WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 10, 1284-1291. Research Article ISSN 2277– 7105

# ANTI-TUBERCULOSIS ACTIVITY OF MURRYA KOENIGII USING TH MICRO PLATE ALAMAR BLUE ASSAY

***Aditya P. Patil and Preeti G. Karade**

Pharmaceutics Department, Appasaheb Birnale College of Pharmacy, Sangli-416416, Maharashtra, India.

Article Received on
28 September 2014,

Revised on 23 Oct 2014,
Accepted on 18 Nov 2014

***Correspondence for Author**
**Aditya P. Patil**
Pharmaceutics Department, Appasaheb Birnale College of Pharmacy, Sangli-416416, Maharashtra, India.

## ABSTRACT

The present study describes the Anti-tuberculosis (anti-TB) activity of oil obtained by hydro-distilation from the leaves of *Murraya koengii* by Micro-plate Alamar blue assay (MABA). The oil of *Murraya koengii* was separated by using Clavenger apparatus. The emeregence of multi-drug resistant and extensively drug resistant strains of *Mycobacterium tuberculosis* has created the problem in treatment. In present study a hope for developing alternate medicine for the treatment of TB. *Murraya koenigii* shows good anti-tuberculosis activity at different concentration were Pyrazinamide, Streptomycin and Ciprofloxacin are used as standard drug.

**KEYWORDS:** *Murraya koenigii, Myobacterium tuberculosis,* Alamar blue Assay, Micro Plate, oil, and Anti-tuberculosis acitivity.

## INTRODUCTION

Today, there is widespread intrest in herbal drugs. This interest primarily stems from the belief that herbal medicines are safe, inexpensive and have no adverse effects. As per the World Health Organization guideline, 80% of the drugs are obtained from herbal plant. These herbal drugs are used in the treatment of Cancer, TB, AIDS, Malaria, Polio, Diabetes, Typhoid, Leprosy, Renal failure, and Kidney failure. *Murraya koengii* which is also called "Currypatta", is native to south East Asia and Australia, It grows wild and is found almost throughout India up to height of 1500 to 1655m. This plant has been reported to possess activities like Anti-dermatophytic, Anti-inflammatory, Immunomodulator, Hepatoprotective, Anti-diabetic, Cytotoxic, Anti-oxidant and Antifungal activity. [2]

Tuberculosis or TB (short for tubercle bacillus), in the past also called Phthisis, Phthisis Pulmonalisoe Consumption, is a common and in many cases fatal, infectious disease caused by various strains of *Mycobacteria tuberculosis*. Tuberculosis mostly attacks on the lungs. It is spread through the air when people who have an active TB infection like Cough, Sneeze, or otherwise transmit respiratory fluids through the air. Most infections donot have symptoms known as latent tuberculosis. Some drugs are like Isoniazide, Rifampicin, Pyrazinamide, Ethambutol, Streptomicin, cyclocerine, amikacin, shows Antitubercular activity. But these drugs are having some side effects like Peripheral neuritis, Neurological manifestation, Mepatitis, Renal failure, Haemolysis, Respiratory syndrome, Jaundice etc. [3]

Hence to overcome the side effects of the other Antitubercular drugs, we extract *Murraya koenigii***,** an Ayurvedic drug having no side effects.

**Curry tree[4]**

The curry tree is native to India, Sri-lanka, Bangladesh and the Andaman Islands. Later spread by Indian migrant, they now grow in other areas of the world where Indian immigrants settled. Widely cultivated, the leaves are particularly associated with the south Indian cuisines.

**Origins**

Curry leaf trees is naturalized in forests and waste land throughout the Indian subcontinent except in the higher part of the Himalayas. From the Ravi river in Pakistan its distribution extends eastwards towards Assam in india and Cittagong in Bangladesh, and southwards to Tamil Nadu in India. The plants were spread to Malaysia South Africa and Reunion Island with south Asian immigrants.

**History**

The use of curry leaves as a flavouring for vegetables is described in early Tamil Nadu literature dating back to the 1$^{st}$ to 4$^{th}$ centuries AD. Its use is also mentioned a few centuries later in Kannada Literature. Curry leaves are still closely associated with south India where the word; Curry" originates from the Tamil Nadu 'Kari' for spiced sauces. An alternative name for Curry leaves throughout India is Kari-patta. Today Curry leaves are cultivated in India, Sri-lanka, South East Asia, Australia and pacific Islands and in Africa as a foods flavouring.

