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RESEARCH ARTICLE

BIOASSAY- GUIDED FRACTIONATION AND ANTI-FUNGAL ACTIVITY STUDIES ON *Pisonia grandis* R.BR.

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ABSTRACT

Pisonia grandis is an herb claimed to be used for treatment of various diseases by local folks. Since, this plant possesses many medicinal properties; there are not many scientific studies carried out on this plant which promoted us to pursue a systematic pharmacological evaluation of *Pisonia grandis* leaves to verify their medicinal properties. Bioassay- guided fractionation of ethanol extract of leaves of *Pisonia grandis* was studied for its anti-fungal activity against microorganisms *Candida albicans, Aspergillus niger, Pencillium citrinum* and *Monascus purpureus* by disc diffusion method. The ethanol extract showed good anti-fungal activity against *Monascus purpureus* comparable to standard clotrimazole.

INTRODUCTION

Pisonia grandis R.Br (*Nyctaginaceae*) is widely distributed throughout India and is a widespread evergreen commonly grown lettuce tree (Wealth of India 1969). Leaves, stem and root of this species are extensively used by the tribals in the preparation of several folk medicines. It has been extensively used in Indian traditional medicine as an anti diabetic (Sunil *et al*., 2009), anti inflammatory agent (Radha *et al*., 2008), and used for wound healing (Prabu *et al*., 2008), dysentery (Will McClatchey 1996) and filariasis (Dukes phytochemical database). It is also analgesic and diuretic (Anbalagan *et al.,* 2002).

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The plant has been studied by different workers with special reference to its pharmacological activity but no isolation of phytochemicals has been reported (Buenz *et al*., 2005). Also no report on the antifungal effects of *Pisonia grandis* exists. This paper reports the anti-fungal effects of its extracts with clotrimazole as reference drug.

MATERIALS AND METHODS

Collection of plant material

The plant material (leaves) was collected during January- March 2009 in the local areas of Coimbatore, Tamilnadu, India. The identity of plant material was confirmed by the taxonomist Dr. C. Kunhikannan, Scientist D, Biodiversity

Division, Institute of Forest Genetics & Tree Breading, Coimbatore. The leaves were dried in shade and cut into small pieces and then used for phytochemical study.

Preparation of leaf extract

Air dried pieces of leaves of *Pisonia grandis* were extracted with 100% ethanol for 6 hour at reflux temperature. The extract was filtered; the filtrate was evaporated to one tenth volume under reduced pressure to get a greenish black pasty solid (sample A).

colorless. Then the $CHCl_3$ and aqueous layers were combined separately. A portion of these two layers were concentrated and subjected to anti-fungal study. The rest of the $CHCl_3$ layer was distilled completely; the residue was dissolved in 10% aqueous ethanol for further extraction with pet-ether. The LLE with pet-ether is continued until the organic layer was colorless. Then the entire organic and aqueous layers were combined separately; distilled under vacuum and the residue was stored for anti-fungal study. Anti-fungal screening results for the above fractionated residues are given in

Table 1. Anti-fungal screening result (Zone of inhibition in mm)

Sample	*Candida albicans*	*Aspergillus niger*	*Penicillium citrinum*	*Monascus purpureus*
leaf ethanol extract (sample A)	----	----	----	25
Concentrated chloroform residue	10	10	----	20
Concentrated aqueous residue	12	12	10	15
Concentrated pet-ether residue	6	12	----	12
Concentrated 10% aqueous ethanol	14	8	----	20
Standard Clotrimazole	20	18	21	25

Table 2. Anti-fungal screening result for column fractions against *Monascus purpureus*

Sample at saturated concentration	Mobile phase in column chromatography	Zone of inhibition (mm)	MIC in µg/ml
Fraction 1	100% $CHCl_3$	10	
Fraction 2	99% $CHCl_3$and 1% MeOH	17	
Fraction 3	98% $CHCl_3$and 2% MeOH	13	
Fraction 4	98% $CHCl_3$and 2% MeOH	31	62.5
Fraction 5	98% $CHCl_3$and 2% MeOH	30	62.5
Fraction 6	98% $CHCl_3$and 2% MeOH	34	15.62
Fraction 7	97% $CHCl_3$and 3% MeOH	30	62.5
Fraction 8	97% $CHCl_3$and 3% MeOH	26	31.25
Fraction 9	97% $CHCl_3$and 3% MeOH	15	
Fraction 10	96% $CHCl_3$and 4% MeOH	18	
Fraction 11	6%,8%,10% MeOH	20	62.5
Fraction 12	88% $CHCl_3$and 12% MeOH	16	
Fraction 13	85% $CHCl_3$and 15% MeOH	13	
Fraction 14	20%,25%, 30% MeOH	8	
Fraction 15	40%, 50% MeOH	14	
Standard Clotrimazole 10µg/disc		25	10

Fractionation and column chromatography Procedure

A small portion of the sample A was set aside for testing its anti-fungal activity and the rest was macerated with equal volume of water and extracted with equal volumes of chloroform ($CHCl_3$). The Liquid liquid extraction (LLE) with $CHCl_3$ was continued until the $CHCl_3$ layer was

Table 1. A column of silica gel (400 g) built in $CHCl_3$ and was eluted with $CHCl_3$, $CHCl_3$- MeOH mixtures of increasing polarity. The homogeneity of the fractions was examined by TLC and similar fractions were combined and tested for anti-fungal activity (Table 2).

Anti-Fungal assay

The anti-fungal activity was assayed by Disc diffusion method. All the residues were dissolved in DMSO and stored as a stock solution. Species cultures were grown on Sabouarud's dextrose agar (www.microbelibrary.org) at 28° C, and each sample impregnated discs were placed on the agar and incubated at 28° C for 48 hrs then the clear zone of inhibition was measured.

RESULTS AND DISCUSSION

Anti-fungal screening results for the leaf ethanol extract (sample A) and for the various fractionated residues are given Table 1. The sample A and the various fractionated residues showed varying degrees of inhibition against all the fungal stains. Sample A possessed maximum anti-fungal activity for *Monascus purpureus* comparable to standard clotrimazole. Hence sample A was selected for column chromatographic analysis and the entire column fractions are tested against for *Monascus purpureus* fungal stains. Table 2 shows anti-fungal screening result for column fractions against *Monascus purpureus*. Since column fractions 4, 5,6,7,8 and 11 showed a higher zone of inhibition than the standard Clotrimazole these fractions were subjected to Minimum Inhibitory Concentration (MIC) study and the results are recorded in the Table 2. This study revealed the potentially active fractions from which the active principles could be isolated and the study is under progress in the same laboratory.

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