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Antifungal Effect of Leaf Extract of *Premna latifolia* Roxb. on Rhizopus and Fusarium Species.

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ABSTRACT

This study aims at validating the medicinal value of *Premna latifolia* from its antifungal properties against medically important fungal strains. The antifungal properties were tested against *Fusarium* and *Rhizopus* which had shown moderate rate of inhibition. The fungal activity of the *P. latifolia* 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.

Key Words: *Premna latifolia*, Antifungal properties, Fusarium and Rhizopus

INTRODUCTION

The plant kingdom plays a crucial role in the advancement of human civilization all over the world. Each plant contains important compounds which can be used in medical field and for the development of different kind of drugs. They provide essential nutrition and possess medicinal potency towards life threatening diseases. Two-third of the world's population depends on the plant- based traditional medical systems for their primary health care. Plants are the essential basis for human day to day life and health care systems. Thus human life without plants cannot be possible (Garg *et al.*, 2021).

The consumption of herbal drugs all over the globe and mainly in India is ubiquitous. Even though the utilization of herbal drugs in India is an ancient practice, attempts to standardize these drugs took place only post independence. Presently, there are some challenges with regard to clinical trial of these drugs. Moreover, groundless use is also widespread in rural areas of India. Further standardization and clinical trial of medicinal plants need to be promoted in India (Samal, 2016). It is in this context, that *Premna latifolia* from Lamiaceae have been selected for the present investigation.

Premna is one of the biggest woody genera in family Lamiaceae (Harley *et al.*, 2004). *Premna latifolia* is an unexploited native plant of India with immense medicinal properties. It contains chemical constituents like iridoids, glycosides, diterpenes, saponins and flavonoids which exhibit anti- inflammatory and antioxidant activities. Different parts of *P. latifolia* are used in the traditional system of medicine for treatment of wound healing, dropsy, boils, fever, and liver complaints. It acts as a substitute for *P.*

serratifolia in the ten herb Ayurvedic medicine "Daśamūla". The leaves and tender shoots of *P. latifolia* are eaten in the form of curries and it is locally known as Erumamunna, Erumunna or Erumunnamaram in Malayalam.

Pathogenic fungi are the causative medium of various diseases and evolved as a crucial public health issue all over the world. Some fungal genera such as *Aspergillus, Penicillium, Fusaria* and *Rhizopus* have the ability to produce secondary metabolites that can have a toxic effect on humans and animals and are therefore named mycotoxins. Moreover mycotoxins are able to withstand various food processing steps and can thus lead to food safety concerns (Salas et al, 2017).

Fusarium is a large cosmopolitan genus of imperfect fungi and its numerous species are important plant pathogen (Nelson et al. , 1981), produce a wide range of secondary metabolites and cause various diseases in human (Austwick 1982). Mycotoxicosis in humans following ingestion of food that has been colonized by fungal organisms, localized infection includes -septic arthritis, endophthalmitis etc. are caused by *Fusarium* species (Gupta et al., 2000). *Fusarium* head blight, foot and root rot are among the major plant diseases.

Rhizopus, is probably the well known genus in the class of Zygomycetes fungi, which commonly live on dead and decaying plant material. Various strains of *Rhizopus* are used for the making of fermented food, beverages etc, moreover it also has several industrial applications, in manufacturing enzymes, metabolites etc. (Lennartsson et al., 2014). Even though these fungi are convenient in many aspects, but some species causes food spoilage, infection in human, plants etc. It is a fast growing type of fungi with white mycelia and black sporangia and is placed in taxonomic order Mucorales and probably the most common genus of that order to contaminate food (Bullerman L. B, 2003). Rhizopus headrot, Rhizopus rot, blight etc. are plant diseases which causes large crop loss and in human, this fungi causes diseases like zygomycosis, allergic infections etc. (Ribes et al., 2000).

Advancement of multi-drug resistance in infectious microbes and non-availability of safe antifungal drugs for widespread fungal infections constrains a search for new antifungal components from other sources including plants (Aqil and Ahmad, 2003). Plants generally produce many secondary metabolites like flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates (Quiroga *et al.,* 2001) which constitute an important source of microbicides, pesticides and many pharmaceutical drugs.

The aim and objective of this project is to investigate the antifungal activity of the solvent extract of the leaf of *Premna latifolia* against *Fusarium* and *Rhizopus* by using disc diffusion method. Extraction of different concentrations is used to analyse the concentration at which the antifungal property is maximum.

