



Research Article

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**Evaluation of Anti-Mycobacterial activity of the Siddha medicine  
Singi Chenduram against Multi-drug resistant tuberculosis using Luciferase  
Reporter Phage assay**

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**ABSTRACT**

Multidrug-resistant tuberculosis (MDR-TB) caused by mycobacterium tuberculosis becomes a global life threatening issue in many developing countries including India. Treatment of MDR-TB with second line anti tubercular drugs is associated with lot of side effects which includes hepatitis, depression and hallucinations. Siddha system of medicine has wide choice of therapeutic formulations which offers ailment against dreadful infectious like TB. As an objective of exploring alternate therapy for the clinical management of MDR-TB with less or no side effects the present investigation aimed at evaluating the anti-mycobacterial activity of the siddha drug Singi Chenduram (SC) using Luciferase Reporter Phage assay. In-vitro anti-mycobacterial activity evaluation of SC carried out at the concentration of 100 and 500µg/ml against standard strains of *M. tuberculosis* H37Rv, clinical isolates of *M. tuberculosis* strains resistant to first line anti-TB drugs (Streptomycin (S), Isoniazid (H), Rifampicin (R) and Ethambutol (E)) in comparison with marketed standard Rifampicin 2µg/ml. The result obtained from the study clearly reveals that the drug SC at both the concentration of 100 and 500µg/ml shows significant percentage reduction in relative light units (RLU) which is a marker indication of its efficacy against. Hence from the study it was concluded that the drugs like SC from the siddha system of Indian traditional medicine will provides clinically significant results in treating MDR-TB and further has to be ascertained by proper clinical evaluation in the infected subjects.

**Keywords:** MDR-TB, Singi Chenduram, Siddha drug, Luciferase Reporter Phage assay, *M. tuberculosis* H37Rv, Rifampicin.

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**INTRODUCTION**

Tuberculosis (TB) is an infection caused by bacterial complex, predominantly *Mycobacterium tuberculosis* and more rarely *Mycobacterium bovis*. Multidrug resistant tuberculosis (MDR-TB) is a clinically infectious condition caused by infection due to *Mycobacterium tuberculosis* which is resistant to Isoniazid and Rifampicin, with or without resistance to other anti-tubercular drugs [1]. Due to inadequate treatment and poor patient compliance, mutant resistant strains are developed which causes multidrug resistant tuberculosis [2]. According to the literature it was concluded that the most important risk factor associated with the development of MDR-TB is previous anti-tuberculosis therapy [3]. In 2007, global burden of tuberculosis was reviewed and it was found that there were

500000 cases of MDR TB reported from high burden countries. Among these cases of MDR TB, number of cases reported from India were 131,000 cases from China were 112000, from Russia 43000, from South Africa 16000 and 15000 cases were from Bangladesh [4].

India is one of the high TB burden countries accounting for one fifth of the global incidence of TB and tops the list of 22 high TB burden countries [5]. The only available source of TB patient related information is from the Government of India's Revised National TB Control Programme (RNTCP) which uses standardized recording and reporting systems spread throughout the country for systematically collecting, analyzing and disseminating data. This recording and reporting system is in alignment with the WHO recommended standard recording and reporting system for National TB Programmes and captures information on TB patients initiated on treatment using the drugs and regimens prescribed by RNTCP [6,7]. India has reported nearly 1.4 million TB cases in the year 2006 contributing to one-fifth of the total global cases. The country continued to report high morbidity and mortality due to tuberculosis and as many as 1.39 million cases were reported in 2006, according to a report on tuberculosis in the South East Asia region by the WHO. 8.6 million new cases of TB reported in the year 2012 and 1.3 million TB death has been reported in the year 2013.

Extensively drug-resistant tuberculosis (XDR-TB) is a form of tuberculosis caused by bacteria that are resistant to some of the most effective anti-TB drugs. XDR-TB strains have arisen after the mismanagement of individuals with multidrug-resistant TB (MDR-TB). The principles of treatment for MDR-TB and for XDR-TB are the same [8]. Treatment requires extensive chemotherapy for up to two years. Second-line drugs are more toxic than the standard anti-TB regimen and can cause a range of serious side-effects including hepatitis, depression and hallucinations. Patients are often hospitalized for long periods, in isolation. In addition, second-line drugs are extremely expensive compared with the cost of drugs for standard TB treatment [9].