This article is about *Murraya koengii*, a tree which produces an aromatic leaf often used in Indian cuisine and its Anti-TB activity.

**Morphology**

**Curry leaf tree**

| Scientific classification | |
|---|---|
| Kingdom | Plantae |
| (Unranked) | Rosids |
| Class | Eudicots |
| Order | Sapindales |
| Family | Rutaceae |
| Genus | *Murraya* |
| Species | *M. koenigii* |
| Binomial Name | |
| *Murrayakoenigii Sprangel*[4] | |

The curry tree (*Murraya koenigii*) is a tropical to sub- tropical tree in the family Rutaceae, which is native to India and Sri Lanka .its leaves are used in many dishes in India and Neighbouring countries. Often used in curries, the leaves are generally called by the name "Curry leaves", through they are also translated as "sweet Neem leaves" in most Indian languages (as opposed to ordinary Neem leaves which are bitter).

**The small flowers are white and fragrant**

It is a small tree, growing 4-6 m (13-20 feet) tall, with a trunk up to 40 cm diameter. The leaves are pinnate, with 11-12 leaflets, each leaflet 2-4 cm long and 1-2 cm broad. They are highly aromatic. The flowers are small, white, and fragrant. The small black shiny berries are edible, but their seeds are poisonous. The species name commemorates the botanist Johann Konig.

**Chemical Constituents**

The leaves of Murrayakoenigii contain Anthroquinone Glycosides, Alkaloid, Carbohydrates, Proteins, Phytosterols, Tannis, Flavonoids, Volatile oil, and Saponins. [9]

It also contains Murrayanine (32%), glycoside scopoline (25%), free glucose (3.5%) and ach (10.4%). Carbazole alkaloid also isolated from this plant viz Mahanimbine, Girinimbine, Isomahanimbine, Murrayazoline, Murrayazolidine, and Mahanine. [8]

## MATERIAL AND METHOD

**The Plant Material**

The leaves of Murrayakoenigii were collected from Sangli district in morning hours. They were stored in sterile polythene bags and transfrrred to the laboratory and stored at $4^0$C till time of use.

**Extraction of Essential Oil**

Extraction is carried out by Hydro-distillation method. Fresh leaves of M. koenigii (100g) were chopped and subjected to Hydro- distillation using a Clevenger – type apparatus for duration of 8 hours. Distilled oil water collected and stored in air tight glass vials and allowed it to freeze.

**Determination of Anti-Tuberculosis Activity**

A variety of methods have been developed to measure the sensitivity of Mycobacterium tuberculosis. [5] In the present study we used Micro-plate Alamer blue assay (MABA) in which Alamar blue was used as the dye. It is rapid and low cost method for the sensitivity study of Mycobacterium tuberculosis. This bioassay may also be used to establish relative Cytotoxicity of agents within various chemical classes. [6] Over the years, a number of improved and high throughput techniques towards screening of Anti-Mycobacterial agents have been developed. The Microplate Alamar Blue Assay is a clorimetric oxidation-reduction based assay. It is a non-radiometric, rapid, comparatively low cost assay producing results

with a high degree of confidence. Moreover, this technique has been used by a number of researchers for testing Anti-Mycobacterium activity of several plants.

**Anti-TB Activity Using Alamar Blue Dye**

1) The anti-Mycobacterialactivity of compounds was assessed against *M. tuberculosis* using micro-plate Alamar Blue assay (MABA).
2) This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.
3) Briefly, 200µl of sterile deionised water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during inhibition.
4) The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilutions of compound were made directly on plate.
5) The final drug concentrations tested were 100 to 0.2µg/ml.
6) Plates were covered and sealed with parafilm and incubated at 37$^{o}$c for five\days.
7) After this time, 25 µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.
8) A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth.
9) The MIC was defined as lowest drug concentration which prevented the color change from blue to pink. [7]

**RESULT AND DISCUSSION**

The anti-TB sensitivity of essential oil from leaves against *Mycobacterium tuberculosis* was observed by using Micro-plate Alamar Blue Assay by observing intensity of color. This method uses thermally stable reagent (Almar Blue reagent) and is non-toxic. The results are shown in the Table no.1. The *Mycobacterium tuberculosis*is sensitive at a concentration of 100, 50, and 25µg/ml.. The bacteria exhibited resistance at a concentration of 12.5, 6.25, 3.125, 1.6 and 0.8µg/ml. Anti-TB activity found from 25 to 100µg/ml.