REVIEW OF LITERATURE

Premna species are widespread and most of them are mainly scattered in the tropical and subtropical regions of Asia, Africa, Australia (Munir, 1984; Harley *et al.,* 2004) but closely related and geographically restricted species are distributed from India to Pacific Islands. *P. latifolia* is an evergreen tree, distributed all over India and other tropical and sub-tropical and coastal areas (Chopra *et al.,* 1992).

According to Khare (2007) the decoction of the leaves, stem, bark, or roots of four species of *Premna, P. herbacea, P. integrifolia, P. latifolia and P. tomentosa* are used in traditional medicinal system for treating asthma, rheumatism, neuralgia, diarrhoea and stomach disorder, hyperglycaemic and obesity. It is also used as a post-delivery tonic for women (Khare, 2007). The leaves and tender shoots of *Premna latifolia* are used in curries and for treating fevers and liver complaints. Leaf showed notable cardiotonic, anti- coagulant, and hepatoprotective properties (Weiss, 1979; Dianita and Ibrahim, 2017). Traditionally *P. latifolia* is used for dropsy, boils (Kumar *et al.,* 2018), fever and liver complaints (Kabra *et al.,* 2015). The use of the stem and bark of *P. latifolia* for wound healing is also reported by Jeevan Ram *et al.,* (2004) (Dianita and Ibrahim, 2017).

A lot of phytochemical work has been done on root bark and leaves of *P. latifolia* from which iridoids, sesquiterpenoids, diterpenoids, furanoid, and flavonoids have been reported (Jena *et al*., 2017). Preliminary phytochemical analysis of ethanol, chloroform, petroleum ether and aqueous extracts of *P. latifolia* and *P. herbacea* showed the strong presence of triterpenoids and alkaloids with trace amounts of carbohydrates and flavonoids (Thirumalai *et al*., 2013; Gowtham *et al*., 2013).

Mohd Nazri *et al*., (2011) evaluated the antifungal activities of dichloromethane and ethanol extracts of *Premna cordifolia* against *Candida albicans*. *P. cordifolia* extracts did not display any activity against the pathogenic fungus. Rajendran, (2010) studied the antimicrobial activity of ethyl acetate, ethanol and aqueous extracts of stem, wood and bark of *Premna serratifolia* against *Aspergillus flavus, Aspergillus niger, Penicillium notatum* and *Candida albicans.* All five extracts exhibited significant antifungal activity. Antifungal activity of crude extracts (hexane, chloroform, ethyl acetate, ethyl alcohol and aqueous) and fractions of *P. serratifolia* root were observed against *Candida albicans, Aspergillus flavus, Epidermatophyton flocossum, Penicillium chrysogenum,* and *Microsporum gypseum*. All the extracts and the fractions were effective in concentration of 33.3mg/ml against the fungi genera studied and possessed significant antifungal activity (Rajendran and Basha, 2010). Antifungal properties of ethanol extract of leaves of *Premna latifolia* was studied against *Candida albicans*. It exhibited a zone inhibition of 8-10 mm against the fungus (Ram *et al*., 2004).

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). In 2008, Lopez *et al*., reported in vitro anti oxidant and anti rhizopus activities of Lamiaceae herbal extracts. Eighty – eight extract of different polarity obtained from 18 Lamiaceae medicinal and aromatic plants were screened for their antioxidant and antifungal properties. Phlomis lychnitis, Salvia pratensis and Calamintha sylvatica caused the highest inhibition on rhizopus.

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.

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 was collected from Chavakkad, Guruvayur, Thrissur district, Kerala. 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 68413) 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 then filtered with the help of muslin cloth and filtered extract were taken and used for antifungal studies and phytochemical analysis.

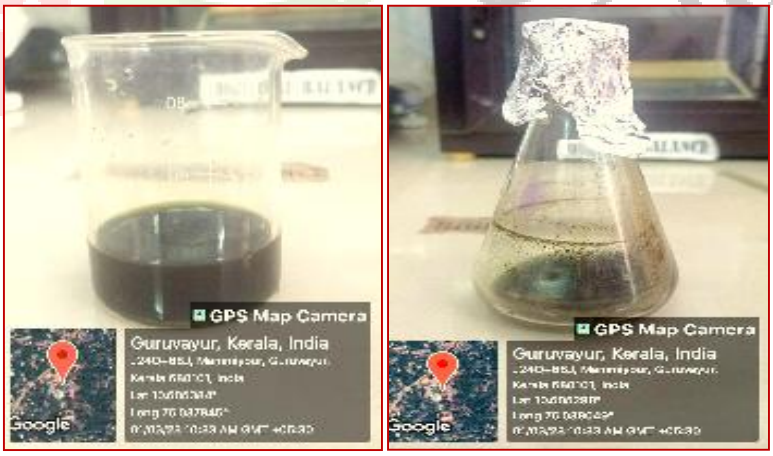

Fig. 1 Extraction Procedure

ANTIFUNGAL ACTIVITY

Fungal strains

Fusarium spp. and Rhizopus spp. *a*re the fungal strains were used for the antifungal analysis.

