Research Article

Anti-Tubercular Activity on Leaves and Roots of *Sida rhombifolia* L.

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Accepted on: 22-03-2013; **Finalized on:** 31-05-2013.

ABSTRACT

Sida rhombifolia L. is a very small perennial or annual plant which grows in tropical and subtropical areas. It belongs to the family Malvaceae. It grows upto 50-120 cm in height. It is commonly called Bala or Atibala. Modern research carried out on the Malvaceae plants reveals that most of the plants belonging to this family are medicinally important as they contain biologically active compounds. It has been reported for the presence of steroids, flavanoids, terpenoids, alkaloids, phenol, saponins, glycosides and tannins. Its leaves, stem, bark and roots are the usable parts. The present study was carried out to find out the susceptibility of *Mycobacterium tuberculosis* to ethyl acetate and ethanolic extracts of leaves and roots of *Sida rhombifolia* L. Luciferase reporter phage (LRP) assay was used to study the inhibition of *Mycobacterium tuberculosis*. The LRP assay showed that ethyl acetate extracts of leaves and roots at concentrations of 100 and 500 µg/ml showed good activity against the Standard strain of *M.tuberculosis* $H37R_V$ and clinical isolate of *M.tuberculosis* resistant to S, H, R and E. The results show that ethyl acetate leaf extract at 100 µg/ml was found to cause 67.18 % reduction in Relative Light Units (RLU) and 500 µg/ml was found to produce 83.61 % reduction in RLU. This shows that the ethyl acetate has anti tubercular activity against *Mycobacterium tuberculosis*. The study can further be extrapolated to isolate and identify the compound responsible for the anti tubercular activity.

Keywords: *Sida rhombifolia* L., Luciferase Reporter Phage (LRP), Ethyl acetate and Ethanol extract, Anti tubercular Activity.

INTRODUCTION

Tuberculosis has been present in humans since antiquity, at the latest. The earliest unambiguous detection of *M. tuberculosis* involves evidence of the disease in the remains of bison dated to approximately 17,000 years ago[1]. Skeletal remains show prehistoric humans (4000 BC) had TB and researchers have found tubercular decay in the spines of Egyptian mummies dating from 3000–2400 BC[2].

Tuberculosis (TB) is an infectious disease caused by the bacterium ***Mycobacterium tuberculosis*.** It retains certain stains after being treated with acidic solution, so it is classified as an acid-fast bacillus (AFB). *Mycobacterium tuberculosis* is an aerobic, gram positive rod shaped bacteria ranging from 1 to 4 µm in length[3].

Tuberculosis remains the largest cause of death in the world from a single infectious disease[4] and accounts for as much as 40% of deaths in human immunodeficiency virus (HIV) co-infected individuals in some developing countries[5]. Infection with conventional *M. tuberculosis* can effectively be cured with a combination of antitubercular drugs. Ominously, multidrug-resistant tuberculosis (MDR-TB) strains have emerged in several countries, with case fatalities ranging from 40 to 60% in immunocompetent individuals and >80% in immunocompromised individuals[6].

Though we have an enormous wealth of medicinal plants throughout the length and breadth of our country, not many detailed attempts have been made to explore this activity of the plant kingdom. Considering the need for newer anti- tubercular drugs and existence of enormous wealth of medicinal plants in our country, it was planned to study the *In vitro* anti-tubercular activity of *Sida rhombifolia* L. which is commonly used in the treatment of respiratory diseases like bronchitis, asthma, tuberculosis, urogenital diseases and in dysenteries in the indigenous system of medicine[7].

MATERIALS AND METHODS

Plant Material

The fresh leaves and roots of *Sida rhombifolia* L. Family: Malvaceae, were collected from Thirunelveli Dist, Tamil Nadu in July 2012. The specimens were authenticated by Taxonomist, Prof.P.Jayraman, Plant Anatomy Research Centre (PARC), Chennai. The herbarium specimen bearing the voucher number PARC/2013/2003 is kept in the Department of Pharmacognosy, College of Pharmacy, Madras Medical College, Chennai.

