Anti-tuberculosis activity of selected medicinal plants against multi-drug resistant *Mycobacterium tuberculosis* isolates

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**Background & objectives:** Emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *Mycobacterium tuberculosis* has further complicated the problem of tuberculosis (TB) control. Medicinal plants offer a hope for developing alternate medicines for the treatment of TB. The present study was done to evaluate *in vitro* anti-tubercular activity of five medicinal plants viz., *Acalypha indica*, *Adhatoda vasica*, *Allium cepa*, *Allium sativum* and *Aloe vera*.

**Methods:** Aqueous extracts of leaves of *A. indica*, *A. vasica*, bulbs of *A. cepa*, cloves of *A. sativum* and pure gel of *A. vera* leaves, were tested *in vitro* for their activity against two MDR isolates (DKU-156 and JAL-1236), reference susceptible strain *M. tuberculosis* H37Rv as well as rapid grower mycobacterial pathogen *M. fortuitum* (TMC-1529) using Lowenstein Jensen (L-J) medium and colorimetric BacT/ALERT 3D system. 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.

**Results:** Extracts of all the five plants *A. indica*, *A. vasica*, *A. cepa*, *A. sativum* and *A. vera* exhibited anti-tuberculosis activity in L-J medium, the proportion of inhibition of these plants extract in respect mentioned above is 95, 32, 37, 72, 32 per cent, respectively for MDR isolate DKU-156 and 68, 86, 79, 72, 85 per cent, respectively for another MDR isolate JAL-1236, while for sensitive *M. tuberculosis* H37Rv, inhibition was found to be 68, 70, 35, 63 and 41 per cent, at 4 per cent v/v concentration in L-J medium. There was no inhibition against rapid grower *M. fortuitum* (TMC-1529). In BacT/ALERT also, extracts of these plants showed significant inhibition against *M. tuberculosis*.

**Interpretation & conclusions:** Our findings showed that all these plants exhibited activity against MDR isolates of *M. tuberculosis*. While the anti-TB activity of *A. vera*, *A. vasica* and *A. sativum* against MDR isolates confirm earlier results, activity of the extracts of *A. indica* and *A. cepa* is reported for the first time. Further studies aimed at isolation and identification of active substances from the extracts which exhibited promising activities, need to be carried out.

**Key words** *Acalypha indica* - *Adhatoda vasica* - *Allium cepa* - *Allium sativum* - *Aloe vera* - anti-tuberculosis activity

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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. 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.

Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases for many centuries. These have been used extensively as pure compounds or as a crude material. Only a few plant species have been thoroughly investigated for their medicinal properties. 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. So far, few plants have been tested against mycobacteria and a few plants which showed anti-TB activity were *Salvia hynargea*, *Euclea natalensis*, etc. The increasing incidence of MDR- and XDR-TB worldwide highlight the urgent need to search for newer anti-tuberculosis compounds/drugs. Therefore, the present study was carried out to check the antibacterial activity of aqueous extracts of five plants against MDR isolates of *M. tuberculosis*, reference susceptible strain *M. tuberculosis* H37Rv as well as fast growing mycobacterial pathogen *M. fortuitum* (TMC-1529). Plants already reported to have anti-tuberculosis activity (*Adhatoda vasica*11,12, *Allium cepa*3,14 and *Aloe vera*15-17) were selected to test their activity further against MDR strains of *M. tuberculosis*, while selection of *Acalypha indica*18 was based on its ethno-medicinal uses in respiratory disorder. *A. cepa* was selected on the basis of knowledge that *A. sativum* has anti-tuberculosis activity; therefore other species of *Allium* might also have anti-tuberculosis activity.

**Material & Methods**

**Collection of plants:** Leaves of *Acalypha indica* L. (*Euphorbiaceae*), *Adhatoda vasica* Nees. (*Acanthaceae*), *Aloe vera* L. (*Aloaceae*) and bulbs of *Allium cepa* L. (*Alliaceae*) and *Allium sativum* L. (*Alliaceae*) were collected between spring and summer season during March to May 2004 from Paliwal Park and Khandari campus Agra, UP. All specimens were identified at Department of Botany, School of Life Sciences, Dr B.R. Ambedkar University, Agra.

