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ANTI-TUBERCULAR ACTIVITY OF THE RHIZOME OF *CURCUMA PSEUDOMONTANA* J. GRAHAM

Gurusiddesh B Hiremath, Basappa B Kaliwal*

Department of Studies and Research in Biotechnology and Microbiology,
Karnatak University, Dharwad, India - 580 003.

Abstract

Tuberculosis is a highly infectious disease, with approximately one third of the world's population including 26 % from India estimated to be infected it. However, this problem has become serious as *Mycobacterium tuberculosis* developed resistance against drugs. Due to this, emergence of multi-drug resistant (MDR) strains of *Mycobacterium tuberculosis* has further complex the difficulty of tuberculosis (TB) control all over the world including India. Medicinal plants offer reliance for developing alternate medicines for the treatment of TB. The present study was performed to evaluate anti-tubercular activity of rhizome of *Curcuma pseudomontana*. The microplate Alamar blue assay (MABA) method is employed for the investigation. The change in the colour in various concentrations 100, 50, 25, 12.5, 6.25, 3.125, 1.6 and 0.8 µg/ml in different solvents like n-hexane, chloroform, ethyl acetate and methanol extracts of the rhizome have been studied. The results of the anti-tubercular assays showed that, hexane, chloroform, ethyl acetate and methanol extracts of rhizome showed activity against *Mycobacterium tuberculosis* H37RV at 100 and 50mg/ml. The overall results provide promising baseline information for the potential use of the crude extracts from the rhizome of *Curcuma pseudomontana* for treatment of tuberculosis.

Keywords: *Curcuma pseudomontana*, *Mycobacterium tuberculosis*, Microplate alamar blue assay.

Introduction

Tuberculosis (TB) is a deadly infectious disease caused by *Mycobacterium tuberculosis*. There were an estimated 8.8 million incident cases of TB (8.5 - 9.2 million) globally in 2010. Most of the estimated number of cases in 2010 occurred in Asia (59%) and Africa (26%). India alone accounted for an estimated one quarter (26%) of all TB cases worldwide[1]. Two of every five Indians are infected with the TB bacillus[2]. Tuberculosis is the major opportunistic infection of HIV/AIDS in developing countries[3]. With an increase in the number of people living with HIV and AIDS in India, the incidence of HIV/TB co-infection is expected to be on the rise. The high incidence of multi-drug resistant TB (MDRTB) in India is yet another issue that poses a challenge to infection control measures[4,5]. The Global Plan to Stop TB 2011-2015 targets that all patients who have been previously treated for TB should be tested for MDRTB using rapid tests by 2015[6].

Author for Correspondence:
Basappa B Kaliwal,
Department of Studies and Research in Biotechnology and Microbiology,
Karnatak University, Dharwad, India – 580 003.
E-mail ID: b_kaliwal@yahoo.com

The recent increase in the widespread existence of MDRTB especially in the developing countries emphasized the need for the development of new drugs to treat this infection. Such new anti-tubercular agents should have novel modes of action and full activity of the pathogen-*Mycobacterium tuberculosis*. The use of herbs and other alternative therapies for the treatment of tuberculosis is on the increase. Natural products continue to take on a more important role in the drug discovery and development process[7], and plants are recognized as a useful source of highly active antimycobacterial metabolites[8, 9]. Anti-TB drugs consist of two groups: essential or first line drugs, which are commonly used for the treatment of TB patients with susceptible Mycobacterium tuberculosis and reserve or second-line anti-TB drugs used for the treatment of multi-drug resistant TB (MDR-TB). Second-line drugs have many more adverse effects that the first-line anti-TB drugs[10].

The traditional healers use the genus Curcuma for the treatment of various disorders whereas *Curcuma pseudomontana* J. Graham is comparatively less known. *C. pseudomontana* belongs to family Zingiberaceae. *Curcuma pseudomontana* J. Graham known as Tavaksheera (Ayurveda) Kachura (Hindi), Raan halada, shindalavana or shindalavani (Marathi), Kattu manjal (Tamil), Kattu manjal (Malayalam). The Savara, Bagata, Valmiki tribes of Andhra Pradesh use tuber extracts to cure jaundice and Bagata tribes use this plant for Diabetes[11]. Jatapu and Kaya tribes apply warm tuber paste to treat body swellings. Women of Jatapu and Savara tribes eat boiled tubers to increase lactation[12]. Khand tribes apply the tuber paste on the head for cooling effect, crushed and boiled rhizome are edible[13]. The Kukus-Mukus eat fresh tubers as a blood purifier[14], The tubers are edible[15], Rhizome past used to apply to wounds and cuts[16, 17]. *C. pseudomontana* rhizome is also beneficial against leprosy, dysentery, cardiac diseases[18]. Since *C. pseudomontana* has shown a wide variety of medicinal importance. Therefore, the present investigation is to study anti-tubercular activity of the rhizome extract of the *C. pseudomontana* by using microplate lamar Blue Assay (MABA) method against *Mycobacterium tuberculosis* H37RV.

