In vitro evaluation of anti-mycobacterial active plant extracts against Mycobacterium tuberculosis using mycobacterium growth indicator tubes (MGIT-960) BACTEC system

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Abstract

The emergence and treatment of Drug resistant Tuberculosis (DRTB) have become a global health issue. Herbal drugs promises other possible options for Tuberculosis treatment.. In the current study, anti-mycobacterial activity of Piper *longum, Cressa cretica* and *Calotropis gigantea* extracts were tested. The methanolic extract of these plants was screened against H37Rv strain using MGIT BACTEC 960 system. The extracts showed inhibitory activity against *Mycobacterium tuberculosis* H37Rv strain. *Piper longum* and *Cressa cretica* showed inhibition at 125µg/mL and *Calotropis gigantea* at 250µg/mL. However, isoniazid and Rifampicin as standard showed higher inhibitory activity against *M. tuberculosis* respectively at 0.05µg/mL and 0.12µg/mL when compared to the crude extract. The present study demonstrates the anti-tubercular potential of *Piper longum* and *Cressa cretica* as a new anti-mycobacterial agent. Although, the action of these compounds and their active principles require further purification and molecular characterization besides in vivo testing to evaluate their capacity as novel anti-TB agents.

Full Text

In 2018, an estimated 10 million new cases of TB and 1.5 million deaths due to TB worldwide were reported. Drug-resistant TB is major concern today globally. In 2018, WHO estimated that there were 4,84000 new cases with drug resistance to rifampicin (WHO TB report 2019). Today there is an urgent need to search for a new anti-tubercular drug, preferably those that can be quickly and easily produced from derivatives of herbal plants. Being a natural product, it is thought that these may possess lower chances of resistance and hepatotoxicity that might play an important role in the chemotherapy of tuberculosis (Arellanes J. et al. 2016). *Cressa cretica* (Rudravanti) traditionally used as anti-tuberculosis agent, widely accepted for the treatment of respiratory diseases due to its bronchodilatory, antitussive, antibacterial, antipyretic, and analgesic effects (Jaafar Noor S. et. al. 2021). It acts both as a bacteriostatic as well as a bactericidal agent. It has been shown to be effective for reducing lung lesions due to tuberculosis and helps to gain weight (Jaafar Noor S. et. al. 2021). *P. longum* is reported as a good herbal remedy for treating respiratory tract infections and also found to increase the circulating antibody titre and antibody-forming cells indicating its stimulatory effect on the humoral immunity (Sunila ES. 2004). The immunomodulatory activity of *P. longum* may be due to the combined action of humoral and cell-mediated immune responses (Sunila ES. 2004). *Calotropis gigantea* used in the traditionally medicinal system for the treatment of various infectious diseases such as leprosy and TB. It is reported to have antimicrobial, anticancer, anti-inflammatory, antidiabetic, hemolytic, antioxidant and larvicidal properties (Kadiyala M. et. al. 2013). Many researchers across the world including in India have reported on the inhibitory properties of these medicinal plants against *Mtb.* (WHO 2006; Askun T. et. al. 2012). In this study, we present the in vitro antimycobacterial activity exhibited by methanolic extracts of these medicinal plant and their therapeutic potential against *Mtb*.

Preparation of Plant material:

Plants were collected from the herbal garden, Jawaharlal Nehru Agricultural University, Jabalpur. The collected plant materials were washed with tap water and then distilled water 2-3 times. The washed plant material was shade dried and crushed in a mechanical grinder to make fine powder and stored at room temperature. Five grams of powdered material was used for extraction with 250 mL of 100% methanol as solvent in soxhlet apparatus at low temperature (15-25°C) continuously for 24-48 hours until the solvent appeared colourless. This concentrated extract was stored at 4^{o}C in a desiccator for all further experiments. Stock solutions of crude extracts were prepared using Dimethylsulfoxide (DMSO) to a concentration of 70mg/mL in accordance with MGIT protocol and sterilized through a syringe filter of 0.2 µm pore size (Yadav R. 2011).

Mtb strain and culture medium:

H37Rv (ATCC 25618) was used as reference strain for the anti-mycobacterial assay, *Mtb* culture with DMSO (1.2%), isoniazid (INH) at MIC99 (0.05µg/mL) and Rifampicin at MIC99 (0.12 µg/mL) were used as controls.

