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## Effects of selective Medicinal Plants against Multi Drug Resistance *Mycobacterium Tuberculosis* Strains

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### ABSTRACT

The increase of multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) demands the search for alternative antimycobacterial drugs. The aim of this study was to evaluate plants used in Indian traditional medicine to treat respiratory diseases for activity against MDR-TB. Six plants (*Taxus baccata*, *Senna alata*, *Andrographis paniculata*, *Adhatoda vasica* Nees, *Acalypha indica* L., *Aloe vera* L.) was selected for activity against *Mycobacterium tuberculosis* and aqueous extracts of these leaves at concentrations from 2%, 4%, 6% were tested *in vitro* for their activity against one MDR isolate (DKU-156), reference susceptible strain *M. tuberculosis* H37Rv as well as rapid grower mycobacterial pathogen *M. fortuitum* (TMC-1529) using Lowenstein Jensen (L-J) medium. Activity in L-J medium was evaluated by percentage inhibition which was calculated by mean reduction in number of colonies on extract containing as compared to extract free controls. Extracts of all the six plants *Taxus baccata*, *Senna alata*, *Andrographis paniculata*, *Adhatoda vasica* Nees, *Acalypha indica* L., *Aloe vera* L exhibited anti-tuberculosis activity in L-J medium, the proportion of inhibition of these plants extract in respect mentioned above is 100, 32, 46, 43, 75, 51 per cent, respectively for MDR isolate DKU-156, while for *M. tuberculosis* H37Rv, inhibition was found to be 86, 46, 40, 71, 69, 51 per cent, and 66, 0, 0, 0, 33, 33 percent against rapid grower *M. fortuitum* (TMC-1529) at 6 per cent concentration in L-J medium. All these plant extract showed promising quantifiable antimicrobial activity against MDR *Mycobacterium tuberculosis* (DKU-156), and can be used for future ethanopharmacological studies and drug development.

**Key words:** *Taxus baccata*; *Senna alata*; *Andrographis paniculata*; *Adhatoda vasica* Nees; *Acalypha indica* L; *Aloe vera* L; Anti-Mycobacterial activity.

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## INTRODUCTION

Tuberculosis is a highly infectious disease with about one third of the world's population including 40 per cent from India estimated to be infected it [1]. In 1993, the World Health Organization stated that tuberculosis was an emerging disease. Each year 8 million people are infected by *Mycobacterium tuberculosis* (*M. tuberculosis*) and 2–3 million patients die from the disease. It is estimated that between 2000 and 2020, nearly 1 billion people will become infected, 200 million will acquire the disease and 35 million will die from tuberculosis[15] The disease spreads more easily in overcrowded settings and in conditions of malnutrition and poverty. New TB cases have been estimated at 9.2 million during 2006, with 1.7 million MTB-related deaths [2]. The resurgence of tuberculosis (TB) as a major disease in many parts of the world, is prompting the search for novel compounds, active against the causative organism, *Mycobacterium tuberculosis* [3].

However, this problem has become serious as *Mycobacterium tuberculosis* developed resistance against both the first line as also the second line drugs. Due to this, there is emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *M. tuberculosis* all over the world including India [1]. Treatment of MDR-TB requires the use of at least three drugs not prescribed previously to the patient and in some cases four or more anti tuberculosis agents are necessary; and still, not all cases are cured. In this scenario, potent new drugs are urgently needed to deal properly with cases of MDR-TB [15].

Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases for many centuries. India is one of the few countries in the world which has unique wealth of medicinal plants and vast traditional knowledge of use of herbal medicine for cure of various diseases [4].

Natural products isolated from plants have played an important role in discovery of drugs against infectious diseases [2,16]. Almost 75% of the approved anti-infective drugs are derived from medicinal plants. According to World Health Organization, more than 65% of the global population uses medicinal plants as a primary health care modality [2].

So far, few plants have been tested against mycobacteria [4]. The increasing incidence of MDR- and XDR-TB worldwide highlight the urgent need to search for newer anti-tuberculosis compounds/drugs [2].