It is a right time to explore the traditional choice of drugs from the origin of siddha system of medicine by considering the facts of deleterious side effects caused by conventional chemotherapeutic agents towards the treatment of MDR-TB. Siddha system of medicine is one of the most conservative medical systems in the world. In the field of medicine Siddhars had enlightened the world to save the human lives from various dreadful life threatening diseases. In Siddha system, the medicines are not only made up of herbs which include minerals, metals and other products of different biological origin.

*Singi chenduram* is indicated as a wonderful drug for *Sayam* in *Yaakoebu loga chenduram* 300. There is no scientific validation behind this formulation against MDR-TB. However it is possible to generate a data based evidence to evaluate this medicine with reference to the authentic drugs for the possible ailment against infectious disease like TB.

The main aim of the present study is to evaluate the anti-mycobacterial activity of the drug *Singi Chenduram* (SC) using Luciferase Reporter Phage assay against standard strains of *M. tuberculosis* H37Rv and clinical isolates of *M. tuberculosis* strains resistant to first line anti-TB drugs.

## EXPERIMENTAL SECTION

### Preparation of *Singi Chenduram* (SC)

*Thaalagam* and *Miruthar singi* were purified separately as per Siddha literature. *Miruthar singi* is bonded with the juice of latex of *vajiram* - *Sathurakkalli* (*Euphorbia antiquorum*) for 6 hours and made into pellets and allowed those pellets to dry. Then it is subjected to calcination process called *Putam*. This process is repeated thrice followed by this calcinated powder of *miruthar singi* was obtained. Then the purified *thaalagam* is grounded well with juice of *Opuntia delenii* along with lemon juice for 12 hours. Followed by this the paste was made into pellets and again allowed to dry. *Kilinjai seyaneer* (prepared according to classical text) was added to it and further subjected to calcination process [10]. Now the calcinated powder of *thaalagam* is also prepared. And finally, mix the above calcinated powder of *miruthar singi* and the above calcinated powder of *thaalagam* with lemon juice and was made into a pellet and allowed to dry it and subjected this into *putam* process. After the completion of *putam* process grind the obtained villai into powder. Now *Singi Chenduram* final formulation is prepared as shown in figure 1 - 3.

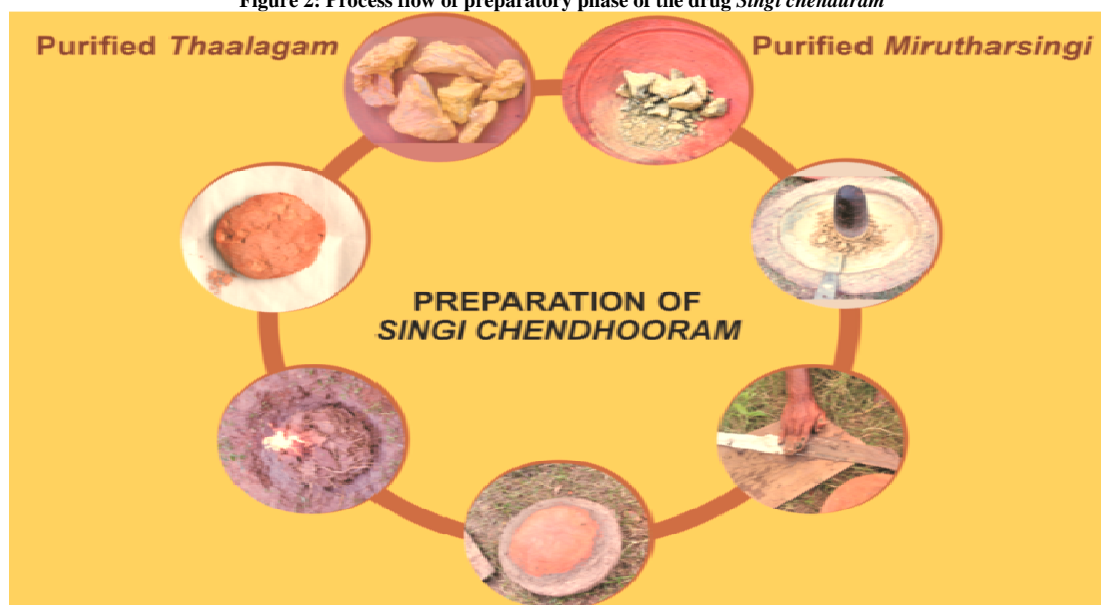
Figure 1: Ingredients and Raw material required for preparation of the drug *Singi chenduram*Figure 2: Process flow of preparatory phase of the drug *Singi chenduram*

