACEAE)-A NEWLY DISCOVERED ENDEMIC SPECIES

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Abstract: This study aims at validating the medicinal value of *Premna rajendranii* from its antifungal properties against medically important fungal strains. The antifungal properties were tested against *Aspergillus niger, Mucor indicus* and *Penicillium vermiculatum* which had shown moderate rate of inhibition. Fusarium spp. and Rhizopus spp. are the isolated fungal strains were used for the same. The fungal activity of the *P. rajendranii* might be due to the presence of various secondary metabolites. Hence, this plant can be used to identify the specific bioactive compounds which may serve as leads in the development of new antimicrobial agents.

Index Terms - Antifungal Analysis, *Premna rajendranii, Aspergillus niger, Mucor indicus, Penicillium vermiculatum,* Fusarium spp. and Rhizopus spp.

INTRODUCTION

Plants are useful for curing human diseases and play a dominant role in healing due to presence of bioactive chemical constituents. These Plants have the potentiality to synthesize a wide variety of chemical compounds that are used to perform important biological functions. They supply major source of molecules with medicinal properties due to presence of natural compounds. (Ajuru *et al.*,2017). Products derived from plants may potentially control fungal growth in diverse situations and in the specific case of disease treatment. Numerous studies have aimed to describe the chemical composition of these plant antimicrobials and the mechanisms involved in microbial growth inhibition, either separately or associated with conventional antimicrobials (Singh, 2015). The presence of antifungal compounds in higher plants has long been recognized by the traditional medicinal practitioners and has been used for the treatment of various human and plant diseases. These plant derived fungicides are easily biodegradable (Fawcett and spencer, 1970) and selective in their toxicity (Beye, 1978). Nowadays concerns have been raised to reduce the use of

synthetic fungicides because of their environmental impact and the potential health risks. Hence, there is a great demand for safer antifungal bio compounds belonging to a wide range of structural classes, which are selective on new targets with fewer side effects (Abad *et al.*, 2008).

Premna is one of the biggest woody genera in family Lamiaceae, and mainly distributed in Old World tropical and subtropical regions from Africa to China, throughout southern Asia, to northern Australia, and various islands in the Pacific and Indian Oceans (Harley *et al.*, 2004; de Kok, 2013). The species of *Premna* are well known for their healing 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). *Premna rajendranii* is a new endemic species reported from Kerala by Kumar *et al.*, (2013) and this was collected from Chinnar wildlife sanctuary, Kerala, and later collected the same specimens from scrub jungles of the Madukkarai Hills, Coimbatore District, Tamilnadu. The detailed studies have revealed that the species is related to *P. mollissima* and *P. corymbosa*, but it shows difference in many characters (Kumar, 2013).

REVIEW OF LITERATURE

The various species of Premna throughout the habitat region resulted in various traditional uses by the local people (Dianita & Ibrahim, 2017). The Premna genus can be used in treating various ailments like rheumatism, asthma, dropsy, cough, fever, boils and scrofulous disease. (Dassanayake & Fosbergeds, 1980; De Paduva *et al.*1999). Traditionally *Premna* species were used as anti inflammatory agent, antibacterial agent, immune system improver, for wound healing and treating skin diseases, for stomach disorders, for migraine, headache, and neuralgia problems, for hypertension, diabetes, liver-and cardiac-related problems (Dianita & Ibrahim, 2017). The study by Abdullah *et al.*, (2021) suggests that the *Premna odorata* Blanco extracts by deep eutectic solvents are promising for the development of treatments against various fungal diseases with a friendly green procedure, low toxicity and new application in pharmaceutical industry.

Although most fungi are harmless to humans, some of them are capable of carrying diseases under specific conditions. Food spoilage caused by microorganisms still widely affects all types of food and causes food waste and loss. Fungal species of Fusarium and Rhizopus are example. It has been estimated that the yearly losses of global food reach upto 40% due to various factors including spoilage by microorganisms (Gustavsson *et al.*, 2011). Food borne disease is another pervasive food safety problem due to the composition of contaminated food products, which has been a significant safety concern to public health *(*Azziz *et al*., 2005).It is mainly the mycotoxins produced by fungi contaminates food (Johannessen *et al.*, 2005).

