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Research Article

Bioassay- Guided Fractionation and Anti-Fungal Activity Studies on *Pisonia grandis* R.Br

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ABSTRACT

Bioassay- guided fractionation of ethanol extract of leaves of *Pisonia grandis* was studied for its anti-fungal activity for the microorganism *Candida albicans, Aspergillus niger, Pencillium citrinum* and *Monascus purpureus* by disc diffusion method. The ethanol extract showed good anti-fungal activity for *Monascus purpureus* compared to standard clotrimazole.

Keywords: *Pisonia grandis***,** *Nyctaginaceae***,** *Monascus purpureus*, Clotrimazole.

INTRODUCTION

Pisonia grandis R.Br (Nyctaginaceae) is widely distributed throughout India and is a widespread evergreen commonly grown lettuce tree[1]. 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 antidiabetic, anti-inflammatory agent, and used in the treatment of an algesia, ulcer, dysentery and snake bite[2-7]**.** The plant has been studied by different workers with special reference to its pharmacological activity but no isolation of phytochemicals has been reported[8]. Also no report on the antifungal effects of *Pisonia grandis* exists. This paper reports the anti-fungal effects of its extracts and 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).

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 colorless. Then the entire $CHCl_3$ and aqueous layer were combined. A portion of these two layers are concentrated separately 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 layer was combined and distilled by vacuum and the residue was stored for anti-fungal study.

A column of silica gel (400 g) built in $CHCl_3$ and was eluted with $CHCl_3$, Chloroform- methanol 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). Three compounds isolated from the column were also tested for their anti-fungal activity.

Anti-Fungal assay : The anti-fungal activity was assayed by Disk diffusion method. Sabouarud's dextrose agar is the most suitable medium because fungal growth is favored by a high sugar concentration and is relatively tolerant to acidity (pH 5.4) [9]. Nutrient agar plates were prepared when the agar medium was amended with complexes separately. Then the medium was warm and poured into petri plates. After solidification of the medium, mycelia discs (6 mm dia) of the test fungi were inoculated at the centre of the plates. Diameter of the inhibition zone for each fungus was measured after 48 hrs of incubation at 28° C.

RESULTS AND DISCUSSION

Table 1 shows the results of anti-fungal activity. The leaf ethanol extract and the various fractionated portion of the leaf extract showed varying degrees of inhibition against all the fungal stains but only the ethanol soluble extract of *Pisonia grandis* possesses maximum anti-fungal activity for *Monascus purpureus* compared to standard clotrimazole. Hence the ethanol extract of the leaves was selected for column chromatographic analysis.

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

Sample	*Candida albicans*	Aspergillus niger	Penicillium citrinum	Monascus purpureus
Concentrated ethanol residue	----	----	----	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 and for isolated compounds against *Monascus purpureus*

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

Table 2 shows anti-fungal screening result for column fractions against *Monascus purpureus* and column fractions 4, 5,6,7,8 and 11 showed a higher zone of inhibition than the standard Clotrimazole. The Minimum Inhibitory Concentration (MIC) values of these column fractions were also shown in Table 2. This study also revealed the potentially active fractions from which the active principles would be isolated in the same laboratory.

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