Original Research Article

Anti-Mycobacterial activity of selected medicinal plants

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Abstract

Background: In recent past *Mycobacterium* developed resistance against both the first line as also the second line drugs and there is emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *M. tuberculosis* all over the world including India. In addition to developing resistance, Anti-TB drug induced hepatotoxicity, which is a common serious adverse drug reaction, is one of the most challenging clinical problems. 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. Therefore, the present study was carried out to check the anti-mycobacterial activity of extracts of various plants viz. *Lantana camara* and *Ocimum sanctum* against standard strain of *M. avium*. **Methods:** Water and Methanolic extracts at concentration of 2%, 4% and 6% of both pants were tested in vitro for their anti-mycobacterial activity against *M. avium* using Lowenstein-Jensen (L J) medium. Percentage inhibition was calculated by mean reduction in number of colonies on extracts containing media as compared to extract free (control) media. **Results:** Out of two extracts of both the plants *Lantana camara* and *Ocimum sanctum*, all four exhibited the inhibitory activity against *M. avium*. At 6% concentration of extract in L J Medium the percentage inhibition of M avium for *Lantana camara* was 63 and 67 for water and Methanolic extract respectively. For same concentration water and Methanolic extract of *Ocimum sanctum* showed 71 and 75 percent inhibition of M avium. **Interpretations and Conclusions:** Our study has shown positive results regarding anti-mycobacterial activity of studied plants. Further studies need to be carried out using different solvents or using fractions of crude extracts to finally conclude the potential of these plants as an anti-mycobacterial treatment.

Key Words: *Lantana camara, M. avium, Ocimum sanctum.*

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Received Date: 12/10/2017 Revised Date: 19/11/2017 Accepted Date: 23/12/2017
DOI: https://doi.org/10.26611/1008433

Access this article online	
Quick Response Code:	Website: www.medpulse.in
	Accessed Date: 27 December 2017

INTRODUCTION

India is highest Tuberculosis (TB) burden country in the world with an estimated 2.2 million new TB cases occurring annually[1]. Tuberculosis is commonest opportunistic infection (OI) in Human Inmmuno-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]. However, this problem has become serious 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. Globally, an estimated 3.3% of new TB cases and 20% of previously treated cases have MDR- TB. Extensively Drug Resistant TB (XDR-TB) had been reported by 105 countries by 2015. An estimated 9.7% of people with MDR-TB have XDR-TB[3]. In addition to developing resistance, Anti-TB drug induced hepatotoxicity, which is a common serious adverse drug reaction, is one of the most challenging clinical problems and main cause of treatment interruption during TB treatment course that causes hospitalization and life threatening event. Among the first line anti-TB drugs, pyrazinamide, isoniazid and

How to cite this article: Geeta Parihar, Rasik Bihari Vyas, Priyanka Soni, Jyotsna Chandwani, Manoj Sharma. Mycobacterial activity of selected medicinal plants. *MedPulse International Journal of Microbiology.* December 2017;4(3): 27-31.
https://www.medpulse.in/Microbiology/

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]. Rifampicin is an important drug for the treatment of TB. However, administration of Rifampicin in combination with antiretroviral therapy, particularly protease inhibitors, is difficult because of drug-drug interactions[7]. Medicinal plants offer a great hope to fulfill these needs and have been used for curing diseases from many centuries. These have been used extensively as pure compounds or as a crude material. 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. Therefore it makes immense sense to explore the empirical wisdom of ancient with the modern research technology[8]. Different plants extracts have been used as traditional medicines against disease including tuberculosis and *Lantana camara* and *Ocimum sanctum* have been reported to have anti-mycobacterial activity[9,10,11,12] Therefore, the present study was carried out to check the anti-mycobacterial activity of extracts of various plants against standard strain of *M. avium*.

MATERIAL AND METHOD

This study was carried out in Department of Microbiology, Jawahar Lal Nehru Medical College, Ajmer, during Feb- August 2017. This was an Observational, descriptive study. Standard Strain of *M. avium* was used as the study material. Plant selection; assay procedure was as follows-

Plant Material: Leaves of *Lantana camara* and *Ocimum sanctum* were collected from Nag Pahad of Pushkar near Ajmer (Rajasthan) India, approximately at Latitude 26.°N and Longitude 74°E. Collection was done during March–May2017. These Plants were botanically authenticated by Botanist and plants judged as mature, were included and plants, which appeared to have viral, bacterial or fungal infections, were discarded. A shoot with leaves and flowers was used for identification and a voucher specimen was kept at the DAV College, Ajmer herbarium.

