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SCREENING OF FOUR INDIAN MEDICINAL PLANTS FOR *IN VITRO* ANTIMYCOBACTERIAL ACTIVITY

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ABSTRACT
In the present study an attempt has been made to find out the *in vitro* antimycobacterial potential of four medicinal plants which, are commonly used to treat respiratory disease in the indigenous systems of medicine. The leaves of *Adhatoda vasica*, leaves of *Aegle marmelos*, whole plant of *Solanum trilobatum*, leaves of *Tectona grandis* were tested. It was found that, the methanolic extracts of Leaves of *Tectona grandis* possessed high antimycobacterial activity at a concentration of 200 μg/mL onwards. Whereas the other plants did not inhibit the growth of *Mycobacterium tuberculosis* even at a concentration of 3200 μg/mL when tested *in vitro*.

INTRODUCTION

Plants are used as medicines since time immemorial. India has a rich heritage of using medicinal plants in traditional medicines such as Ayurveda, Siddha and Unani besides folklore practices. The earliest mention of the medicinal uses of plants is found in the Rigveda which is one of the oldest repositories on human knowledge (Chopra *et al*., 1958). Fairly comprehensive information of the curative properties of some of the herbs has been recorded in "Charaka samhita" and "Sushirutha samhita". The plant kingdom is a virtual goldmine of biologically active compounds and it is estimated that only 10-15 percent of 2, 50,000-7, 50,000 of existing species of higher plants have been surveyed (Congress, 1983).

Tuberculosis is one of the oldest killer diseases of mankind. It is an airborne communicable disease caused by transmission of aerosolized droplets *of Mycobacterium tuberculosis* affecting almost all the organs of the body, the lungs being most commonly involved. It is estimated that 3 million people die from tuberculosis each year the majority of them are in developing countries. Around 8 million people become infected with tuberculosis every year. The WHO report on Tuberculosis (WHO Fact Sheet No.104), estimated that between 2000 and 2020, nearly one billon people will be newly infected, 200 million people will get sick and 35 million will die from it, if effort to control is not further intensified. Tuberculosis kill's more adults in India than any other infectious disease. Every year, 2 million people in India develop Tuberculosis and nearly 0.5 million die from it, more than 1000 per day and one every minute (RNTCP status report – 2001), In India approximately 50% of the population is reported to be tuberculin positive. Every year about 0.4 million deaths and one million new cases of Tuberculosis are reported (TRC Bulletin, 1994).

Many workers have reported the anti microbial potential for various plants of foreign origin (Baker *et al.,* 1995; Contell *et al.,* 1998; Elmer *et al.,* 1998; Wacheter *et al*., 1998; Contell *et al.,* 1999a; Cantell *et al.,* 1999b; Cantell *et al.,* 1999c; Houghton *et al.,* 1999). But, though we have an enormous wealth of medicinal plants throughout the length and breadth of our country, no detailed attempt has been made to reveal this most in needed activity of the plant kingdom. Considering the need for newer antimycobacterial drugs and the existence of enormous wealth of medicinal plants in our country, it was planned to study the *in vitro* antimycobacterial activity of some of the medicinal plants which are commonly used in the treatment of respiratory disease like bronchitis, haemoptysis and asthma in the indigenous systems of medicine.

The present communization aims to find out the *in vitro* antimycobacterial activity of four medicinal plants *Adhatoda vasica Nees*. Syn: *Adhatoda zeylanica, Aegle marmelos* Linn. *Solanum trilobatum* Linn. *Tectona grandis* Linn., which are used for treating respiratory diseases in the indigenous system of medicine in India and also for the treatment of tuberculosis by rural hereditary medicinal practitioners of Tamil Nadu.

MATERIALS AND METHODS

Procedure for plant analysis

Selection of the plants

The plant materials used in the study are 1. Leaves of *Adhatoda vasica;* 2. Leaves of *Aegle marmelos;* 3. Whole plant of *Solanum trilobatum;* 4. Leaves of *Tectona grandis*

Procurement of plants

The plant materials were collected from the Botanical garden of Sri Ramachandra Medical University, Chennai, Tamil Nadu, India, during the month of April and May of 2009. They were identified by Prof.Chamundeeswari Pharmacognist Sri Ramachandra Medical University. The voucher specimens are identified and deposited there.

Solvents and chemicals

Methanol was used for the extraction purpose. The commercial grade solvent was purchased (Ranbaxy Fine Chemicals Ltd., New Delhi, India) distilled and the purified forms was used for extraction procedure in this study.

Preparation of crude extract

The leaves of *A. vasica* and *A. mamelos* and the whole plants of *S. trilobatum* and leaves of *T. grandis* were dried in shade and coarsely powdered and extracted by cold percolation method.

Total methanolic extract

500 g of each of the four plant materials were shade dried, coarsely powdered and soaked in 1L methanol in different aspirator bottles and exhaustively extracted at room temperature for 72 hr. The solvent was decanted, filtered and distilled off in Rotovac apparatus. The combined methanol extracts were completely dried from solvent under reduced pressure using high vacuum conditions. The extracts were tested for the antimycobacterial activity *in vitro*.