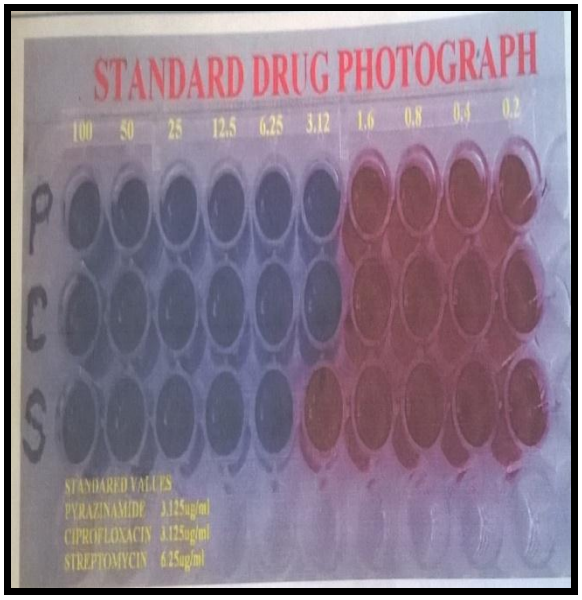


**Figure 3Standard Drug Photograph**

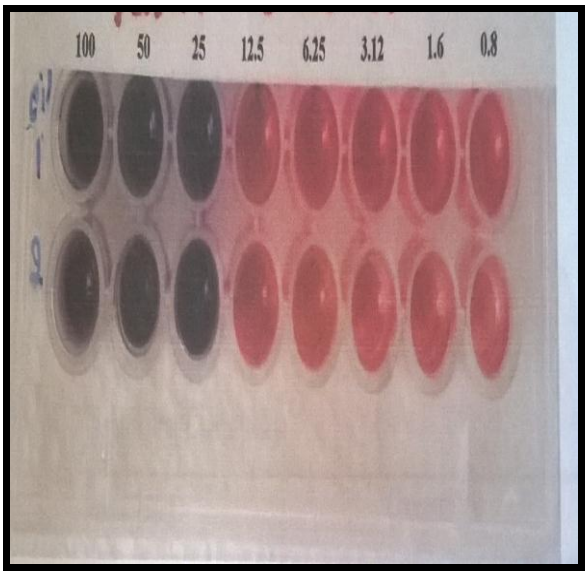


**Figure 4 Result Photograph**

The intensity of color decreases as the concentration decreases. Here the standard drug was taken as Pyrazinamide, Streptomycin and Ciprofloxacin (Figure 3) which were compared With result (Figure 4).

**Anti-TB Results**

**Table No:-1 Standard Drug Concentration**

| Standard drug | Concentration |
|---|---|
| Pyrazinamide | 3.125µg/ml |
| Streptomycin | 6.25µg/ml |
| Ciprofloxacin | 3.125µg/ml |

**Table No:-2. Result of Anti-Tubuerculosis Activity of Test Oil**

| Sl no. | Samples | 100 µg/ml | 50 µg/ml | 25 µg/ml | 12.5 µg/ml | 6.25 µg/ml | 3.12 µg/ml | 1.6 µg/ml | 0.8 µg/ml |
|---|---|---|---|---|---|---|---|---|---|
| 1 | MC | S | S | S | R | R | R | R | R |

**NOTE**

S- Sensitive

R- Resistance

Strain used: M. Tuberculosis (H37 RV strain)

MC – Murraya koenigi

The standard values for the Anti-TB test which was performed here are.

## CONCLUSION

An exclusive literature survey did not afford any information regarding anti-tuberculosis activity of this plant. Thus in the present study an attempt is made to explore anti-tuberculosis potential of oil from the leaves of Murraya koenigi. It gives good result at three different concentration (100, 50, 25µg/ml) and it may used as an alternative drug in case of XDR and MDR tuberculosis.

## ACKNOWLEDGEMENT

The authors are greatly thankful to Appasaheb Birnale College of Pharmacy, Sangli, and Maharashtra, India for providing access to facilities and necessary infrastructure to carry out research work.

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