Therefore, the aim of the present study was to evaluate the antimycobacterial activity of aqueous extracts of six plants against MDR isolates of *M. Tuberculosis*. Plants were chosen based on their traditional uses in India, information obtained from the siddha medicine literature [5] and ethnobotanical information [6-9] that is, they have been used in traditional medicine for the treatment of TB or symptoms of this disease in our environment.



## MATERIAL AND METHODS

### Plant Description

Aloe vera L. is a xerophytic herb belongs to family Aloaceae. *Taxus baccata* is a tree belongs to the family Taxaceae, *Acalypha indica* L, *Andrographis paniculata* are herbs belongs to the family Euphorbiaceae, Acanthaceae. *Adhatoda vasica* Nees., *Senna alata* are shrubs belongs to the family Acanthacea, Cesalpinaceae respectively [5].

### Plant Material

Leaves of *Taxus baccata*, *Senna alata*, *Andrographis paniculata*, *Adhatoda vasica* Nees, *Acalypha indica* L., *Aloe vera* L were collected and authenticated by faculty from the Department of Botany, Mahatma Gandhi Memorial college Manipal University of Mangalore.

### Preparation Of Plant Extract

Leaves were washed with double distilled water twice and fresh juice of the leaves are prepared by crushing leaves in mortar and pestle by using sterile distilled water in ratio 1:1 [1].

### *Mycobacterial* Strains

*M. tuberculosis* H37Rv as control, MDR-TB (DKU-156), and fast growing mycobacterial pathogen *M. fortuitum* (TMC-1529) were used for Anti-*Mycobacterial* assay [1].

### Anti- *Mycobacterial* Assay

Antimicrobial assays are performed in Lowenstein Jensen (L-J) medium, The plant extract is incorporated in the medium at concentration of 2 per cent, 4 per cent and 6 per cent (2 ml, 4 ml and 6ml of fresh plant extract is dissolved into 100 ml of culture medium) prior to inspissation. For comparison, extract free control slants were used [1]. The ten-fold dilution of standard 1 mg/ml *M. tuberculosis* suspension are made and 0.01ml of this suspension is streaked on L-J medium with the help of 4mm (external diameter) loop in the presence and absence of plant extracts. The medium is incubated at 37°C for 60 days [3]. Reading was taken weekly. Each test is done in Triplicate [1]. Percentage inhibition is calculated by mean reduction in number of colonies on extract containing as compared to extract free controls [5]. The number of colonies grown on the L-J media is counted on extract containing and extract free control L-J slants after 42 days of incubation at 37°C were recorded (Table -1).

Table 1- Results of Anti-tuberculosis Assay Using Extracts in L-J Medium

Botanical name	Part used	extract	Mycobacterial strains	L-J proportion method						
				Mean cfu on media			% Inhibition			
				control	Plant extract			Plant extract		
				2%	4%	6%	2%	4%	6%	
<i>Taxus baccata</i>	Leaf	water	<i>M.fortuitum</i>	3+	3+	2+	1+	0	33	66
			<i>M.tuberculosis</i>	35	17	7	5	51	80	86
			MDR –TB	28	5	1	0	82	96	100
<i>Senna alata</i>	Leaf	water	<i>M.fortuitum</i>	3+	3+	3+	3+	0	0	0
			<i>M.tuberculosis</i>	35	29	23	19	17	34	46
			MDR –TB	28	25	21	19	11	21	32
<i>Andrographis paniculata</i>	Leaf	water	<i>M.fortuitum</i>	3+	3+	3+	3+	0	0	0
			<i>M.tuberculosis</i>	35	28	24	21	20	31	40
			MDR –TB	28	19	18	15	21	35	46
<i>Adhatoda vasica</i>	Leaf	water	<i>M.fortuitum</i>	3+	3+	3+	3+	0	0	0
			<i>M.tuberculosis</i>	35	17	13	10	51	60	71
			MDR –TB	28	22	19	16	21	29	43
<i>Acalypha indica</i>	Leaf	water	<i>M.fortuitum</i>	3+	3+	3+	2+	0	0	33
			<i>M.tuberculosis</i>	35	29	17	11	17	51	69
			MDR –TB	28	18	13	7	36	54	75
<i>Aloe vera</i>	Gel	water	<i>M.fortuitum</i>	3+	3+	3+	2+	0	0	33
			<i>M.tuberculosis</i>	35	27	23	17	23	34	51
			MDR –TB	28	19	15	9	32	46	68

