

ANTI-TUBERCULAR ACTIVITY OF METHANOL AND AQUEOUS EXTRACT OF LEAVES OF *TINOSPORA CORDIFOLIA* (WILD) MIERS

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ABSTRACT

Medicinal plants have known to be the potential source for the treatment of many ailments. The present study is designed to investigate the *in vitro* activity of methanol and aqueous extract prepared by soxhlet and microwave assisted extraction of the leaves of *Tinospora cordifolia* against *Mycobacterium tuberculosis* H73Rv strain using Micro plate Alamar Blue Assay (MABA). The results revealed that the methanol and aqueous extract prepared by both methods showed sensitivity against *M. tuberculosis* strain at a concentration 100 µg/ml. The present investigation suggests that methanol and aqueous extract of the leaves of *Tinospora cordifolia* possess remarkable anti-tubercular activity. Further studies for isolation of phytoconstituents responsible for antitubercular activity is under process.

KEYWORDS: *Mycobacterium tuberculosis*, methanol extract, aqueous extract, *Tinospora cordifolia*, *in vitro* MABA, H37Rv.

1. INTRODUCTION

Tuberculosis (TB), infectious diseases in lungs caused by *Mycobacterium tuberculosis* affects the health burden in the world. In 2014, nearly 80% of reported TB cases occurred in 22 countries and India has the largest number of cases, 23% of the global total. [1] In India, nearly 50% of patients have been reported to be tuberculin test positive and one person dies from TB every minute. [2] However, Due to the usage of antibiotics, challenge of multidrug resistant TB has increased drastically. Therefore, there is a need for discovery of new anti-TB drugs, which are cost effective and safe. Medicinal plants have known to be the potential source for the treatment of number of diseases. India has rich wealth of medicinal plants and usage of herbal medicines as traditional knowledge for curing several diseases is well documented. [3] In this regard, one plant viz., leaves of *Tinospora cordifolia* was investigated.

In traditional system of medicine, *Tinospora cordifolia* Wild Miers commonly named as “Guduchi” belonging to family Menispermaceae is known for its immense application in the treatment of various diseases.

A variety of active components derived from the plant like alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides have been isolated from the different parts of the plant body, including root, stem, and whole plant. The plant is of great interest to researchers across the globe because of its reported medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities [4, 5, 6].

Hence, the present study is designed to investigate the *in vitro* activity of methanol and aqueous extract prepared by soxhlation and microwave assisted extraction from the leaves of *Tinospora cordifolia* against *Mycobacterium tuberculosis* H73Rv strain using Micro plate Alamar Blue Assay (MABA).

2. MATERIAL AND METHODS

2.1 Plant material

The leaves of *Tinospora cordifolia* was collected from local market in Bangalore, Karnataka, India and it was identified and authenticated by CCRAS. A voucher specimen (TOCOP/01/2020-21- *Tinospora cordifolia*) was deposited in The Oxford College of Pharmacy, Bangalore. The leaves were dried in shade and powdered coarsely, passed through sieve no. 40 and stored in airtight container for further use.

2.2.1 Preparation of extract by soxhlet technique

Coarsely powdered leaves of *T.cordifolia* 20 g, each were subjected to extraction in soxhlet extractor with methanol and boiled with distilled water (150 ml) each respectively. The two extracts of leaves were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 9.79 and 14.52 % w/w respectively with reference to the air-dried leaf sample. [7]

2.2.2 Preparation of extract by Microwave assisted extraction(MAE)technique

Coarsely powdered leaves of *T.cordifolia* 20 g, each were subjected to extraction in Microwave extractor (Ragotech Company Microwave extractor with Power 3, set at temperature 75⁰C for 15 minutes with continuous stirring) with methanol and water [150 ml] respectively. The two extracts of leaves were concentrated by rotary vacuum evaporator and evaporated to dryness. The yield was found to be 4.76 and 10.92 % w/w respectively with reference to the air-dried leaf samples. [8]

2.3.1 Chemicals

All chemicals and reagents used in this study were at least of analytical grade.

2.4 Anti-tubercular activity

The *in vitro* activity of methanol and aqueous extract of leaves by both method of *Tinospora cordifolia* have been evaluated against *Mycobacterium tuberculosis* H73Rv strain using Micro plate Alamar Blue Assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.

