Evaluation of Anti- mycobacterial Activity of Ocimum sanctum and Lantana camara Against M. Tuberculosis Standard Strain

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ABSTRACT

Background: Tuberculosis (TB) is a major global health problem causing ill-health among millions of people and leading cause of death worldwide. Situation has worsened in recent past as, Mycobacterium developed resistance against both the first line & 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. Anti-TB drug induced hepatotoxicity, is another cause of concern. Medicinal plants have been used for curing diseases from many centuries worldwide. India is one of the countries in the world which has vast traditional knowledge for use of herbal medicine for cure of diseases. Therefore, the aim of the present study was to evaluate the anti-tubercular activity of water and methanolic extract of Lantana camara & Ocimum sanctum against M. tuberculosis standard strain.

Methods: The Water & Alcoholic plant extracts were added in the LJ medium at concentration of 2 %, 4% & 6 %. Anti-mycobacterial activity against M. tuberculosis 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: Each extracts of both the plants showed the inhibitory activity for M. tuberculosis. At 6% concentration of extract in L J Medium the percentage inhibition of M. tuberculosis for Lantana camara was 48 & 56 for water and Methanolic extract respectively. For same concentration water & Methanolic extract of Ocimum sanctum showed 62 & 69 percent inhibition of M. tuberculosis.

Interpretations & Conclusions: Studied plants exhibited positive results regarding anti-mycobacterial activity of their extracts. Further studies, using more specific methods should be carried out on the plants to explore the constituent responsible and mechanism of responsible for anti-tuberculosis activity.

KEYWORDS

Lantana camara, M. tuberculosis, Ocimum sanctum

INTRODUCTION

Tuberculosis (TB) is a major global health problem. It causes ill-health among millions of people each year and ranks alongside the AIDS as a leading cause of death worldwide [1]. Tuberculosis is commonest opportunistic infection (OI) in Human Immuno-deficiency 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 [2]. India is highest Tuberculosis (TB) burden country in the world with an estimated 2.2 million new TB cases occurring annually [3]. 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. [1]. 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 [4,5]. Different studies reported that 1–31% of TB patients experience drug related hepatotoxicity following TB treatment [6].

Since ancient time, orthodox pharmaceutical medicines were the only solution to sustain life and counter interactions. Despite the dramatic advances and advantages of conventional medicine, or biomedicine it has been established that herbal medicine has offered a great solution to cure disease of human being. 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. [7].

Plant extracts have been used as traditional medicines against disease including tuberculosis and *Lantana camara & Ocimum sanctum* and many plant secondary metabolites have been reported to have anti-mycobacterial activity[ 8,9,10,11,12,13 ]

Therefore, the aim of the present study was to evaluate the anti-tuberculosis activity of extracts of water and methanolic extract of *Lantana camara & Ocimum sanctum* against *M. tuberculosis* standard strain.

**MATERIAL AND METHOD**

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

**A. Collection of Plant Material & Extract Preparation**

*Lantana camara & Ocimum sanctum* were the plant material used. Leaves of these plants were handpicked. Only mature plants included. No plant which appeared to have viral, bacterial or fungal infections was included. 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. A shoot with leaves and flowers was used for identification and a voucher specimen was kept at the DAV College, Ajmer herbarium.

**B. Extract Preparation**

All the leaves were washed using distilled water to remove the adhering dust. These washed leaves were then shed dried at room temperature away from direct sunshine. Completely dried leaves were pulverized to make a fine powder weighed and stored at room temperature [14].

**Hot water Extraction**

10 gm finely powdered plant material was weighed and kept in a beaker with 100 ml distilled. The mixture was then heated on a hot plate with continuous stirring at 30°–40° C for 20 minutes to obtained water extract. This extract was filtered using Whatman filter paper No. 42 (125mm). The water extract was preserved in refrigerator for further use.

**Solvent (Alcohol) extraction**

Soxhlet extraction was the method used to prepare crude plant extract [15]. Alcohol was the solvent used in this study. 10gm of powdered plant material was uniformly packed into a thimble and extracted with 100ml of different solvents separately. The process of extraction continues for 24 hours or till the solvent in Siphon tube of an extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30°-40°C till all the solvent got evaporated. Dried extract was kept in refrigerator at 4°C for further use.

Further, after obtaining the desired extracts, 0.5g dried extract was dissolved in water or alcohol by adding respective solvent to make up to 50 ml to make it 10,000 ppm. This solution was considered as 1% stock solution and used after appropriate dilution as required.

**C. Preparation of Culture Media**

All culture media were prepared in Department of Microbiology, JLN Medical College, Ajmer. At every point, Manual of Standard Operating Procedures (SOPs) were followed [16]. 2%, 4%, and 6% extract containing culture media was prepared. The plant extract 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 extract stock solution
was dissolved into 100 ml of culture medium). Further, Inspissation in inspissator at 85°C for 85 min was done [17,18].