***In vitro* antimicrobial activity**

Preparation of medium

Antimycobacterial test were done on Lowenstein-Jensen medium (Himedia). The extracts were dissolved in dimethyl sulfoxide (DMSO) and added to the medium before inspissations. The extracts were added to the medium in separate flasks to give final concentrations of 12.5, 25, 50, 100, 200, 400, 800, 1600, and 3200 μg/mL of the medium. The medium is distributed in the about 7 mL quantities into sterile McCartney bottles and inspissated once about 85ºC for 50 minutes.

Preparation of bacterial suspension

About 2/3 loop full (3 mm internal diameter) of the mycobacterial growth from fresh culture was taken in a sterile Bijou bottle containing 10 glass beads (3 mm) and 1 mL of distilled water. The contents were mixed well in vortex mixture for about 1 minute.

In vitro antimycobacterial activity of total methanolic extract

One loop full (3 mm) of the bacterial suspension thus obtained was inoculated on to one slope of Lowenstein-Jensen medium containing no extract (control) and to the slopes of Lowenstein-Jensen medium containing various concentrations of the different plant extracts. All the slopes were incubated at 37ºC for 4 weeks. At the end of every week all the inoculated slopes were examined for the growth. Presence of 20 or more colonies was considered as a growth. The lowest concentration of the plant extracts which inhibited the growth of *mycobacterium tuberculosis i*.e. less than 20 colonies or no growth (The Minimum Inhibitory Concentration or MIC) was noted (Manual of Laboratory Methods Bacteriology Department, Tuberculosis Research Centre, Indian Council of Medicinal Research, 1987). 15 strains of M. tuberculosis isolated from the patients attending Poonamallee TB centre (District TB centre), Chennai-600 056, Tamil Nadu, India. The isolated pathogens were carried out as per the standard procedure by the Microbiology Division, and processed in ACS Medical College and Hospital a unit of Dr.MGR University Chennai-600 095, Tamil Nadu, and India

RESULTS AND DISCUSSION

500 g each of shade dried, and coarsely powdered *A.vasica* (leaves) when extracted with methanol yielded 10 g of greenish brown pasty mass while *A. marmelos* (leaves) when extracted with methanol yielded 12 g of pale brown residue; *S. trilobatum* (whole plant) when extracted with methanol yielded 13.5 g brownish mass and *T. grandis* (leaves) when extracted with methanol yielded 11 g dark greenish yellow residues respectively (Table 1).

Table 1: Percentage yield of total methanolic extracts of plants (500g each)

Plant	Weight of dried extract (in g)	Yield %	Colour	Consistency
Adhatoda vasica	10	0.6	Greenish brown	Paste
Aegle marmelos	12	0.8	Pale brown	Paste
Solanum trilobatum	15.5	1.1	Brown	Paste
Tectona grandis	11	0.9	Dark greenish yellow	Paste

Of the four extracts tested for their activity against 15 strains (different patients) of *M. tuberculosis*, only the total methanolic extract of *T. grandis* showed activity at concentration of 200 μg/mL onwards. Whereas the other plants did not inhibit the growth of *M. tuberculosis* even at concentration of 3200 μg/mL when tested *in vitro* (Table 2).

Table 2: *In vitro* antimycobacterial activity of total methanolic extrats number of strains tested-15

Concentration inμg/mL	Plants			
	Adhatoda vasica	*Aegle marmelos*	*Solanum trilobatum*	*Tectona grandis*
12.5	NA	NA	*NA*	*NA*
25	NA	NA	NA	NA
50	NA	NA	NA	NA
100	NA	NA	NA	NA
200	NA	NA	NA	**A**
400	NA	NA	NA	**A**
800	NA	NA	NA	**A**
1600	NA	NA	NA	**A**
3200	NA	NA	NA	**A**

A = Active; NA = Not Active

In the present study, an attempt was made to find out the antimycobacterial potential of four medicinal plants which

are commonly used to treat respiratory diseases and for treating Tuberculosis in indigenous systems of medicine. These plants are leaves of *Adhatoda vasica,* leaves of *Aegle marmelos*, whole plant of *Solanum trilobatum*, leaves of *Tectona grandis*. It was found that, of these four plants only leaves of *Tectona grandis* possessed high antimycobacterial activity.

Tectona grandis contains tannin, which are used as anti-inflammatory agents and also used topically for treatment of burns (Shah *et al*., 1995). Leaves have also been used in indifenous medicine. Extracts of the leaves showed complete inhibition of *Mycobacterium tuberculosis*.

The results suggest the presence of either good antimycobacterial potency of leaf extract of *Tectona grandis* contain high concentration of an active compounds like Tectoleafquinone (1, 4, 5, 8, - tetrahydroxy-2 isopentadienyl anthraquinone), betulinac acid, tectoquinone (2-methyl anthraquinone), Juglone (5 hydroxy methyl 2, 4 napthaquinone; data base on medicinal plants used in Ayurveda).

Further study on the fractions of active components in *Tectona grandis* leaves and the maximal specious may provide better understandings of the antimycobacterial activity. In since *Tectona grandis* appear to be most promising, bioassay–guided fractionation is currently underway with a goal of elucidating their active antimycobacterial compound.

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