Anti- mycobacterial Activity of Aloe vera Against M. tuberculosis & M. avium Standard Strain

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ABSTRACT

Background: The infectious killer disease, tuberculosis (TB), is the leading cause of death worldwide from a single human pathogen, claiming more adult lives than diseases such as Acquired Immunodeficiency Syndrome (AIDS), Malaria, Diarrhoea, Leprosy and all other tropical diseases combined. Due to indiscriminate use of Antimicrobial drugs, microorganisms have developed resistance to many antibiotics that has created immense clinical problems in the treatment of infectious diseases. Different studies reported that 1–31% of TB patients experience drug related hepatotoxicity following TB treatment. In the present scenario of emergence of drug resistance in pathogenic organisms there is a need to developed alternative antimicrobial drugs for treatment of infectious diseases. Plant extracts have been used as traditional medicines against disease including tuberculosis and many plant secondary metabolites have been reported to have anti- mycobacterial activity. Therefore, the aim of the present study was to evaluate the anti-mycobacterial activity of Aloe vera against M. tuberculosis & M. avium Standard strains.

Methods: Fresh Aloe vera gel was added in the LJ medium at concentration of 2 %, 4% & 6 %. Anti-mycobacterial activity against Mycobacterium tuberculosis H37Ra (MTCC 300) and Mycobacterium avium (MTCC 1723) was calculated in-vitro as Percentage inhibition, which was calculated by mean reduction in number of colonies on extracts containing media as compared to extract free (control) media.

Results: Aloe vera showed the inhibitory activity against both Mycobacterium tuberculosis H37Ra (MTCC 300) and Mycobacterium avium (MTCC 1723). 2%, 4% & 6% of Aloe vera pure gel containing LJ medium exhibited 25, 35 & 56% percentage inhibition against M. tuberculosis and against M. avium it was 20%, 26% & 33% respectively.

Interpretations & Conclusions: Studied plant Aloe vera exhibited positive results regarding anti-mycobacterial activity. Further studies are required, to get greater insight into the drugs of herbal origin, attempts should be made to identify, purify and evaluate the active principles in the plant products.

KEYWORDS Aloe vera, M. avium, M. tuberculosis
INTRODUCTION
Tuberculosis: An overview
The infectious killer disease, tuberculosis (TB), is the leading cause of death worldwide from a single human pathogen, claiming more adult lives than diseases such as Acquired Immunodeficiency Syndrome (AIDS), Malaria, Diarrhoea, Leprosy and all other tropical diseases combined.[1]
The organism usually responsible is the tubercle bacillus, *Mycobacterium tuberculosis* (MT), discovered by Robert Koch in 1882. However, *M. bovis*, which infects cattle, may also infect man and *M. africanum* is a cause of TB in West Africa. Furthermore, a number of normally non-pathogenic mycobacteria, especially *M. avium*, *M. intracellulare* and *M. scrofulaceum*, cause opportunistic infectious disease in patients with AIDS. [2]

Tuberculosis (TB) is a major global health problem. It causes ill-health among millions of people each year and ranks alongside the human immunodeficiency virus (HIV) as a leading cause of death worldwide. In 2014, there were an estimated 9.6 million new TB cases: 5.4 million among men, 3.2 million among women and 1.0 million among children. There were also 1.5 million TB deaths (1.1 million among HIV-negative people and 0.4 million among HIV-positive people), of which approximately 890 000 were men, 480 000 were women and 140 000 were children. [3]

India is highest Tuberculosis (TB) burden country in the world with an estimated 2.2 million new TB cases occurring annually [4]. Tuberculosis (TB) is principally a disease of poverty, with 95 per cent of cases and 98 per cent of deaths occurring in developing countries.[5]

Tuberculosis is commonest opportunistic infection (OI) in Human Immunodeficiency Virus (HIV) infected individuals. *M. tuberculosis*, *M. avium* and *M. kansasii* which have recently emerged as major opportunistic infections among Acquired Immuno Deficiency Syndrome(AIDS) patients[6].