Figure 3 : Final finished formulation of the drug *Singi chenduram***Mycobacterial strains**

Standard strain of *M. tuberculosis* H37Rv and clinical isolates of *M. tuberculosis* strains resistant to first line anti-TB drugs, Streptomycin(S), Isoniazid (H), Rifampicin (R) and Ethambutol (E) and were used for this study. These strains were grown and maintained on Lowenstein Jensen (L-J) medium in the Department of Bacteriology, National Institute for Research in Tuberculosis (NIRT- ICMR), Chennai, Tamil Nadu, India.

**Anti-Mycobacterial screening by Luciferase Reporter Phage assay**

Luciferase reporter phage PhAE129, a D29 derived mycobacteriophage, constructed in the laboratory of W.R. Jacobs was used in this study and it was propagated with *M. smegmatis* mc2 155 to get high titer by harvesting with Mycobacteriophage buffer (MP) from lacy plates and stored at 4°C until use.

Luciferase reporter phage (LRP) assay was carried out at National Institute for Research in Tuberculosis, Chetput, Chennai, Tamil Nadu, India by using standard protocol as adopted. 800 µl of bacterial suspensions equivalent to McFarland #2 standard were added to 400 µl of G7H9 with and without the test compound. For each sample, two sample-free controls and single drug concentration (100 and 500 µg/ml) were prepared, and incubated for 72 hr at 37°C. After incubation, 50 µl of the high-titer phage phAE129 and 40 µl of 0.1 M CaCl<sub>2</sub> were added to all the vials and incubated at 37°C for another 4 h. After incubation, 100 ml of the mixture was transferred from each tube into a star tube and equal amount of working D-luciferin (0.3 mM in 0.05 M sodium citrate buffer, pH 4.5) solution was added. The relative light unit (RLU) was measured after 10s of integration in the luminometer (Monolight 2010). The percentage reduction in RLU was calculated for each test sample and compared with the control. Anti-mycobacterial activity is indicated by fifty percentage reduction in relative light units (RLU) in the presence of compound in comparison with compound free control [11].

**RESULTS**

Test drug SC at the concentration of 500 µg/ml exerted maximum RLU percentage reduction of about 73.56% and 63.51 percentages reduction was observed at the concentration of 100 µg/ml when compared with standard rifampicin which exerts 97.49 % against *Mycobacterium tuberculosis* H37Rv as shown in Table 1 and Figure 4.

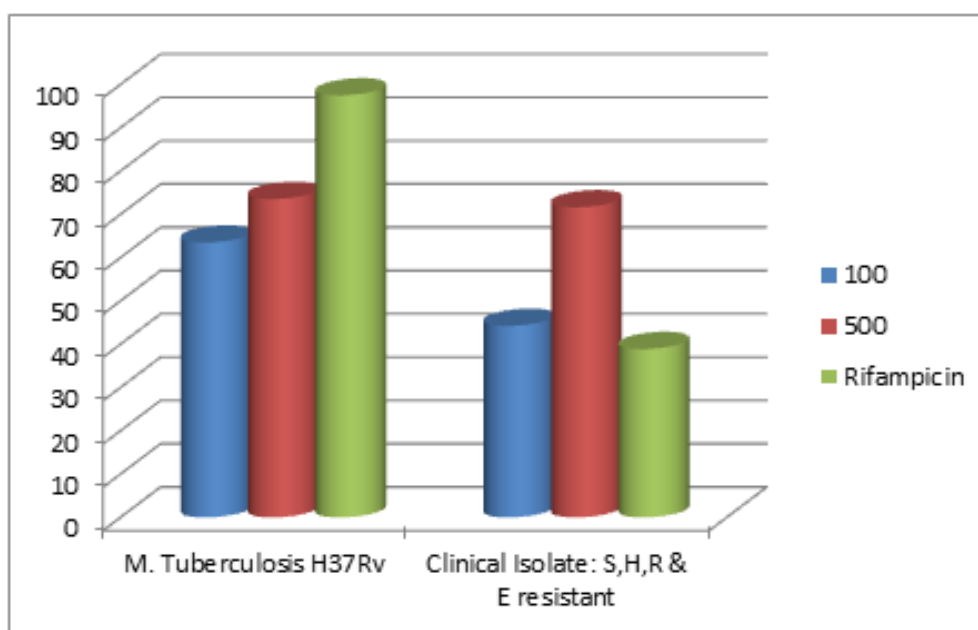
The anti- mycobacterial efficacy of the drug SC were further evaluated against clinical isolates of *M. tuberculosis* strains resistant to first line anti-TB drugs, Streptomycin(S), Isoniazid (H), Rifampicin (R) and Ethambutol (E) and the results of the study shows that SC at the concentration of 500 µg/ml exerted maximum RLU percentage reduction of about 71.65% and 44.26% percentages reduction was observed at the concentration of 100 µg/m when compared