Materials and methods

Procurement of plant materials

Fresh and healthy rhizomes of *Curcuma pseudomontana* J. Graham was collected from the Western Ghats, Karnataka, India, during September 2012. The plant was authenticated by taxonomist Prof, G.R. Hegde, Department of Botany, Karnataka University Dharwad, India. A voucher specimen was deposited at the herbarium of the Department for future reference. The rhizome was washed cleanly, sliced and dried in a hot air oven at 50^0C for 72 hours and powdered.

Extraction

About 50 g of dry rhizome powder was defatted using *n*-hexane, followed by chloroform, ethyl acetate and methanol at room temperature and at atmospheric pressure, for 48 h with shaking at 100 rpm/min speed. The extracts were filtered and concentrated by using a rotary evaporator (Buchi Rotavapor R-124).

The anti-tubercular activity of the rhizome was assessed against *Mycobacterium tuberculosis* H37RV using the Microplate Alamar Blue Assay (MABA) method. This Methodology is non-toxic, uses a thermally stable reagent and shows correlation with proportional and BACTEC radiometric method. The samples were dissolved in DMSO, and final drug concentrations tested were 100 to 0.8µg/ml.

Preparation of Medium

Middle brook 7H9 Broth Base was prepared by mixing the ingredients mentioned in Table1 and the Middlebrooks OADC growth supplement was prepared by mixing the ingredients mentioned in Table 2. Prepared media were stored below 8˚C at pH 6.6 +/-0.2, protected from direct light. 2.35gm of Middle Brook 7H9TB broth base was suspended in 450ml distilled water which contains 5 ml glycerol sterilized by autoclaving at 15psi pressure at 121˚C for 15 minutes. Cooked to 400˚C and enriched with dextrose to a final concentration of 0.5% of bovine albumin fraction V.

Anti-tubercular screening

Microplate Alamar Blue assay

200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received

100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.8 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The MIC was defined as lowest drug concentration which prevented the color change from blue to pink[19, 20].

Results and Discussion

World Health Organization declared TB as a global health crisis because of the increase in HIV co-infection and the appearance of MDR-TB strains. No new anti-tubercular drug has been introduced in the 30 years, hence, there is an urgent need to develop novel, safe, effective and affordable drug for treating resistant forms of TB. There has been a renewed attention in phytochemicals as sources of novel therapeutics in the past decade, hence, plant have been investigated for various pharmacological effects including anti-tubercular activity. Phytochemicals may become the base for new drug development by providing a pharmacophore, which could be used for the development of new drug with novel mechanism of action.

In the present study, the anti-tubercular activity of rhizome of *Curcuma pseudomontana* was assessed against *Mycobacterium tuberculosis* H37RV by the Microplate Alamar Blue Assay (MABA) method. The change in the colour in various concentrations 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.6 µg/ml, 0.8 µg/ml in different solvents like n-hexane, chloroform, ethyl acetate and methanol extracts of the rhizome have been shown in theand compared to standard drug Pyrazinamide- 3.125µg/ml, Streptomycin-6.25µg/ml, Ciprofloxacine-3.125µg/ml (Table 3). Here MIC is defined as the lowest drug concentration, which prevents the colour change from blue to pink. Higher concentration of hexane, chloroform, ethyl acetate and methanol at 100 and 50mg/ml shows sensitivity by the *Mycobacterium tuberculosis* H37RV. Similar reports shows that aqueous and ethanolic extracts of *C. long* and *Z. Officinal* rhizome as has been studied for the anti-tubercular activity but these plants did not exhibit any inhibition of *M. Tuberculosis* H37RV growth[21]. The extracts of *A. sativum* showed anti-tubercular activity at 50 µg/ml of aqueous and 100 µg/ml of ethanolic extracts. *A. cepa* tissue extracts showed inhibition at 100 µg/ml onwards to the higher concentrations. *S. aromaticum* -flower bud aqueous extract showed no inhibition but ethanolic extract inhibits *Mycobacterium tuberculosis* at 200 µg/ml. The aqueous extract of *C. verum* -bark inhibits at 100 µg/ml whereas the ethanolic extract having an antitubercular effect at 200 µg/ml[21]. The ethanolic extract of *Allium cepa* inhibits at 500µg/ml. While the extracts of *A. vasica* and *A. indica* did not inhibit the growth of *Mycobacterium tuberculosis* even at 500 µg/ml[22]. 70% EtOH extract of *Capparis tomentosa* inhibits at 125 µg/ml, n-hexane extract of *Securidaca longepedunculata* inhibits at 125 µg/ml, Ethyl acetate, 70% alcohol and aqueous extracts of *Tabernaemontana elegans* showing inhibition at 15, 125 , 125 µg/ml respectively. The n-hexane, dichloromethane and ethyl acetate extracts of *Zanthoxylum capense* showing inhibition at 125, 62,125 µg/ml respectively[23]. When compared other medical other plant which used for study of anti-tubercular activity, rhizome extracts of *Curcuma pseudomontana* provide promising baseline information for the potential use of the crude extracts for treatment of tuberculosis.