Extract Preparation: Leaves of all plants were washed using distilled water to remove the adhering dust, then shed dried at room temperature away from direct sunshine. The dried leaves were pulverized, weighed and stored at room temperature.

Hot water extraction: 10 gm of dried finely powdered plant material was taken in a beaker and 100 ml of distilled water was added into it. The mixture was than heated on a hot plate with continuous stirring at 30°-40° C for 20 minutes thus obtained water extract was filtered through Whatman filter paper No. 42 (125mm) and used for further studies. The water extract was preserved in refrigerator.

Solvent extraction: Crude plant extract was prepared by Soxhlet extraction method. 10gm of powdered plant material was uniformly packed into a thimble and extracted with 100ml of different solvents separately. Solvent used in this study was alcohol. The process of extraction continues for 24 hours or till the solvent in Siphon tube of an extractor become colourless. 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 future use in phytochemical and / or biological analysis.

For biological / antibacterial investigations 0.5g dried extract was dissolved in water or alcohol and solution was 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.

Mycobacterial Strains: Reference strain *M Avium* (MTCC 1723) was obtained from MTCC, IMTECH Chandigarh. The revival of lyophilized strains was done as per the standard operating procedures (SOPs) provided by MTCC, IMTECH, Chandigarh and ATCC Guidelines using L-J Medium and Middlebrook 7H9 broth medium. The identification process of the growth comprise the phenotypic identification of cultures of Acid- fats bacilli grown on the solid medium based on the combination of observation of colony morphology, results of bio-chemical tests and Z-N Staining.

Assay Protocol

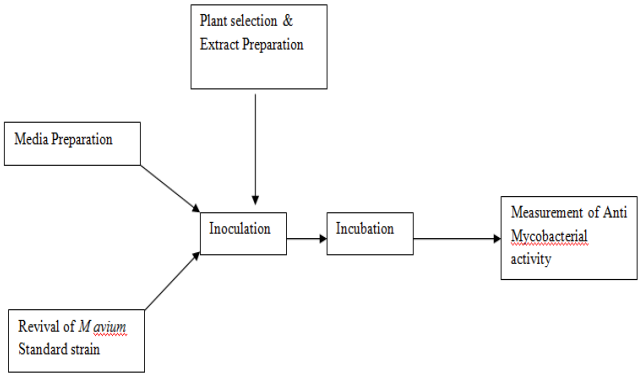

Figure 1:

All culture media was prepared in Department of Microbiology, JLN Medical College, Ajmer, by following the Manual of Standard Operating Procedures (SOPs) [14]. The plant extract was incorporated in the medium at concentration of 2 per cent v/v and 4 per cent and 6 per cent v/v (2 ml, 4 ml and 6 ml of 1% fresh plant extract stock solution was dissolved into 100 ml of culture medium) prior to inspissation in inspissator at 85°c for 85 min[15,16].

Determination of Colony forming units (cfu) on Lowenstein-Jensen (L-J) Medium: *M. avium* suspension of 1 mg/ml, equivalent to MacFarland 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[16]. The medium set inoculated with the standard bacterial suspension incubated at 37°C for 21 days, reading was taken weekly[17]. For comparison, extract free control slants were used[15]. 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[15] 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[16].

OBSERVATION AND RESULTS

Table 1: Adverse effects of Anti-TB drugs [13]

Drug	Adverse effects
Isoniazid	Skin rash, hepatitis
Rifampicin	Abdominal pain, nausea, vomiting, hepatitis, thrombocytopenic purpura
Pyrazinamide	Arthralgia, hepatitis
Streptomycin	Vestibular and auditory nerve damage, renal Damage
Ethambutol	Retrobulbar neuritis, ocular side effects
Thioacetazone	Skin rash, Exfoliative dermatitis
Para-aminosalicylic Acid	Anorexia, nausea, vomiting, hypersensitivity Reactions
Kanamycin	Vertigo, auditory nerve damage, nephrotoxicity
Ethionamide	Diarrhoea, abdominal pain, hepatotoxicity
Cycloserine	Dizziness, headache, depression, psychosis, Convulsions

Table 2: Results of anti- mycobacterial activity using plant extracts in Lowenstein Jensen (L-J) medium