Medda *et al*., (2015) studied the biosynthesis the leaf extract of *Aloe vera* and the antifungal activity against Rhizopus sp. According to Surapuram *et al.*, (2014) the natural product extracts identified to inhibit *A. niger* and *R. stolonifer* with high potency are leading candidates for antifungal identification. Isolation of the active compounds from these extracts could lead to improved antifungals for use in agriculture to

preserve food crops as well as in the pharmaceutical industry for treatment of mycoses. Gakuubi *et al.*, (2017) evaluated the antifungal activity of essential oil (EO) of *Eucalyptus camaldulensis* Dehnh. against five *Fusarium* spp. commonly associated with maize and the findings confirm the fungicidal properties of *E. camaldulensis* essential oils and their potential use in the management of economically important *Fusarium* spp. and as possible alternatives to synthetic fungicides.

Antifungal assay revealed that the methanol extract as well as the methanol fraction and ethyl acetate fraction of *Premna hispida* inhibited the growth of the entire test microorganisms *(Candida albicans* and *Aspergillus niger)* in varying degrees due to the presence of secondary metabolites in substantial amounts in the plant (Onuegbu *et al.*, 2019). The antifungal activities of methanolic extracts of *P. paucinervis* leaf, stem and bark were carried out by Francis *et al.*, (2018) against three pathogenic fungi, namely *Aspergillus niger*, *Mucor indicus* and *Penicillium vermiculatum.* Bark methanolic extract exhibited maximum inhibitions against *A.niger* 15.5±0.5 (mm) and *M.indicus* 14.5±0.5 (mm) than leaf and stem methanolic extracts.

The review presented here has dealt with several aspects of genus *Premna* from Pharmacognostic, Pharmacological, Phytochemical and Phylogenetic perspectives. It is obvious that some members of *Premna* have been studied in depth, while others are yet to be studied. The literature study has revealed that the efficacy of the plant extracts is unexplored. When more data are available by enhanced research endeavours, as for example from phytochemistry, abiological assays and classical and molecular analysis, they cumulatively will contribute to improved drug design, promotions of pharmaceutical outcomes especially relating to antifungal assay of health care.

MATERIALS AND METHODS

Collection and authentication

The plant material for our investigation was collected from the scrub jungles of the Madukkarai Hills, Coimbatore District in Tamilnadu, and authenticated by **Dr. S. John Britto S.J**, at the Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli. The voucher Specimen (RHT 68887) was deposited in the Rapinat Herbarium.

Fig. 1 Madukkarai-Place of Collection

Fig. 2 *Premna rajendranii*

Extraction

The 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 tube and solvent (Methanol) was added to it such that plant powder soaked in it and shaken well. The solution then filtered with the help of muslin cloth and filtered extract was taken and used for antifungal analysis.

Fig. 3 Extraction Procedure

ANTI FUNGAL ACTIVITY

Fungal strains

Aspergillus niger, Mucor indicus and *Penicillium vermiculatum* are the purchased fungal strains were used for the antifungal analysis. Fusarium spp. and Rhizopus spp. are the isolated fungal strains were used for the same.

Isolation of the Fungi

Fusarium spp. was isolated from soil and root sample of tomato plants collected from the fields and Rhizopus spp. was isolated from stale bread. Potato dextrose agar is a nutrient rich medium for growing a wide range of fungi therefore it is used10-4 at a dilution for pour plating method of Ofunne (1999). For fungal isolation from plant, the roots were washed under tap water, chopped into 2 cm small pieces and surface sterilized in 0.5% NaOCl for two minutes then rinsed twice with triple distilled water and placed on PDA and finally kept in an incubator at 27°C under dark conditions. All the procedure was carried out into laminar hood under sterilize condition. After five days of incubation, small colonies of fungus appeared, which were picked with a sterilized tooth pick and transferred to fresh PDA plates

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

The antifungal activities of methanolic extracts of *P. rajendranii* leaf and stem were carried out against three pathogenic fungi, namely *Aspergillus niger, Mucor indicus* and *Penicillium vermiculatum*. Leaf extract of *P. rajendranii* revealed inhibition activity with inhibition zone of 14±0.05, 12±0.06, 11±0.01 against *A. niger, M. indicus* and *P. vermiculatum* respectively. Stem extract showed activity with inhibition zone of 12.5±0.6, 9.8±0.5, 10.33±1.1 against *A. niger, M. indicus* and *P. vermiculatum* respectively **(Fig. 4)**.