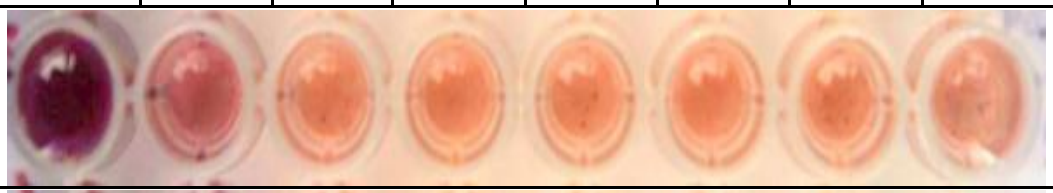
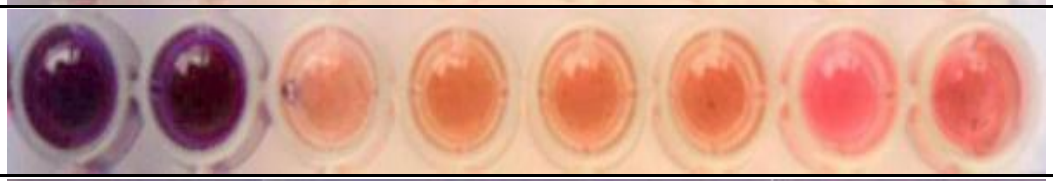
Table No. 01: Middlebrook 7H9 Broth Base

Ingredients	Quantity (grams/litre)
Ammonium sulphate	0.50
Disodium phosphate	2.50
Monopotassium phosphate	1.00
Sodium citrate	0.10
Magnesium sulphate	0.05
Calcium chloride	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Ferric ammonium citrate	0.04
L- Glutamic acid	0.50
Pyridoxine	0.001
Biotin	0.0005

Table No. 02: Middlebrook Oadc Growth Supplement

Ingredients	Quantity
Bovine albumin fraction V	2.50 grams
Dextrose	1.00 grams
Catalase	0.002 grams
Oleic acid	0.025 grams
Sodium chloride	0.425 grams
Distilled water	50.00 ml

Table No. 03: Anti-tubercular activity of the rhizome extract of the *C. pseudomontana* using different solvents

Final Drug Concentration (µg/ml)									
S/n	Extracts	100	50	25	12.5	6.25	3.125	1.6	0.8
		S	S	R	R	R	R	R	R
1	**n-Hexane**								
2	**Chloroform**								
3	**Ethyl acetate**								
4	**Methanol**								
5	**Pyrazinamide**								
6	**Streptomycin**								
7	**Ciprofloxacine**								

***S-sensitivity, R-resistance**

Plants have provided many medicinal drugs in the past and remain an impending source of therapeutic agents to this day[24]. The use of plants with medicinal action has grown, in spite of all the advances made in medicine, even in developed countries, due to several factors such as the assurance of the populations that use them, their ease of possession, and their low cost[25]. Public scepticism about the ability of allopathic medicines to be free from adverse effects, or to cure chronic conditions, have contributed to consumer demand for high quality herbal medicinal products[24]. Hence, indigenous plants are used worldwide as medicines, particularly in the developing countries. A growing number of chemical and pharmacological studies are proving the effectiveness and the existence of the medicinal properties in a great variety of plants. Anti-mycobacterial activity has been found in several species of plants and some classes of natural compound such as the terpenoids[26] and physalines[27, 28] were indicated as responsible for

the biological activity against *Mycobacterium tuberculosis.*

A significant number of plant species have been described in Ayurveda for the treatment of TB, leprosy and TB related disorders. Recently in a review[29] on Indian medicinal plants active against TB reported total 255 plant species mentioned in Ayurveda. It is not surprising that there are differences in the antibacterial effects of plant groups, due to the phytochemical differences between species. Plants produce a great diversity of substances that could be active in many fields of medicine[30]. Biochemical aspect of the synthesis of secondary metabolites depend on the plant genetic, taxonomy, the stage of development, the season, the presence of parasites and others. The variations could also be the result of abiotic factors such as relief, altitude, geological substrate characteristics[31]. 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[32].

Conclusion

This is the first report of anti-tubercular activity of rhizome of *Curcuma pseudomontana*. Rhizome extract exhibited significant anti-tubercular activity against *Mycobacterium tuberculosis* H37 RV. Further studies using more specific methods are required to explore the constituents responsible for the activity and the mechanism of this activity, which might prove important and improved therapies for the treatment, and prevention of tuberculosis.

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