C. Mycobacterial Strains
Culture strain of Mycobacterium tuberculosis H37Ra (MTCC 300) was 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-fats bacilli grown on the solid medium was confirmed to be M. tuberculosis, using combination of observation of colony morphology, results of biochemical tests & Z-N Staining.

Estimation Colony forming units (cfu) on Lowenstein-Jensen (L-J) Medium:
M. tuberculosis 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 [18]. 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 [17] 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 [18].

OBSERVATION AND RESULTS

Table 1. Results of anti-mycobacterial activity using plant extracts in Lowenstein Jensen (L-J) medium

<table>
<thead>
<tr>
<th>Plant Botanical Name</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</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lantana camara</em></td>
<td>Leaf</td>
<td>Water</td>
<td>55</td>
<td>0</td>
<td>2% 4% 6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanolic</td>
<td>55</td>
<td>0</td>
<td>13 31 48</td>
</tr>
<tr>
<td><em>Ocimum sanctum</em></td>
<td>Leaf</td>
<td>Water</td>
<td>55</td>
<td>0</td>
<td>19 44 56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Methanolic</td>
<td>55</td>
<td>0</td>
<td>19 40 62</td>
</tr>
</tbody>
</table>

Average growth and percentage inhibition of *M. tuberculosis* on extract containing and extract free control L-J slants after 42 days of incubation at 37°C were recorded (Table 1). Inhibition of *M. tuberculosis* standard strain was observed for Water & Alcoholic extracts of both medicinal plants. Among Water and alcoholic extract of both the plants *Lantana camara & Ocimum sanctum*, all four showed the inhibitory activity for *M. tuberculosis*.

*Ocimum sanctum* exhibited anti-mycobacterial activity against *M. tuberculosis*. The percentage of inhibition was 19, 40 and 62 for water extract and 28, 57, and 69 for alcoholic extracts in 2%, 4% & 6% concentration of extract containing LJ medium. The percentage of inhibition in 2%, 4% 6% of extract containing LJ media for Water Extract of Lantana camara was 13, 31 and 48 respectively and for LJ media containing Alcoholic Extract it was 19, 44 and 56 respectively.

*Ocimum sanctum* exhibited more anti-mycobacterial activity compared to the *Lantana* in both Water & Alcoholic Extract. Alcoholic extract was more effective than Water Extract for both plants.
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 [19]. In addition to this, the development of adverse effects of chemotherapy for TB is the most common reason leading to interruption of therapy.

In this study, 2%, 4%, 6% of extract containing media for Water Extract of Lantana camara showed inhibition, was 13%, 31%, 48% respectively and for media containing Alcoholic Extract it was 19%, 44%, 56% respectively. Similar findings were reported in the study done by Claude Kirimuhuzya et al [14] where the methanol extract showed anti-mycobacterial activity, with zones of inhibition of 18.0–22.5 mm and MIC values of 20 µg/ml for H37Rv strain using agar well diffusion method & Agar dilution method on Middlebrook 7H11.

In our study Ocimum sanctum exhibited anti-mycobacterial activity as percentage inhibition 19, 40, 62 for water extract and 28, 57, 69 for alcoholic extracts in 2%, 4% & 6% concentration of extract containing LJ medium. Our finding were consistent with Khushboo Jethva et al [20], which showed that 1000µg/ml extract exhibited anti-mycobacterial activity as Zone of inhibition of 13 mm and 14 mm for water and alcoholic extract respectively, using Agar diffusion cup method. Vikrant Arya et al [21] have also reported anti-mycobacterial activity of Ocimum sanctum.

Thus in the present study, we concluded that percentage Inhibition exhibited by Ocimum sanctum was more when compared to the Lantana for both Water & Alcoholic Extract. Lantana camara exhibited less activity than Ocimum sanctum.

Present study indicates that, Alcoholic extract was more effective than Water Extract in both plants. A study done by Claude Kirimuhuzya et al[14] also showed that water extract was less effective then Alcoholic extract. Similarly Pooja gupata et al [22] also reported that water extract of A. galangal was ineffective on H37Rv, while Alcoholic extract of the same plant showed the anti-mycobacterial activity. Similar type of anti-tubercular activity was reported in study of Rakesh Ranjan Pradhan et al [23] & Bernaitis L et al [18] and the also Alcoholic extract of plants exhibited more anti-tubercular activity then water extract.

CONCLUSION
This is not campaign against synthetic drugs, for that would be a serious mistake – some complaints respond well to herbal remedies while others react better with the synthetic ones. The sole objective, of both types of medicine, is to restore the patients to good health. It is desirable, therefore that research in both fields should continue without conflict.

Hence, our study is an attempt to give scientific account of medicinal plant extracts for their anti-tuberculosis activity. Here, not just holy and traditionally known plant (Ocimum sanctum), but also rapidly growing notorious plant (Lantana camara) are showing effects inhibiting mycobacteria. This study will result in a useful reference for all those who are concerned by the increasing drug resistance of present antibiotics.

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

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