1+ 1-19, 2+ 20-100, 3+ >100colonies,  
*M. fortuitum* (TMC-1529), *M. tuberculosis*( H37Rv), MDR-TB (DKU-156)

### RESULTS AND DISCUSSION

The increase of resistance to conventional antibiotics by microorganisms has necessitated the search for new, efficient and cost effective ways for the control of infectious diseases [2].

All these *Taxus baccata*, *Senna alata*, *Andrographis paniculata* , *Adhatoda vasica* Nees, *Acalypha indica* L., *Aloe vera* L plants exhibited anti tubercular activity against *M. tuberculosis* H37Rv multi-drug resistant isolate (DKU-156), but only three(*Taxus baccata*, *Acalypha indica* L., *Aloe vera* L )of these plants exhibited inhibition of *M. fortuitum* (TMC-1529) growth. The proportion of inhibition of these plants extract in respect mentioned above is 100,32,46,43,75 51 per cent, respectively for MDR isolate DKU-156, while for *M. tuberculosis* H37Rv, inhibition was found to be 86,46,40,71,69, 51 per cent,and 66,0,0,0,33,33 percent against rapid grower *M. fortuitum* (TMC-1529) at 6 per cent concentration in L-J medium.

*Taxus baccata* has anti tubercular activity against *M. tuberculosis* H37Rv while *A. vera*, *A. vasica* against MDR isolates of *M. tuberculosis* up to the concentration of 4% [1,8] this is supported by previous studies also, from our study *Taxus baccata* showed inhibitory property against MDR – TB and *M. tuberculosis* H37Rv. The aqueous extract appears to have less anti mycobacterial activity than the methanolic extract. This is interesting in that the traditional method of treating a bacterial infection was by administering a decoction of the plant or a part there of by boiling it in water [16], but in vitro studies an organic solvent result is better [17]; in vivo a high dose of aqueous extract may effective.

Although *Senna alata*, *Andrographis paniculata* plant extracts were previously reported to have strong antibacterial [11, 12], they showed weak activity against MTB in this study. In choosing the plant extraction method, we anticipated that aqueous extract may contain many chemical compounds with antimicrobial activities such as alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins, and triterpenoids [14]. The anti tubercular property of *A. vasica* is believed to be the presence of bromhexine and ambroxol [6]. Although further studies are required to determine whether these compounds act synergistically with any of the antituberculosis drugs [6].

A key target for antimycobacterial chemotherapy is cell wall biosynthesis. Due to the complex lipoglycan calyx on the cell surface, which provide a significant physical barrier to intracellular acting, compounds many antibiotics do not work on MTB [2]. This may explain the lack of activities showed by some of the plant extracts against MTB in this study. The novel bioactive compounds which could act synergistically with the classic tuberculosis drugs within the host macrophages might have direct access to dormant organisms [12]. It is now widely accepted that a physiological state of non replicating persistence of the tubercle bacillus (NRP-TB) is responsible for the long treatment duration for tuberculosis [13].

All these plant extract showed promising quantifiable antimicrobial activity against MDR *Mycobacterium tuberculosis* (DKU-156), and can be used for future ethano-pharmacological studies and drug development.

## CONCLUSION

This study compile some of antitubercular plants from Siddha medicine to give scientific account on usage of anti-tubercular plants. Various phytoconstituents like alkaloids, flavonoids, tannins, xanthonenes, triterpenes, quinones etc. were involved in anti-tubercular activity<sup>18</sup>. This study made an attempt to give scientific account of use of medicinal plants extracts in tuberculosis treatment.

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