Preparation of Extracts

Extraction is the preliminary step involved in the phytochemical studies. It brings out the metabolites into the extracting solvent depending upon their polarity[8].

Phytochemical Analysis

The leaves and roots were air dried, powdered and passed through a sieve No.22 and stored in air tight containers and were extracted successively with each of petroleum ether, chloroform, ethyl acetate and ethanol in a Soxhlet extractor for 18-20 hrs. The extracts were concentrated to dryness under reduced pressure using a rotary vacuum evaporator. All the extracts were

subjected to qualitative chemical tests for the identification of various phytoconstituents (Table 1).

Table 1: Phytochemical studies

Extracts	Leaves	Roots
Petroleum ether	Steroids, Terpenoids	Steroids, Terpenoids
Chloroform	Alkaloids, Phenol	Alkaloids, Phenol
Ethyl acetate	Saponins, Glycosides, Tannins and Flavanoids	Saponins, Glycosides, Tannins and Flavanoids
Ethanol	Alkaloids, Glycosides, Tannins, Saponins, Terpenoids and Flavanoids	Alkaloids, Glycosides, Tannins, Saponins, Terpenoids and Flavanoids

Antitubercular Assay

Anti-mycobacterial activity of the *Sida rhombifolia* L. extracts was evaluated by Luciferase reporter phage (LRP) assay against Standard strain of *M. tuberculosis* $H37R_V$ and Clinical isolate of *M.tuberculosis* resistant to Streptomycin, Isoniazid, Rifampicin and Ethambutol (S,H,R & E) at two different concentrations (100 and 500 µg/mL). The Luciferase reporter phage assay methodology is rapid, inexpensive and less laborious for high throughput screening of compounds for their anti mycobacterial activity compared to BATEC methodology which is costly, cumbersome and uses radioactive reagents. A compound is considered as an anti-tubercular agent if fifty percent reduction in relative lights units (RLU) is observed when compared to the control using luminometer[9].

Microbial strain for anti-Mycobacterium tuberculosis Assays

Standard strain of *M.tuberculosis* $H37R_V$ and clinical isolate of *M.tuberculosis* resistant to S,H,R & E maintained at National Institute for Research in Tuberculosis, Chennai were used for the anti mycobacterial assay.

Luciferase reporter phage (LRP) assay

Standard strain $H37R_V$ and a clinical isolate of *M.tuberculosis* resistant to S, H, R & E were grown in Middlebrook 7H9 complete medium 12 with and without extracts of *Sida rhombifolia* L. for 3 days at 37°C.

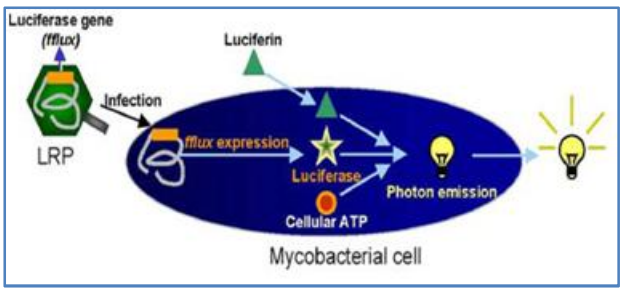

Figure 1: LRP Instrumentation

Luciferase Reporter Phage Assay[10] was done using concentrations of 100 and 500 µg/ml of *Sida rhombifolia* L. extracts. Fifty-microliter bacterial suspension equivalent to MacFarlands No.2 standard was added to 400 µl of G7H9 with and without the test compound. For each sample, two drug-free controls and two drug concentrations were prepared and this set up was incubated for 72 h at 37°C. After incubation, 50 µl of the high titer Luciferase reporter phage (phAE129) and 40 µl of 0.1 M $CaCl_2$ were added to all the vials and this setup was incubated at 37°C for 4 h. After incubation, 100 µl of the mixture was taken from each tube into a luminometer cuvette and an equal amount of working D-luciferin (0.3 mM in 0.05 M sodium citrate buffer, pH 4.5) solution was added. The RLU was measured after 10s of integration in the Luminometer. Duplicate readings were recorded for each sample and the mean was calculated. The percentage reduction in the RLU was calculated for each test sample and compared with control. The experiment was repeated when the mean RLU of the control was less than 1000[11].