Drug Resistant TB & Drug Reactions
Situation has worsened in recent past as, *Mycobacterium* 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. [3].

Anti-TB drug induced hepatotoxicity, is another cause of concern. Among the first line anti-TB drugs, pyrazinamide, isoniazid and rifampicin have all been associated with hepatotoxicity and the risk is enhanced when these drugs are used in combination [7,8]. Different studies reported that 1–31% of TB patients experience drug related hepatotoxicity following TB treatment [9].

Due to indiscriminate use of Antimicrobial drugs, microorganisms have developed resistance to many antibiotics that has created immense clinical problems in the treatment of infectious diseases.[10]

In the present scenario of emergence of drug resistance in pathogenic organisms there is a need to developed alternative antimicrobial drugs for treatment of infectious diseases.[11]

Medicinal Plants: A great Hope
Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases from 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. [12]. Plant extracts have been used as traditional medicines against disease including tuberculosis and many plant secondary metabolites have been reported to have anti-mycobacterial activity[13,14,15,16,17,18,19,20].

*Aloe vera* L

Family:*Xanthorrhoeaceae*, Sub family: *Asphodeloideae*, Genus:*Aloe*, Species:*Aloe vera*

The species is frequently cited as being used in herbal medicine since the beginning of the first century AD. Extracts from *Aloe vera* are widely used in the cosmetics and alternative medicine industries, being marketed as variously having rejuvenating, healing, or soothing properties. In the pharmaceutical industry, *Aloe vera* has been used for the manufacture of tropical products such as ointments and gel preparations, as well as in the production of tablets and capsules[21,22]. Important pharmaceutical properties that have recently been discovered from both the *Aloe vera* gel and whole leaf extracts includes the ability to improve bioavailability of co-administered vitamins in human subjects [23]. The biological activities include promotion of wound healing, antifungal activity, hypoglycemic or antibiotic effects, anti-inflammatory, anticancer, immunomodulatory and gastro protective properties.

Gupta R et al (2010) and Bernaitis L (2013)- Used fresh Juice of leaves of *Aloe vera* as extract in their study and reported anti-mycobacterial activity[19,20]

Therefore, the aim of the present study was to evaluate the anti-mycobacterial activity of *Aloe vera* against *M. tuberculosis* & *M. avium* Standard strains

**MATERIAL AND METHOD**

This Observational, descriptive study was done in Department of Microbiology, Jawahar Lal Nehru Medical College, Ajmer, during January - September 2017.

**C. Mycobacterial Strains**

Culture strain of *Mycobacterium M. tuberculosis H37Ra* (MTCC 300) and *M. avium* (MTCC1723) were obtained from The Microbial Type Culture Collection and Gene Bank (MTCC), a national Institute funded jointly by the Department of Biotechnology (DBT) and the Council of Scientific and Industrial Research (CSIR), Government of India. The revival of lyophilized strains was done as per the standard operating procedures (SOPs) provided by MTCC, IMTECH, Chandigarh & ATCC Guidelines using L-J Medium & Middlebrook 7H9 broth medium. The growth of cultures of Acid-fast bacilli grown on the solid medium was confirmed to be *M. tuberculosis*, using combination of observation of colony morphology, results of bio-chemical tests & Z-N Staining.

**Plant Material**

*Aloe vera* leaves were the plant material used. Leaves were handpicked. Mature plants included, plant which appeared to have viral, bacterial or fungal infections were excluded. Plants were picked from Nag Pahad of Pushkar near Ajmer (Rajasthan) India, approximately at Latitude 26.°N and Longitude 74°E. Duration of collection was done during March–May2017. Botanical authentication was done by Botanist of our team from D A V College, Ajmer.
**Preparation of fresh Aloe vera gel**

Leaves of *Aloe vera* were washed to remove the adhering dust for 2-3 times. The outer layer or Green Rind was removed using a knife. Inner leaf gel was blended using a blender, thus a semisolid gel was obtained. This extract gel filtered using Whatman filter paper No. 42 (125mm). The gel was preserved in refrigerator for further use.