Table1. Antifungal assays of *Premna rajendranii*

	A.niger	*M. indicus*	*P.vermiculatum*
Sample 1	12.5±0.6	9.8±0.5	10.33±1.1
Sample 2	14±0.05	12±0.06	11±0.01

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

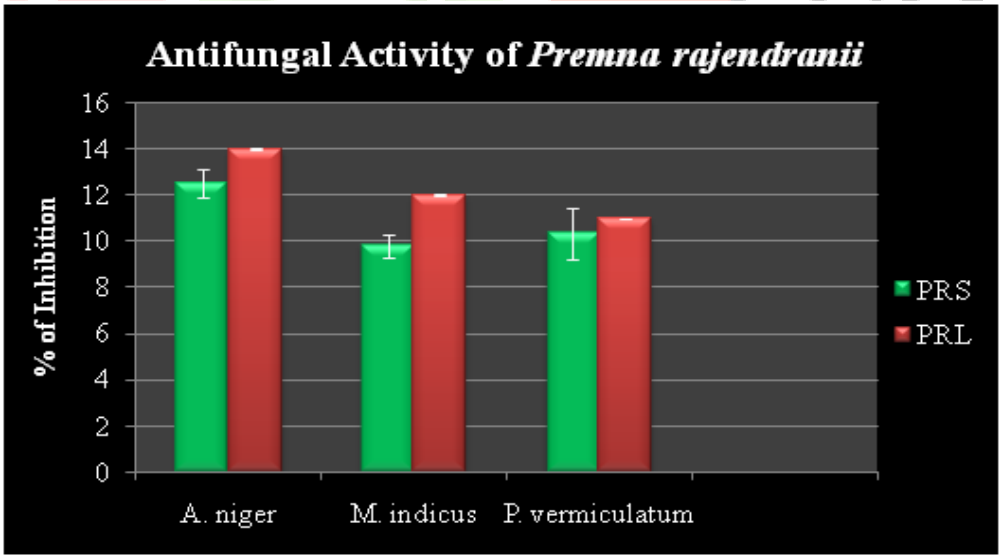


Fig 4. Antifungal activity of *P. rajendranii* extracts (leaf and stem)

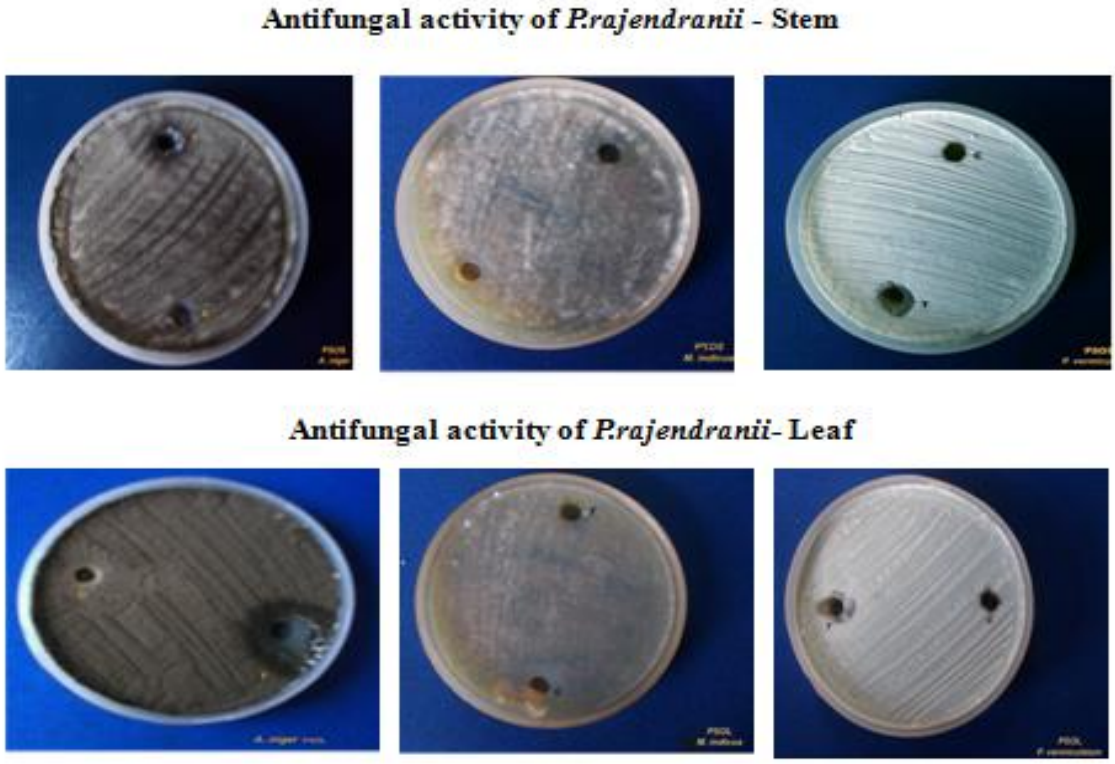


Fig. 5. Antifungal activity of *P. rajendranii* extracts (leaf and stem)

Leaf extract of *P. rajendranii* revealed inhibition activity with inhibition zone of 9.67±1.5, 11±1, against *Fuasrium sp.* and *Rhizopus* sp. respectively.