**Extract preparation:** Fresh juice of leaves of *Aloe vera* was used as extract, while water extracts of other plants were prepared by crushing fresh leaves or bulbs in mortar and pestle by using sterile distilled water in ratio 1:2 (*Adhatoda vasica*) and 1:1 (remaining three plants).

**Mycobacterial strains/isolates:** Reference drug susceptible strain *M. tuberculosis* H37Rv as control, multi-drug resistant isolates DKU-156, JAL-1236 and fast growing mycobacterial pathogen *M. fortuitum* (TMC-1529) were obtained from Mycobacterial Repository Centre, Department of Microbiology and Molecular Biology at National JALMA Institute for Leprosy and other Mycobacterial Diseases (ICMR), Agra. Susceptibility profile of the strains against standard anti-tuberculosis drugs was as follows: DKU-156 resistant to RIF (rifampicin), INH (isoniazid), EMB (ethambutol), STR (streptomycin); JAL-1236 resistant to RIF, INH, STR, KAN (kanamycin), and OFL (ofloxacin).

**Assay protocol:** Antimicrobial assays were performed in Lowenstein Jensen (L-J) medium and Middlebrook 7H9 broth in BacT/ALERT 3D system (Sigma-Aldrich, St. Louis, UAS).

(i) Determination of Colony forming units (cfu) on Lowenstein-Jensen (L-J) - The ten-fold dilution of standard 1 mg/ml *M. tuberculosis* suspension were streaked on L-J medium for determining cfu in the presence and absence of plant extracts. An *M. tuberculosis* suspension of 1 mg/ml is equivalent to MacFarland standard-1. One loopful (6 µl) of this suspension was streaked on the L-J slants using 3 mm external diameter loop. Reagents of L-J media included potassium di hydrogen phosphate anhydrous (Qualigens), magnesium sulphate anhydrous (Qualigens), magnesium citrate (Loba Chemie), L-asparagine (Hi-media, Mumbai), glycerol (Fisher Scientific, Mumbai), and malachite green (Hi-Media, Mumbai).

The plant extract was incorporated in the medium at concentration of 2 per cent v/v and 4 per cent v/v (2 ml and 4 ml of fresh plant extract was dissolved into 100 ml of culture medium) prior to inspissation. The medium set inoculated with the standard bacterial suspension and incubated at 37°C for 42 days. Reading was taken weekly. For comparison, extract free control slants were used. Susceptibility testing of MDR isolates was also performed against standard drugs like: rifampicin and isoniazid in the same batch of media for comparison of cfu on drug free controls. Each test was done in duplicate. Percentage inhibition was calculated...
by mean reduction in number of colonies on extract containing as compared to extract free controls.

(ii) Middlebrook 7H9 broth in BacT/ALERT 3D system - Exposure of mycobacterial suspension (0.2 ml, 1mg/ml) to the millipore (0.22 μm) filtered plant extract (4% v/v) was done for 15 min at room temperature. The resultant mixture was inoculated into Mycobacterial Process (MP) bottles containing Middlebrook 7H9 broth supplemented with reconstitution fluid (Oleic acid, glycerol, & bovine serum albumin) in colorimetric BacT/ALERT 3D system (BioMerieux, France). The bottles were loaded in the instrument’s incubation module at 37° C. As per the prescribed method for determining susceptibility to anti-TB drugs, the relative delay of 3.5 days in positivity of drug (plant extract)-containing bottles as compared to that in drug-free control was considered as the criterion for susceptibility to particular drug (plant extract in this case) containing medium\textsuperscript{21,22}. An isolate was considered to be susceptible to (inhibited by) a drug if the drug-containing bottle was not flagged positive within 3.5 days of the positive signal in drug-free control bottle. This has been correlated with more than 90 per cent inhibition of growth by a drug (anti-microbial agent) as compared to that in the drug free medium\textsuperscript{21}.