Plant Botanical Name	Part Used	Extract Type	Control Drug Free Media	Isoniazide Drug Media	L-J proportion method Mean cfu on media			% Inhibition		
					Plant Extract			Plant Extract		
					2%	4%	6%	2%	4%	6%
Lantana camara	Leaf	Water	75	0	45	36	28	40	52	63
		Methanolic	75	0	40	31	25	47	59	67
Ocimum sanctum	Leaf	Water	75	0	41	29	22	45	61	71
		Methanolic	75	0	31	24	19	59	68	75

Average growth and percentage inhibition of *M. avium* on extract containing and extract free control L-J slants after 21 days of incubation at 37°C were recorded (Table 2). Inhibition of *M. avium* isolates was observed for Water and Alcoholic extracts of both medicinal plants. Out of four extracts of both the plants *Lantana camara* and *Ocimum sanctum*, all four exhibited the inhibitory activity against *M. avium*. The percentage of inhibition in 2%, 4% 6% of extract containing media for Water Extract of *Lantana camara* was 40, 52, 63 respectively and for media containing Alcoholic Extract it was 47, 59, 67 respectively. *Ocimum sanctum* showed anti-mycobacterial activity as the percentage of inhibition was 45, 61, 71 for water extract and 59, 68, 75 for alcoholic extracts in 2%, 4% and 6% concentration of extract containing LJ medium. Inhibition exhibited by *Ocimum sanctum* was a bit higher compared to the Lantana in both Water and Alcoholic Extract. *Ocimum sanctum* exhibited more activity than *Lantana camara* for both plants Alcoholic extract was more effective than Water Extract.

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 anti-mycobacterial agents to combat this problem [18].In addition to this, The development of adverse effects of chemotherapy for TB is the most common reason leading to interruption of therapy. In the present study, the percentage of inhibition in 2%, 4% 6% of extract containing media for Water Extract of *Lantana camara* was 40, 52, 63 respectively and for media containing Alcoholic Extract it was 47, 59, 67 respectively. Our study was similar to the study done by Claude Kirimuhuzya *et al*[19] in which 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 and Agar dilution method on Middlebrook 7H11. Another sudy of U Dibua *et al*[20] reported activity against mycobacteria using agar well diffusion method where *Lantana camara* had MIC of 0.89 mg/ml for *M avium* complex. Similarly Girish k.[21] reported anti-mycobacterial activity of *Lantana camara* against *M. avium* with MIC ranging between 8 and 32 µg/ml. In our study *Ocimum sanctum* showed anti-mycobacterial activity 45, 61, 71 for water extract and 59, 68, 75 for alcoholic extracts in 2%,4% and 6% concentration of extract containing LJ medium. Our finding were consistent with Khushboo Jethva *et al*[22], 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*[13] have also reported anti-mycobacterial activity of *Ocimum sanctum*. In the current study, we concluded Inhibition exhibited by *Ocimum sanctum* was a bit higher compared to the *Lantana* in both Water and Alcoholic Extract. *Ocimum sanctum* exhibited more activity than *Lantana camara*. In this study, For both plants Alcoholic extract was more effective than Water Extract. A study done by Claude Kirimuhuzya *et al*[19] Was similar to our study which also showed that water extract was less effective then Alcoholic extract. Similarly Pooja gupata *et al*[23] also reported that water extract of *A galanga* was ineffective on *H37Rv*, while Alcoholic extract of the same plant showed the anti-mycobacterial activity. Similar findings were reported in study of Rakesh Ranjan Pradhan *et al*[24] and Bernaitis L *et al*[16] where Alcoholic extract of plants exhibited more anti- bacterial activity then water extract.

CONCLUSION

Hence, this study is an attempt to give scientific account of medicinal plant extracts for their anti-mycobacterial activity. Not just plants traditionally known (*Ocimum sanctum*) but also rapidly growing notorious plant (*Lantana camara*) showing effects inhibiting mycobacteria. This study will result in a valuable reference for all those who are concerned by the increasing drug resistance of present antibiotics, and will be useful to Phytochemists and to those interested in the medical aspect of plants. Further studies should be carried out on the plants in order to isolate, identify, and characterize the bioactive compounds. Further toxicological activity of these bioactive compounds should also be done.

ACKNOWLEDGEMENT

We are grateful to Principal and Controller, J L N Medical College, Ajmer (Rajasthan) for granting permission to conduct this study and use the laboratory of Department of Microbiology. We also thank Dr Narendra Bhojak, Green Chemistry Research Center, P.G. Department of Chemistry, Govt. Dungar College, Bikaner (Rajasthan) for his able guidance and allowing us access to his department for extraction of plant material.

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Source of Support: None Declared
Conflict of Interest: None Declared