Table: 2 Antifungal activity of *Premna rajendranii* Leaf

Sl. No.	Sample fungal strains	*P. rajendranii* - leaf
1	Fuasrium sp.	9.67±1.5
2	Rhizopus sp.	11±1

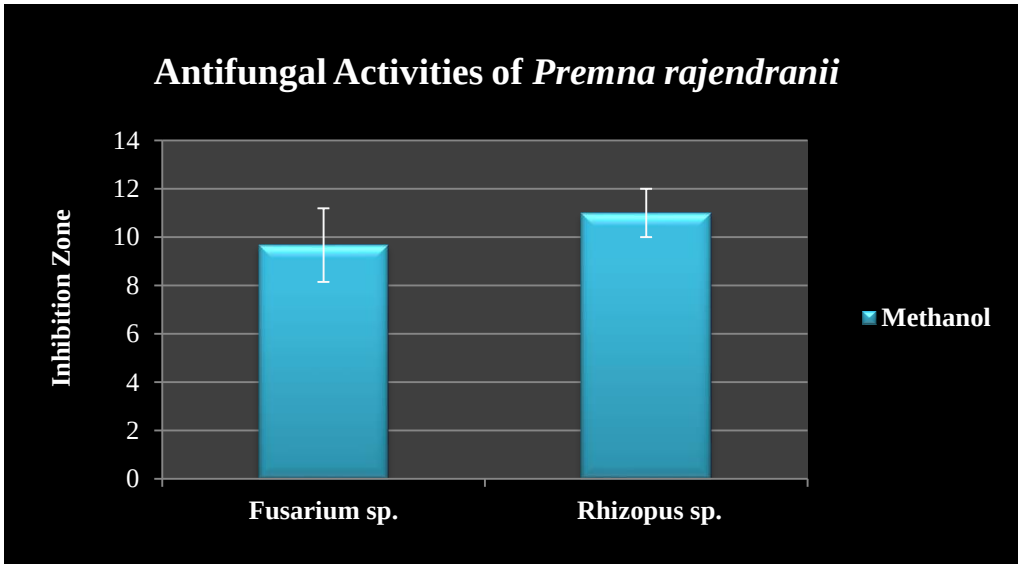
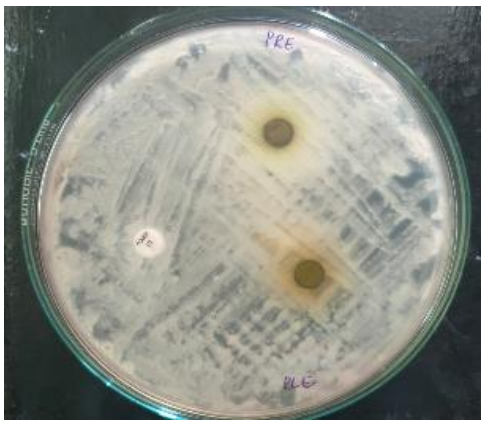


Fig.6 Antifungal activity of *Premna rajendranii* against *Fuasrium sp.* and *Rhizopus* sp.



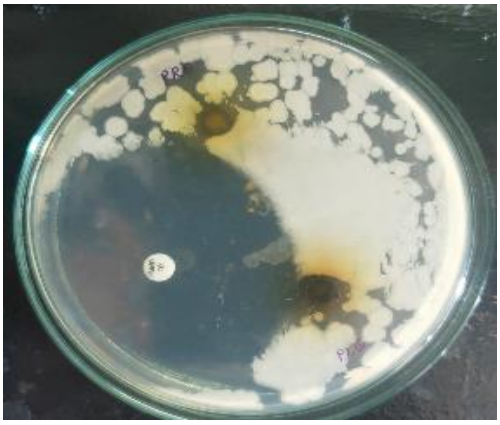


***Fig.7 Premna rajendranii* against Fusarium sp.** ***Fig.8 Premna rajendranii* against Rhizopus sp.**

CONCLUSION

Genus *Premna* is widely known for its medicinal effects and has been used in Indian traditional system of medicine. Based on the available data, there are thirty two species and six varieties of *Premna* reported from India. Out of these, *P. rajendranii* was selected for the present study. The study on the stem and leaf of *P. rajendranii* for its 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. rajendranii* is necessary for elucidating the active principles and their mode of action.

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