Results

Average growth and percentage inhibition of \textit{M. tuberculosis} H37Rv, MDR isolates and rapid grower \textit{M. fortuitum} (TMC-1529) by L-J proportion method on extract containing and extract free control L-J slants after 42 days of incubation at 37°C were recorded (Table). Inhibition of \textit{M. tuberculosis} isolates was observed for all these medicinal plants, but none of these plants exhibited any inhibition of \textit{M. fortuitum} (TMC-1529) growth.

Anti-tuberculosis activity of all five plants was also tested in Middlebrook 7H9 broth using BacT/ALERT 3D system. For \textit{M. tuberculosis} H37Rv, extract-free bottle flagged positive after 3.99 days whereas those

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<th>Botanical name/ Family</th>
<th>Common name</th>
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<th>Extract</th>
<th>Isolate code</th>
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<td>\textit{M. tuberculosis} H37Rv</td>
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LE, leaf; BU, bulb; CL, clove; 3°, confluent growth
containing plant-extracts remained negative up to 10 days indicating the inhibition of growth.

Similarly, for MDR isolates of *M. tuberculosis*, the time-delay for positive signal in extract-containing bottles as compared to extract-free bottle was more than 8 days in case of *A. indica*, while for *A. cepa* and *A. sativum*, this delay was more than 30 days, indicating the inhibition of mycobacterial growth. However, bottles containing *A. vasica* and *A. vera* extracts flagged positive within 3.5 days of the positive signal in extract-free bottle, indicating that mycobacterial growth was not inhibited by these extracts.

**Discussion**

Tuberculosis has been a major health problem for developing countries including India. Due to increase in MDR and XDR strains of *M. tuberculosis*, there is an urgent need of finding newer anti-mycobacterial agents to combat this problem.

In the present study, water extracts of selected medicinal plants (*Acalypha indica*, *Adhatoda vasica*, *Allium cepa*, *Allium sativum* and *Aloe vera*) were observed to have anti-tuberculosis activity against two MDR *M. tuberculosis* isolates and drug-susceptible reference strain *M. tuberculosis* H37Rv and poor/no activity against rapid grower *M. fortuitum* (TMC-1529). These MDR isolates were earlier found to be resistant against at least rifampicin and isoniazid, in addition to some other first line and second line drugs. As inhibition of growth by these extracts was observed in both the systems, inference about their anti-tuberculosis activity appears to be meaningful. However, more studies using more isolates/strains of *M. tuberculosis* as well as fractions of crude extract/ purified/semi-purified principles of the above plants are needed to conclude about the anti-tuberculosis potential and promise of these plants for their ultimate use in the treatment of drug resistant tuberculosis.

Dry weights of 4 ml extracts of various plants were found to be 2.8 mg (*Acalypha indica*), 2 mg (*Adhatoda vasica*), 3.3 mg (*Allium cepa*), 8 mg (*Allium sativum*), and 0.2 mg (*Aloe vera*). Even though our procedure has indirect calculation of dry weight, it would be better to quantify the dry weight of each extract, dilute them in water and obtain MICs for each one.

Previous studies using radiometric BACTEC have reported anti-mycobacterial activity of *Eucalea natalensis* and its active principle. There are reports about anti-tuberculosis activity of *A. vera* against reference susceptible strain H37Rv while *A. vasica* and garlic have been tested against clinical isolates which were resistant to streptomycin and isoniazid, respectively. We observed activity of extracts of *A. vera*, *A. vasica* and garlic against MDR isolates of *M. tuberculosis*. In addition, anti-TB activity of *A. indica* and *A. cepa* against susceptible *M. tuberculosis* H37Rv as well as MDR isolates of *M. tuberculosis* is perhaps being reported for the first time. Further studies should be carried out using various fractions of crude extracts of these plants as well as their semi-purified/ purified principle responsible for anti-tuberculosis activity (specially for MDR and XDR isolates of *M. tuberculosis*) to find out the minimum inhibitory concentration (MIC) in suitable broth based media as MICs defined in broth are more accurate. Though we did not find any significant activity of these extracts against *M. fortuitum* (TMC-1529), these may still have activity against other slow growing non-tuberculous mycobacteria (NTMs) which respond to anti-TB drugs but mostly at higher concentration levels.

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**References**


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