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Full Length Research Paper

Anti-tuberculosis activity of commonly used medicinal plants of south India

A.Sivakumar and G. Jayaraman*

School of Bio Sciences and Technology, Vellore Institute of Technology University, Vellore, 632014 Tamil Nadu, India.

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Tuberculosis (TB) has been a major health problem in developing countries including India. Due to increase in multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis,* there is an urgent need of finding newer anti-mycobacterial agents to combat this problem. Natural sources provide numerous examples of interesting secondary metabolites with anti mycobacterial activity, indicating that natural products could be a rewarding field for the discovery of new anti-TB leads. In the present study, aqueous extracts and ethanolic extracts of selected medicinal plants used as spices (*Allium sativum, Allium cepa, Syzygium aromaticum, Cinnamomum verum*) were observed to have anti-TB activity against *M. tuberculosis* H37Ra and (*Zingiber officinale, Camellia sinensis, Curcuma longa, Elettaria cardamomum*) had poor/no activity against *M. tuberculosis* H37Ra. The growth and minimum inhibitory concentration (MIC) (µg/ml) in which the aqueous and ethanolic extracts of medicinal plants inhibits *M. tuberculosis* H37Ra by microplate Alamar blue assay (MABA) method after 7 days of incubation at 37℃ were reco rded, and according to our results, *A. sativum, A. cepa, S. aromaticum, C. verum* could be used as adjuvant therapy for TB.

Key words: Anti mycobacterial activity, medicinal plants, microplate alamar blue assay (MABA).

INTRODUCTION

Tuberculosis (TB) is an ancient disease that remains a significant global health problem. Nearly half a million cases have the multidrug-resistant (MDR) form of the disease. World Health Organization (WHO) plan to eliminate TB by 2050, but MDR and extensively drug-resistant (XDR) forms of TB are the biggest challenges for the WHO's goal to eliminate TB. MDR-TB is a form of TB that is difficult and expensive to treat, and fails to respond to standard first-line drugs. Many countries have developed plan to address MDR-TB, but the response globally is still insufficient. XDR-TB occurs when resistance to second-line drugs develop on top of MDR-TB. The recent increase of TB is associated with the emergence of the human immunodeficiency virus (HIV) and the rapid spreads of MDR-TB strains worsen the situation (Billo et al., 2005). For the past several decades, there are no new anti-TB drugs which hit the market. So, there is an urgent need to develop newer anti-TB drugs with unique drug targets. In the past, medicinal plants were used in curing diseases for many centuries. Most healthy individuals are able to control the infection with a vigorous immune response, halting the progression of the disease, but not necessarily eradicating the organism (McKinney, 2000). There could be several factors that cause resistance to *Mycobacterium tuberculosis* so that it could not develop into active TB in 90% of infected population (Sivakumar et al., 2010). Malnutrition is an important risk factor for the development of TB. Malnourished individuals have an increased likelihood of primary or latent infection progressing to active disease.

Population groups at highest risk for poor nutrition are also at high risk for TB (Paras et al., 2006). Particularly, vitamins, alkaloids, terpinoids and polyphenols are important for proper functioning of immune system and detoxification. It has been reported that there is a high oxidative stress during early stages of TB and anti-oxidants such as green tea extract can play a vital role by reducing stress through adjuvant therapy (Guleria et al., 2003). Only a few plant species have been thoroughly

*Corresponding author. E-mail: gjayaraman@vit.ac.in. Tel: +91 416 220 2608, +91 9944 796904.

investigated for their medicinal properties (Heinrich and Gibbons, 2001). India is one of the few countries in the world which has unique wealth of medicinal plants and vast traditional knowledge of the use of herbal medicine for the cure of various diseases (Gupta and Tandon, 2004; Sharma, 1998). India is represented by rich culture, traditions and natural biodiversity; and offer unique opportunity for the drug discovery researchers. (Raju Gautam, 2007). So far, few plants have been tested against mycobacterium. The increasing incidence of MDR- and XDR-TB worldwide, highlight the urgent need to search for newer anti-TB compounds/drugs. Therefore, the present study was carried out to check the antibacterial activity of aqueous and ethanoilc extracts of eight plants against *M. tuberculosis* H37Ra. Few plants have already reported to have anti-TB activity (Grange, 1996; Gupta and Chopra, 1954; Jain, 1993; Ratnakar and Murthy, 1996; Bruce, 1967; Gottshall et al., 1949; Reynolds, 1999). Naturally occurring pure compounds as well as extracts from higher and lower forms of plants, microorganisms and marine organisms have indicated that inhibitory activity against *M. tuberculosis* is widespread in nature. Many compounds isolated using preliminary functional assays have been provided from investigators interested in phytochemical biodiversity. Usually, their potential pharmaceutical worth remains unknown since data to show that these compounds are adversely affecting mycobacterial survival mechanisms in humans, or have been derived from medicinal plants are lacking (Adewole et al., 2004). Research studies for *in vitro* screening of plant remedies are important for validating the traditional use of herbal treatments, and for providing leads in the discovery of new active principles for possible future development as anti-TB drugs (McGawa et al., 2008). These studies also help in promoting the value of the India's traditional medicine. Commonly used spice/beverage plants in south India like *Zingiber officinale, Allium sativum, Allium cepa, Syzygium aromaticum, Cinnamomum verum, Camellia sinensis, Curcuma longa* and *Elettaria cardamomum* were selected to test their activity against strains of *M. tuberculosis* H37Ra through microplate Alamar blue assay (MABA).