RESULTS AND DISCUSSION

The result of the anti tubercular activity by Luciferase reporter phage assay is presented (Tables 2&3) against $H37R_V$ a Standard strain of *Mycobacterium tuberculosis* and clinical isolate of *M.tuberculosis* resistant to S, H, R & E.

Table 2: Antitubercular activity of leaf and root extracts of *Sida rhombifolia* L. against standard strain of *M. tuberculosis* $H37R_V$

S. No.	Extract	% Reduction in RLU	
		100 µg/ml	500 µg/ml
Leaf Extracts			
1	Ethyl Acetate	45.69	61.72
2	Ethanol	0	0
Root Extracts			
3	Ethyl Acetate	14.67	84.75
4	Ethanol	0	0

From the table 2 it can be seen that at both the concentrations tested i.e. 100 µg/ml and 500 µg/ml, the ethanolic extract of both the leaves and roots did not show any characteristic activity against Standard strain of *M. tuberculosis* $H37R_V$. The ethyl acetate extract of both leaves and roots showed good activity. The percentage reduction in RLU was 45.69 % and 61.72 % for ethyl acetate extract of leaves at 100 µg/ml and 500 µg/ml respectively. For the roots, the ethyl acetate extract showed 14.67 % and 84.75 % inhibition at the concentration of 100 and 500 µg/ml respectively.

In the case of clinical isolate of *M.tuberculosis* resistant to S, H, R & E, the ethanolic extract of both the leaves and roots at lower concentration of 100 µg/ml did not show any characteristic activity, whereas at 500 µg/ml there was some activity. The ethyl acetate extract of both

leaves and roots showed good activity. The percentage reduction in RLU was 67.18 and 83.61 for ethyl acetate extract of leaves at 100 µg/ml and 500 µg/ml respectively. For the roots, the ethyl acetate extract showed 29.02 % and 62.37 % inhibition at the concentration of 100 µg/ml and 500 µg/ml respectively.

Table 3: Antitubercular activity of leaf and root extracts of *Sida rhombifolia* L. against clinical isolate of *M.tuberculosis* resistant to S, H, R & E.

S. No.	Extract	% Reduction in RLU	
		100 µg/ml	500 µg/ml
Leaf Extracts			
1.	Ethyl Acetate	67.18	83.61
2.	Ethanol	0	3.61
Root Extracts			
3.	Ethyl Acetate	29.02	62.37
4.	Ethanol	0	29.94

The maximum inhibitory activity was seen in the Ethyl acetate extract of the leaves which is 83.61 % inhibition at 500 µg/ml concentration and at 100 µg/ml concentration it showed 67.18 % inhibition. This is even more significant as this activity was noted against a clinical strain which was resistant to S, H, R and E.

The results suggest good anti mycobacterial potency of the ethyl acetate leaf extract of *Sida rhombifolia* L. This extract has been reported to contain high concentrations of active compounds like alkaloids such as ephedrine, si-ephedrine, cryptolepine, quinazolines, beta-phenethylamines, vasicinol, vasicinone, vasicine, choline, betaine, tryptomine, sterculic acid, malvalic acid and linoleic acid and any of these could have contributed to the antitubercular activity[12].

CONCLUSION

The anti mycobacterial screening showed that the ethyl acetate extract of *Sida rhombifolia* L. leaves has the potential to cure tuberculosis and is a promise for future therapeutic interventions. To our knowledge it is for the first time that it has been possible to demonstrate experimentally in the laboratory the promising antitubercular activity of *Sida rhombifolia* L. leaves.

Further study on the activity of the fractions of the ethyl acetate extract of *Sida rhombifolia* L. leaves may provide better understanding of the anti tubercular activity. The bioassay–guided fractionation of this extract is currently underway in our laboratory with the goal of identifying the active compounds which may be responsible for the anti tubercular activity.

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Source of Support: Nil, **Conflict of Interest:** None.