**C. Preparation of Culture Media**

All culture media were prepared in the Department of Microbiology, JLN Medical College, Ajmer. At every point, Manual of Standard Operating Procedures (SOPs) were followed [24].

**Material used for Preparation of Lowenstein-Jensen (LJ) Medium**

Note: One batch of LJ medium is 1600 ml of solution.

- Warning Blender (mixer) with Sterile Jar marked of 1000 ml capacity, along with sterile lid.
- Sterile funnel with double layer of gauze fixed over mouth.
- 1 x 1lit stainless steel jars.
- 2 x 1 litres sterile round, flat bottomed flask.
- 500 ml methylated spirit.
- Sterile McCartney bottles (28 ml universal containers) (~300 per batch of medium).
- Sterile Mineral salt solution, 600 ml with malachite green.
- Fresh hen’s eggs (24 to 28 eggs per batch of 1000 ml fluid). Quality of media depends on the freshness of the hen’s eggs.
- Inspissator, thermostatically maintained at 85°C.
- Clean bench surfaces and a Bunsen burner

**Mineral SALT SOLUTION**

**Ingredients:**
- Potassium dihydrogen phosphate anhydrous (KH2PO4) A.R. 2.4 g
- Magnesium sulphate (MgSO4.7H2O), A.R. 0.24 g
- Magnesium citrate 0.6 g
- Asparagine 3.6 g
- Glycerol (reagent grade) 12 ml
- Malachite green, 2% solution* 20 ml
- Malachite green solution 2%
- Malachite green dye 2.0 g
- Distilled water 100 ml

Dissolve the dye in distilled water by grinding the dye with water using a mortar and pestle. Filter and store in refrigerator. Dissolve the ingredients in order in about 300 ml distilled water by heating. Add glycerol, malachite green solution and make up 600 ml with distilled water. Autoclave at 121°C for 30 minutes to sterilize. Cool to room temperature. This solution keeps indefinitely and may be stored in the refrigerator.

**Preparation of LJ Medium with anti-TB drug Isoniazid & Plant Extracts- 2%, 4%, 6%**

Isoniazid (H): Isoniazid 0.2 μg/ml, Drug potency = 1g to 1g substance. Potency Factor = 1

HIMEDIA Product No CMS7169

Stock solution preparation: Weigh out 20mg of Isoniazid powder in 40ml of sterile distilled water to obtain a concentration of 500μg/ml Isoniazid solution. Label with date of preparation, as ‘H Stock solution’.
Working solution: Prepare the working solution 1 ml of stock solution (500μg/ml) + 24ml of sterile distilled water (=25ml of 20μg/ml). Sterilize by filtering through a 0.22 μ membrane filter. Do not store this solution.

Addition to LJ plain medium: Add 1ml per 100ml LJ medium prepared.

Addition of Plant Extracts: 2%, 4%, 6%
2%, 4%, and 6% extract containing culture media was prepared. The Aloe vera gel was incorporated in the medium at concentration of 2 per cent v/v and 4 per cent & 6 per cent v/v (2 ml, 4 ml & 6 ml of 1% fresh plant gel stock solution was dissolved into 100 ml of culture medium. Further, Inspissation in inspissator at 85°C for 85 min was done [19,20].

Estimation Colony forming units (cfu) on Lowenstein-Jensen (L-J) Medium:

*M. tuberculosis* H37Ra (MTCC 300) and *M. avium* (MTCC 1723) suspension of 1 mg/ml, equivalent to Mac-Farland standard-1 was prepared. Ten-fold dilution of standard 1 mg/ml suspension was streaked on L-J medium for determining cfu in the presence and absence of plant extracts. A 0.01 ml of this suspension was inoculated on each L-J slant [20]. Susceptibility testing of this strain was also performed against standard drug 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.
The medium set inoculated with the standard bacterial suspension incubated at 37°C for 42 days, reading was taken weekly. For comparison, extract free control slants were used. Blank slants were also incubated to check the sterility/ quality of the medium. Susceptibility testing of this strain was also performed against standard drug isoniazid [19] 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 [20].