Inoculum and extract preparation

The inoculum was prepared from solid culture (L–J slants), as described by (Askun T *et al.* 2012). 500 µl of 1:5 dilution of 0.5 McFarland bacterial suspension of *Mtb*-H37Rv was inoculated in MGIT (BD, USA), tubes containing test compounds and controls (Askun T *et al.* 2012). The bacterial growth was monitored as per the manufacturer's instructions. 100µl of extract was added individually in MGIT tubes, containing *Mtb* and allowed for incubation at 37°C in the BACTEC system. The growth units (GU-400) were monitored for six days. For the minimum inhibitory concentration (MIC) evaluations, a 1% bacterial suspension of 0.5 McFarland was prepared and cultured as a control without any extract in MGIT tube. All the extract preparations were tested at two-fold decreasing concentration from 1mg/mL to 0.12mg/mL. The MIC of the extract determined in comparative to the growth units of the control (GU–400) as the lowest extract concentration that equals or lower than GU–400. Growth or inhibition of H37Rv was observed in extract containing and extract free control after 42 days of incubation at 37°C were recorded.

The preliminary in-vitro screening revealed that the methanolic extract of *Piper longum* and *Cressa cretica* inhibit the H37Rv with a MIC value of 125µg/mL individually. *C.gigantea* inhibited at 250µg/mL but to a lesser extent than. *P longum* and *C. cretica* at all concentrations tested. All 3 methanolic extracts showed growth inhibition against *Mtb*- H37Rv at MIC between 125µg/mL to 250µg/mL. On the other hand, both isoniazid and rifampicin showed relatively higher inhibitory activity against *Mtb*-H37Rv even at 0.05µg/mL and 0.12µg/mL as compared to the crude extract (Table 1).

In many developing countries rural and tribal population largely rely on conventional medicines obtained from medicinal plants (Askun T. et. al. 2012). According to the United Nations conference on trade and development, more than 33% of medicines are produced by industries that are plant-derived. More than 20,000 types of medicinal plants are named by WHO for their medicinal properties (WHO 2006).

In general, medicinal plants do not confer resistance while exerting their biological activity (Yadav R. 2011). Previously reported medicinal plants for the TB treatment are *Acalyphaindica, Adhatodavasica, Allium cepa*, *Allium sativum, A. Indica* and *Aloe vera* etc. (Gupta R et. al. 2010). Based on ethnomedicinal values and uses, the present study was carried out to identify the *in-vitro* antimycobacterial potential of *P. longum, C. cretica* and *C. gigantiea*. There are several reports available using MGIT for the determination of MIC value of available anti TB drugs in contrast this study uses MGIT for plant extract.

P. longum has been indicated to an effective remedy for the treatment of respiratory tract infections and in the treatment of TB. *C. Cretica* possesses a variety of biological properties such as antipyretic, anti-microbial, antioxidant, immunomodulatory, anti-inflammatory, hypoglycaemic and anti-cardiovascular properties and also reported as an anti-tubercular activity for its bronchodilatory properties (Ali AS 2016). *C. gigantiea* has been used for syphilis, leprosy, tuberculosis and lupus bacterial infections (Askun T et. al. 2012). In the present studies, the MIC value of *P. longum and C. Cretica* was found to be 125µg/mL against *Mtb* while it was 250µg/mL for *C. gigantiea.* To the best of our knowledge the MIC values of these compounds are reported for the first time. The methanolic extract of *P. longum, C. cretica* and *C. gigantiea* showed appreciable anti-mycobacterial activity. The active compound in these medicinal plant and their combine action could be the reason for enhanced anti-mycobacterial activity. This study shows that these plants have potential for antimycobacterial activity. The benefits of plant derivative compounds using as anti-mycobacterial agent includes fewer side effects, better patient acceptance due to a long history of use, reduced costs and cultivability rendering them renewable in nature (Gupta P et. al. 2014).

The limitation of the study is that it was done on crude extract and hence the MIC is very high compared to the in use anti TB drugs.

This study has revealed that of *P. longum, C. cretica* and *C. gigantiea* have the potential to be an effective anti TB drug. These plants have potential of anti-tubercular agents as all 3 showed activity against *Mtb*. However, these crude extracts and their active component needs to be purified and their active principles characterized both in vitro as well as in vivo study for identifying the clinical potential.

Declarations

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Conflict of Interests: *None declared*

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Contributors:

VT, JB and SR conceived the design of the study. VT, NP, and HT carried out the experiments. VT, JB and RS were involved in drafting and revision of the manuscript. All the authors have read and agree to the manuscript.

Ethical clearance:

The Institutional Ethical Committee (IEC) of ICMR–NIRTH, Jabalpur approved the study with a reference number NIRTH/IEC/539/2018 dated 5/06/2018

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Tables

Table 1. Susceptibility testing and minimum inhibition concentration (MIC) of three crude extracts of medicinal plants against *Mtb*-H37Rv using MGIT BACTEC 960 system

Name of Plants/drugs	Susceptibility activity (µg/mL)	MIC of the methanolic crude extracts(µg/mL)
Cressa Cretica	< 150	125
Piper longum	< 150	125
Calotropis gigantea	< 300	250
Isoniazid (INH)	< 25	0.05
Rifampicin (RIF)	< 25	0.12