Briefly, 200µl 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. The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration, which prevented the color change from blue to pink. The drug Pyrazinamide was used as positive standard for comparison. [9]

3. RESULTS AND DISCUSSION

Medicinal plants provide numerous examples of interesting secondary metabolites indicating that natural products could be a helpful for the discovery of new anti- TB agents. Medicinal plants produce an excessive diversity of phytochemicals that could be active in medicinal field. There is an increasing demand of medicinal plants all over the world because the allopathic medicines have more side effects.[10] A diversity of methods has been developed to measure the sensitivity of *M. tuberculosis*. It is interesting to mention that some medicinal plants may not contain phytochemicals, which inhibit the growth of *M. tuberculosis*, but may have stimulant or modulatory effects on the immune system. [11] In present study the anti-tubercular activity was assessed by Micro-plate Alamar blue assay (MABA), using Alamar blue as the dye. The (MABA) Assay is a colorimetric oxidation-reduction based assay in which the Alamar blue dye accepts electrons and changes from the oxidized, non-fluorescent blue state to the reduced fluorescent pink state. In addition to mitochondrial reductase, other enzymes such as the diaphorases, dihydrolipoamine dehydrogenase, quinoneoxidoreductase and flavin reductase located in the cytoplasm and the mitochondria may be able to reduce Alamar blue reagent.[12] The anti-tubercular potential of methanol and aqueous extract of leaves prepared by soxhlet and microwave assisted extraction of *T.cordifolia* which inhibited the growth of *M. tuberculosis* H37Rv strain at given in the Table 1. A blue color in the well was interpreted as no bacterial growth and pink color was scored as growth [Fig 1 and Fig 2].

The qualitative visible change in color indicates on presence or absence of viable cells. Standard drug used were Pyrazinamide, Streptomycin, Isoniazid, Ethambutol and Rifampicin. Methanol extract and aqueous extracts prepared by both methods had antitubercular activity with an MIC value of 100 µg/ml. The MIC values for standard were found to be Pyrazinamide- 3.125 µg/ml; Streptomycin- 0.8 µg/ml; Isoniazid-1.6 µg/ml; Ethambutol-1.6 µg/ml and Rifampicin- 0.8 µg/ml respectively.

4. CONCLUSION

TB has become a serious infection as *Mycobacterium tuberculosis* develops resistance against both first and second-line anti-TB drugs. Due to this, there is an urgent need for novel anti-tuberculosis agents, which should be sensitive. Hence, the present investigation have conclude that methanol and aqueous extract from leaves of *Tinospora cordifolia* have shown anti-mycobacterial activity when compared with standard drugs such as pyrazinamide, streptomycin, Isoniazid, Ethambutol and Rifampicin. Further isolation of phytoconstituents responsible for the antitubercular activity is under process.

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6. ETHICAL ISSUES

There is none to be applied.

7. CONFLICT OF INTEREST

None to be declared.

8. REFERENCES

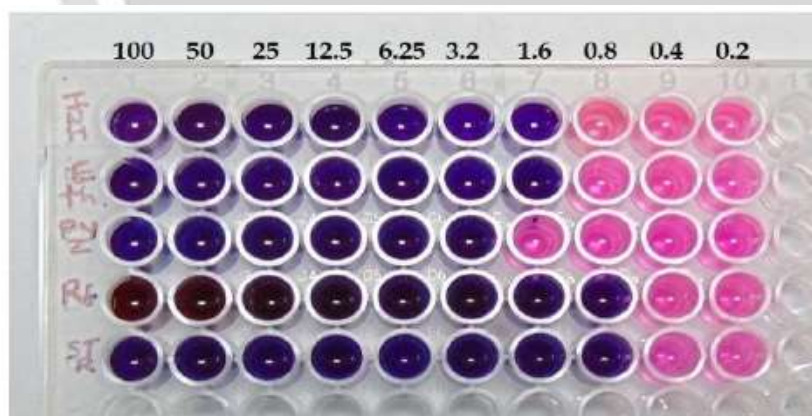
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Table.1 : Anti-tubercular activity of methanol and aqueous extract prepared by soxhlet and microwave extraction of leaves of *T.cordifolia*

Sl. No.	Sample	100 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$	25 $\mu\text{g/ml}$	12.5 $\mu\text{g/ml}$	6.25 $\mu\text{g/ml}$	3.12 $\mu\text{g/ml}$	1.6 $\mu\text{g/ml}$	0.8 $\mu\text{g/ml}$
01	TC-01	S	R	R	R	R	R	R	R
02	TC-02	S	R	R	R	R	R	R	R
03	TC-03	S	R	R	R	R	R	R	R
04	TC-04	S	R	R	R	R	R	R	R

S – Sensitive; R – Resistance. Strain used: *M. tuberculosis* (H37RV strain).

TC-01: Methanol extract soxhlet; TC-02: Aqueous extract soxhlet; TC-03: Methanol extract MAE and TC-04: Aqueous extract MAE

Fig 1: Anti-tubercular activity of methanol and aqueous extract of *T.cordifolia* leaves**Fig.2: Antitubercular activity of standard drug**

MIC values of Pyrazinamide- 3.125 $\mu\text{g/ml}$; Streptomycin- 0.8 $\mu\text{g/ml}$; Isoniazid- 1.6 $\mu\text{g/ml}$; Ethambutol-1.6 $\mu\text{g/ml}$ and Rifampicin- 0.8 $\mu\text{g/ml}$.