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

Antifungal Activity

The present examination has investigated the antifungal properties of methanol leaf extract of *Premna latifolia*. The antifungal activity of the leaf extract was quantitatively assessed by the presence or absence of inhibition well zone and by measuring the diameter of the inhibition zone around the discs. Clear inhibition zones were found after 24 hours of incubation at 37°C. The results of antifungal activity of the plant extracts are presented in **Table 1**; **Fig. 2**. The results show the antifungal activity of plant extract tested against two important fungal strains.

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

Table: 1 Antifungal activity of *Premna latifolia*- Leaf

Sl. No.	Sample fungal strains	*P. latifolia*
1	Fusarium sp.	12±1
2	Rhizopus sp.	13.3±0.6

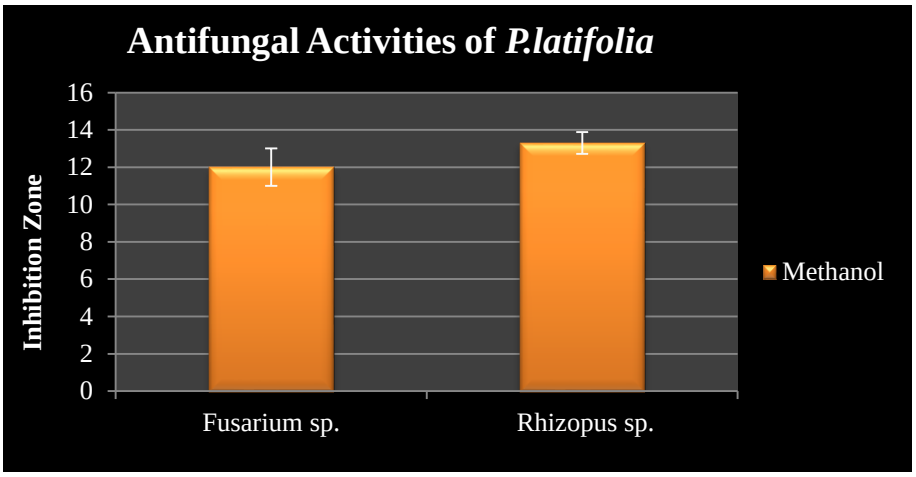

Fig. 2 Antifungal activity of *Premna latifolia*

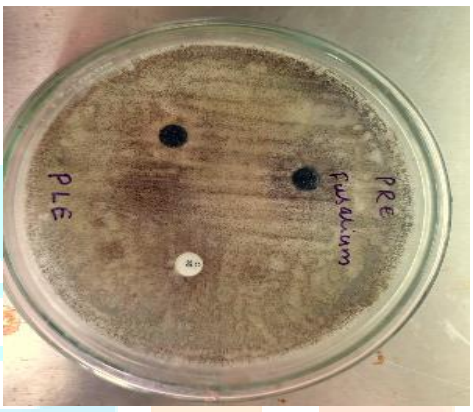

Fig.3 P.latifolia against Fusarium sp.

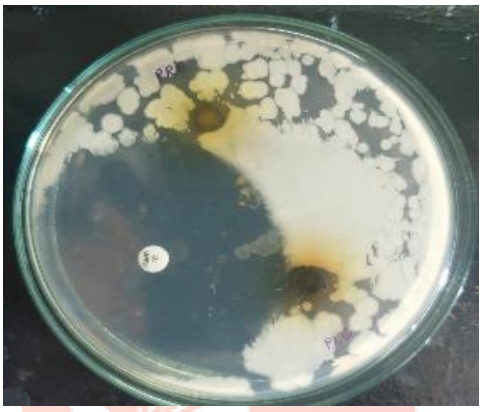

Fig.4 P.latifolia against Rhizopus sp.

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. latifolia* was selected for the present study. The study on the leaf of *P. latifolia* 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. latifolia* is necessary for elucidating the active principles and their mode of action.

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