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. 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. The plant has been studied by different workers with special reference to its pharmacological activity but no isolation of phytochemicals has been reported. 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 Breeding, 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). 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. The plant has been studied by different workers with special reference to its pharmacological activity but no isolation of phytochemicals has been reported. 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.

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 (CHCl₃). The Liquid liquid extraction (LLE) with CHCl₃ was continued until the CHCl₃ layer was colorless. Then the entire CHCl₃ and aqueous layer were combined. A portion of these two layers are concentrated separately and subjected to anti-fungal study. The rest of the CHCl₃ 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 CHCl₃ and was eluted with CHCl₃, 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.

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**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 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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