**OBSERVATION AND RESULTS**

Table.1. Results of anti- mycobacterial activity using *Aloe vera* gel in Lowenstein Jensen (L-J) medium

<table>
<thead>
<tr>
<th>Mycobacterium Strain</th>
<th>Part Used</th>
<th>Extract Type</th>
<th>Control Drug Free Media</th>
<th>Isoniazide Drug Media</th>
<th>L-J proportion method Mean cfu on media</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. tuberculosis</em></td>
<td>Leaf</td>
<td>Pure Gel</td>
<td>55</td>
<td>0</td>
<td>41 36 24</td>
<td>25 35 56</td>
</tr>
<tr>
<td><em>M. avium</em></td>
<td>Leaf</td>
<td>Pure Gel</td>
<td>75</td>
<td>0</td>
<td>60 55 50</td>
<td>20 26 33</td>
</tr>
</tbody>
</table>

Average growth and percentage inhibition of *M. tuberculosis H37Ra* (MTCC 300) and *Mycobacterium avium* (MTCC 1723) on extract containing and extract free control L-J slants after 42 days of incubation at
37°C were recorded (Table 1). Inhibition of *Mycobacterium tuberculosis* H37Ra (MTCC 300) and *Mycobacterium avium* (MTCC 1723) standard strains was observed.

*Aloe vera* showed anti-mycobacterial activity against both strains.

2%, 4% & 6% of *Aloe vera* pure gel containing LJ medium exhibited 25, 35 & 56% percentage inhibition against *M. tuberculosis*.

Similarly the LJ medium 2%, 4% & 6% of *Aloe vera* pure gel exhibited 20, 26 & 33% percentage inhibition against *M. avium*.

Thus, *Aloe vera* exhibited more antibacterial activity against *Mycobacterium tuberculosis* (MTCC 300), when compared to *M. avium* (MTCC 1723) for same concentration in LJ Medium.

**DISCUSSION**

TB has always been a major health problem especially in developing countries like India. An increase in emergence of MDR and XDR strains of *M. tuberculosis* has lead to urgent need of finding newer antimycobacterial agents to combat this problem [25]. In addition to this, the development of adverse effects of chemotherapy for TB is the most common reason leading to interruption of therapy.

2%, 4% & 6% of *Aloe vera* pure gel containing LJ medium exhibited 25, 35 & 56% percentage inhibition against *M. tuberculosis*.

While 2%, 4% & 6% of *Aloe vera* pure gel containing LJ medium exhibited 20, 26 & 33% percentage inhibition against *M. avium*.

Similar findings were reported in a study of Bernaitis L et al in which 2%, 4% & 6% of *Aloe vera* pure gel containing LJ medium exhibited 23, 34 & 51% percentage inhibition against *M. tuberculosis*.

This is also consistent with the findings of Gupta R et al., where 10 & 41% inhibition was reported for 2 & 4% gel containing medium.

**CONCLUSION**

The present scenario of the treatment of infections is becoming difficult. While there has been remarkable progress in the identification of specific allopathic drugs for treating the majority of the infections, there are many bumps and hurdles along the road. Biggest problem is the emergence of organisms and viruses that have evolved to be drug resistant.

So this is the need of the hour to explore the medicinal properties of herbal products used traditionally by practitioners in Indian medicine.

Also, to get greater insight into the drugs of herbal origin, attempts should be made to identify, purify and evaluate the active principles in the plant products.

Further studies, using more specific methods should be carried out on the plants to explore the constituent responsible and mechanism of responsible for anti-tuberculous activity. Also toxicological activity of these bioactive compounds should also be done.

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