with standard Rifampicin which exerts 38.90 % as shown in Table 1 and Figure 4. The percentage reduction exhibited by SC in evaluation against *M. tuberculosis* strains resistant to first line is significantly much higher than the standard drug rifampicin.

**Table 1:** Effect of SC on percentage reduction of RLU against *Mycobacterium tuberculosis* H37Rv and Clinical isolate resistant to S, H, R & E

Strain	% Reduction of RLU		
	100µg/ml	500µg/ml	Rifampicin 2µg/ml
<i>Mycobacterium tuberculosis</i> H37Rv	63.51	73.56	97.49
Clinical isolate: S, H, R & E resistant	44.26	71.65	38.90

**Figure 4:** Percentage reduction of RLU by SC in Luciferase Reporter Phage assay against *Mycobacterium tuberculosis* H37Rv and Clinical isolate resistant to S, H, R & E



## DISCUSSION

MDR-TB becomes global health hazard associated with higher rates of failure and mortality than drug susceptible TB, especially in human immunodeficiency virus (HIV) co-infected patients [12,13]. From a microbiological perspective, resistance is caused by a genetic mutation that makes a drug ineffective against the mutant bacteria. Patients with a large bacillary load have an increased risk of developing resistant bacteria because more spontaneous mutations occur in a large population of bacteria. An inadequate treatment regimen then allows for the selection of a drug-resistant strain to become the dominant strain in a patient infected with TB [14]. Controlling large bacterial load seems to be a hall mark phase in patient with TB and thus it may be claimed from the results of the study that treatment with SC have significantly higher chances of controlling the bacterial load hence chances of drug resistance may be greatly reduced. This property of SC could be due to the presence of nanoparticle and related components present with in the formulation .Presence of nano particle in SC was already reported through systematic standardization study carried out and documented previously in the year 2015 [15].

Results obtained from Luciferase Reporter Phage assay clearly shows that SC has promising activity against *Mycobacterium tuberculosis* H37Rv in which SC at the concentration of 500µg/ml exerts significantly higher activity than 100µg/ml when compared with standard drug rifampicin, similarly the results of SC against clinical isolates of *M. tuberculosis* strains resistant to first line anti-TB drug reveals that SC at both concentration exerts significantly higher percentage reduction than the standard drug rifampicin this shows the better therapeutic efficacy of the drugs than the rifampicin.



Lead oxide being a major component of the formulation *Singi chenduram* has undergone a major transition from its basic metallic state to another inorganic complex form during preparatory phase of the formulation may renders a potential medicinal property like Anti-TB. This transition of lead from one form to another will be achieved only through the prescribed procedure as described by siddhar in the vedic literature [16].

It was evident from the literatures that nanoparticle has better membrane permeability and access to the cell wall of the mycobacterium and the rough predicated mechanism of the SC against S, H, R & E resistant *Mycobacterium* may be due to hindrance in expression of efflux proteins that actively pumps out a broad range of chemotherapeutic agents from the interior of the cell but still the molecular level study has to be carried out to confirm the efflux protein inhibition potential of the SC in future.

### CONCLUSION

Hence from the results it was concluded that *Singi Chenduram* has shown significantly higher percentage reduction at the concentration of 100 and 500 µg/ml in both the strains of *Mycobacterium tuberculosis H37Rv* and clinical isolate: S, H, R & E resistant *Mycobacterium*. Further clinical study has to be carried in subjects with MDR-TB to justify the efficacy of the drug for clinical management of TB.

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