

ANTITUBERCULAR ACTIVITY OF LEAVES OF *RHINACANTHUS NASUTUS* (L.)

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ABSTRACT

The antitubercular effect of n-hexane ,chloroform, ethanol extracts was prepared from leaves of *Rhinacanthus nasutus* (k) was evaluated against *Mycobacterium tuberculosis* using Microplate Alamar Blue assay (MABA).The Minimum inhibitory concentration was taken to assess antitubercular activity .The results showed that ethanolic extract have more significant Antitubercular activity as compared to n-hexane, chloroform extracts. Pyrazinamide and Streptomycin is taken as standard drugs.

KEYWORDS: *Rhinacanthus nasutus* (k), MIC, Antitubercular activity.

INTRODUCTION:

Rhinacanthus nasutus more commonly known as *Rangchita*, *Nagamalli* which belong to the family Acanthacea, is a shade loving perennial shrub.^{1, 2, 3}

Plant has been recently used in the treatment of hepatitis, diabetes, hypertension and skin diseases in Taiwan⁴. The decoction

of its roots or whole plant is drunk for treatment of some cancer forms. It has long been used in Thai traditional medicine for disease like pruritic rash, ring worm, abscess, pain, skin disease, and plant extract has inhibitory activity against different species of bacteria and fungi.⁵

The aim of our study was to investigate the Antitubercular activity of n-hexane, chloroform and Ethanolic extracts of *Rhinacanthus nasutus* leaves by using Microplate Alamar Blue assay.

MATERIALS AND METHODS

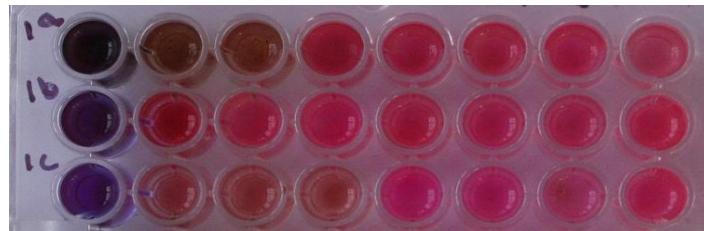
Plant Materials: The fresh leaves of *Rhinacanthus nasutus* were collected from Tirumala Hills, Tirupati and Chittoor district of Andhra Pradesh in the month of July - October and identified by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V.University and Tirupati.

Preparation of Extract: The powdered Leaves of *Rhinacanthus nasutus* was extracted with n-Hexane, Chloroform, Ethyl acetate and Ethanol successively by Soxhlation method and concentrated over water bath and evaporated under reduced pressure. The n-Hexane, Chloroform and Ethanolic extracts was chosen for Anti tubercular activity.^{1,2}

Chemicals: n-Hexane, Chloroform, Ethanol, 96 well plate, Middlebrook 7H₉ broth with *M. tuberculosis* H37 RV strain, Deionized water, Almar Blue reagent, 10% Tween 80, Incubator, Micropipettes.

EXPERIMENTAL PROCEDURE:¹⁰

- 1) Briefly, 200 μ l of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.
- 2) The 96 wells plate received 100 μ l of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.
- 3) The final drug concentrations tested were 100 to 0.2 μ g/ml.
- 4) Plates were covered and sealed with parafilm and incubated at 37°C for five days.
- 5) After this time, 25 μ l of freshly prepared 1:1 mixture of Almar Blue reagent and 10% Tween 80 was added to the plate and incubated for 24 hrs.
- 6) A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.
- 7) The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.



Microplate Alamar Blue assay

Phytochemical analysis:^{3, 6, 7}

The n-Hexane, Chloroform and Ethanol extracts were subjected to Thin Layer Chromatography using TLC plates (0.1 mm thick silica gel) eluted with n-hexane: Ethyl acetate (8:2) and chloroform :benzene (9:1) respectively. The spots were identified under long UV light by using UV cabinet.

RESULTS AND DISCUSSION:¹⁰

Traditionally medicinal plants have been used in folk medicine throughout the

world to treat various diseases; especially tuberculosis.^{11, 12}

We evaluated preventive effects of n-hexane, chloroform and ethanolic extracts of leaves of *Rhinacanthus nasutus* (L.) using Microplate Alamar Blue assay method. N-hexane, Chloroform and Ethanolic extracts was 12.5, 6.25, 3.125 $\mu\text{g}/\text{ml}$ respectively and compared to standard drug Pyrazinamide- 3.125 $\mu\text{g}/\text{ml}$, Streptomycin- 6.25 $\mu\text{g}/\text{ml}$

| S.No | Type of Extract | MIC ($\mu\text{g}/\text{ml}$) |
|------|-----------------|------------------------------------|
| 1. | N-Hexane (1b) | 12.5 |
| 2. | Chloroform (1a) | 6.25 |
| 3. | Ethanol (1c) | 3.125 |

MIC- Minimum inhibitory concentration

CONCLUSION:

This study reveals significant Antitubercular effect of N-hexane, Chloroform and ethanol extracts from leaves of *Rhinacanthus nasutus* (k). Further studies using more specific methods are required to

explore the constituents responsible for the activity and the mechanism of this activity which might prove important and improved therapies for the treatment and prevention of tuberculosis.

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