MATERIALS AND METHODS

Collection of plants

Z. officinale -rhizome, *A. sativum* -bulb, *A. cepa* -tissue, *S. aromaticum* -flower bud, *C. verum* -bark, *C. sinensis* -leaves, *C. longa* –rhizome and *E. cardamomum* -seed pods were collected in the month of June, 2011 from in and around Vellore and Vellore market. All plant specimens were identified at Department of Medical Biotechnology, School of Bio Sciences and Technology, Vellore Institute of Technology (VIT) University, Vellore.

Extract preparation

Powdered leaves of *C. sinensis* was boiled with water to collect extract, while water extracts of other plants were prepared using mortar and pestle with sterile distilled water in ratio 1:1 and passed through the millipore (0.22 µm) membrane filter, vacuum concentrated and lyophilized to give fine powdered samples. For ethanolic extract preparation, the dried plant parts were powdered and soxhlet apparatus was used.

Mycobacterial strains

Reference drug susceptible strain *M. tuberculosis* H37Ra (MTCC-300) was obtained from MTCC, IMTECH Chandigarh. *M. tuberculosis* H37Ra was revived in Loewenstein-Jensen medium (Fluka prod. no. 63237). Rifampicin (Sigma Prod. No. R3501) was purchased.

MABA assay protocol

A stock solution of Rifampicin was prepared in dimethyl formamide (10 mg/ml), aliquoted, and stored at -20℃. Alamar dye (Invitrogen) was purchased. Anti-mycobacterial bioassay was performed using the MABA (Collins and Franzblau, 1997). Briefly, representative colonies of *M. tuberculosis* H37Ra from Lowenstein-Jensen (LJ) slope were suspended in 1 ml distilled water and the turbidity was adjusted to match McFarland tube No.1 (10^7 CFU/ml) and further diluted to 1:25 in 7H9 (Middlebrook 7H9 [Becon Dicinson] supplemented with 0.2% glycerol, 0.1% Casitone and 10% albumin-dextrose, pH 6.8), and used as inoculums. 100 µl of the bacterial suspension were added to each well of a micro titer plate together with the plant extracts in Middlebrook 7H9 medium to the final volume of 200 µl, and the final concentration of the aqueous and ethanolic extracts were 50, 100 and 200 µg/ml. A growth control and a sterile control wells were also included. Plates were covered and sealed with parafilm and incubated at 37℃. Afte r incubation for about 7 days, 20 µl of Alamar blue dye were added to the wells. The plates were re-incubated overnight at the same temperature. A color change from blue to pink indicated bacterial growth. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of the drug that showed no color change. For standard and tests, the MIC values of Rifampin were determined. The acceptable MIC ranges of drug were 0.0047 to 0.0095 µg/ml.

RESULTS AND DISCUSSION

TB has been a major health problem in 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 (Renu et al., 2010). The Failure in the compliance of the treatment produced MDR-TB strains (Adelina et al., 2007). The association with HIV epidemic, the increasing emergence of MDR-TB and XDR-TB have worsened the situation and posed a serious health threat. Therefore, potent new anti-TB drugs with novel modes of action and low toxicity are urgently needed to combat the threat of TB. Natural sources provide numerous examples of interesting secondary metabolites with anti-mycobacterial activity, indicating that natural products could be a rewarding field for the discovery of new anti-TB leads (Xuan et al., 2011). In the present study, water and ethanolic extracts of selected medicinal plants (*A. sativum* -bulb, *A. cepa* -tissue, *S. aromaticum* -flower

Table 1. Anti-mycobacterial activity of plant extracts in MABA.

Medicinal plant	MIC (µg/ml) values for aqueous extracts	MIC (µg/ml) values for ethanolic extracts
Z. officinale	Nil	Nil
A. sativum	50	100
A. cepa	100	100
S. aromaticum	Nil	200
C. verum	100	200
C. sinensis	Nil	Nil
C. longa	Nil	Nil
E. cardamomum	Nil	Nil
Rifampicin (standard)	0.0095	0.0095

The data represents mean of three replicates.

bud, *C. verum* -bark) were observed to have anti-TB activity against *M. tuberculosis* H37Ra and (*Z. officinale* -rhizome, *C. sinensis* -leaves, *C. longa* -rhizome, *E. cardamomum* -seed pods) had poor/no activity against *M. tuberculosis* H37Ra. The growth and MIC (µg/ml) in which the aqueous extracts of medicinal plants inhibits *M. tuberculosis* H37Ra by MABA method after 7 days of incubation at 37℃ were recorded (Table 1). Inhibit ion of *M. tuberculosis* was observed in many of these medicinal plants, but some of these plants did not exhibit any inhibition of *M. tuberculosis* H37Ra growth.

As shown in Table 1, the extracts of *A. sativum* showed anti-mycobacterial activity at 50 µg/ml of aqueous and 100 µg/ml of ethanolic extracts. Renu et al. (2010) observed that *A. sativum* showed various inhibitory effects on the *M. tuberculosis* H37Rv they used. *A. cepa* tissue extracts showed inhibition at 100 µg onwards to the higher concentrations. *S. aromaticum* -flower bud aqueous extract showed no inhibition but ethanolic extract inhibits *M. tuberculosis* at 200 µg. The aqueous extract of *C. verum* -bark inhibits at 100 µg whereas ethanolic extract having an anti-mycobacterial effect at 200 µg/ml.

It is not surprising that there are differences in the anti-bacterial effects of plant groups, due to the phytochemical differences between species. To better evaluate the plants growing naturally in south India that are potentially useful resources, additional studies are necessary from the medicinal stand points. Plants produce a great diversity of substances that could be active in many fields of medicine (Kuete et al., 2010). There has been an increase in demand for the phytopharmaceuticals all over the world because of the fact that the allopathic drugs have more side effects (Vikrant, 2011). So, according to our results, *A. sativum* -bulb, *A. cepa* -issue, *S. aromaticum* -flower bud and *C. verum* -bark could be used as adjuvant therapy for TB.

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