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Antimycobacterial activity of methanol extract from the stem bark of *Alangium salvifolium* against multi-drug resistant mycobacterium tuberculosis

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ABSTRACT

Tuberculosis (TB) remains the 2nd leading cause of the major infectious disease in humans and causes considerable morbidity worldwide among millions of people every year. The present study aimed to screen the susceptibility of *Mycobacterium tuberculosis* to the stem bark of *Alangium salvifolium* using Luciferase reporter phage assay (LRP). Powdered stem bark of *Alangium salvifolium* was extracted with methanol, subjected to phytochemical analysis and TLC. Susceptibility of *M. tuberculosis* was studied by LRP assay at various concentrations (50, 100 and 200 µg/ml). The strains used for the present study are one reference strain *M. tuberculosis* H37 Rv and 2 clinical isolates one sensitive and the other resistant strain of *M. tuberculosis*, to Isoniazid, Streptomycin, Ethambutol and Rifampicin. The presence of the phytoconstituents viz., alkaloids, tannins, terpenoids, glycosides and flavonoids in the methanol extract are confirmed by preliminary phytochemical analysis. TLC analysis of methanol extract showed 12 well separated spots with Rf value ranging from 0.2 to 0.9. Methanol extract of *A. salvifolium* exhibited maximum antimycobacterial activity at higher concentration (200 µg/ml). The proportion inhibition of methanol extract of *A. salvifolium* against *Mycobacterium tuberculosis* H37Rv strain were 53.26%, 82.87% and 94.56% at the concentration of 50, 100 and 200 µg/ml respectively. The tested extract showed good microbial sensitivity against Multi drug resistant (MDR) strains. Further phytochemical analysis and identification of lead molecule(s) based on bioassay guided fractionation from the selected plant could serve as complementary alternative therapy for treating tuberculosis.

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INTRODUCTION

Tuberculosis (TB) remains the 2nd leading cause of the major infectious disease in humans next to human immunodeficiency virus (HIV) and causes considerable morbidity worldwide in millions of people every year. In many Asian and African countries, about 80% of the population shows positive in tuberculin tests, while in US population shows only 5–10% (Morris *et al.,* 2009). Additionally, drug-resistant strains of *M. tuberculosis* emerges increased pressure on current chemotherapy regimes. The morbidity caused by multi drug resistant TB in 2012 reported by WHO is 450.000 and mortality rate of about 170.000 deaths (WHO, 2013). Current anti-tuberculosis treatments lead to poor patient compliance due to long course of

treatment and toxic side effects of combination of antibiotics. There is a need to identify a novel anti-tubercular drug because of rapid increase in the incidence of multi drug resistant TB and anti-tubercular agent has not been introduced for the past 30 Yrs Gautam *et al.,* 2007). Herbal medicines provide hope to overcome the search. Traditional practitioner treats several diseases using herbal medicines over the world. More number of plants has been screened for antitubercular activity against *M. tuberculosis* in the past few years (Mmushi *et al.,* 2010; Luo *et al.,* 2011). To target tubercle bacilli and drug resistance antitubercular drug, there is a need to identify new safe and herbal drugs. Development of new potential anti-tuberculosis compounds from herbal sources have shown good antimicrobial activity and also inhibits the mechanism of resistance or modulation of the immune response (Hassan Farsam *et al.,* 2000).

Alangium salvifolium Wang belonging to the family Alangiaceae is a tall deciduous thorny tree belonging to the family Alangiaceae, medicinally used in India, China and Phillipines (Kirtikar Basu, 1987; Chopra *et al.,* 1956). The plant exhibits antimicrobial, diuretic, anti inflammatory, cardiac activity, anti-fertility, anticancer, antidiabetic, emollient, anthelmintic, antiepileptic and antiprotozoal activities. Externally applied to treat leprosy, inflammation and rheumatism. It is applied externally and internally to treat rabid dog bite (Praveen Kumar *et al.,* 2010; Xavier *et al.,* 2005 and Jain *et al.,* 2010). The stem bark shows anti-tubercular activity. However, the anti-tubercular activity of the plant has not investigated scientifically.

MATERIALS & MEHODS

Plant material

The plant specimen for study was obtained from Attur, Tamil Nadu, authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre, Tambaram, Chennai.

Extraction

Stem bark of *Alangium salvifolium* were dried, pulverized and the powdered stem bark (100 g) was extracted by percolation for 4 successive days with methanol (500 ml). The extract was filtered and evaporated by rotary evaporator at a temperature of 40^{o}C under pressure and dried at room temperature. For further testing, the dry extract was stored at room temperature.

Preliminary chemical analysis

The phytoconstituents present in the methanol extract was identified using the standard procedure (Harborne, 1987).

Thin Layer Chromatography

The total methanol extract of the stem bark of *A. salvifolium* was subjected to TLC analysis to support preliminary phytochemical analysis using the solvent system toluene: formic acid: ethyl acetate: (5:1:4). The TLC plate spotted with methanol extract was developed after drying by exposing to ammonia vapour. Rf value calculated for well separated and clear spots using the formula,

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

Mycobacterial strains

The mycobacterial strains used are the standard strain *M. tuberculosis* H37Rv, resistant strain to first line anti tubercular and sensitive strains to SHRE for the present study were grown in Lowenstein Jensen (LJ) medium in NIRT- ICMR (National Institute for Research in Tuberculosis), Chennai, Tamil Nadu.

Luciferase Reporter Phage assay for evaluating anti-tubercular activity

Luciferase reporter phage PhAE129, D29 derived mycobacteriophage constructed in the laboratory of W R Jacobs was propagated with *M. smegmatis* mc^2 155 and stored at 4^{o}C till use.

By using standard protocol, LRP assay was carried at National Institute for Research in Tuberculosis. To 400 µl of G7H9, 100 µl bacterial suspensions equivalent to McFarland #2 standard with and without test compound was added. Stock solution (10 mg/ml) was prepared by dissolving 1 g of methanol extract in small amount of DMSO and diluted to 100 ml with distilled water in a 100 ml volumetric flask and sterilized by filtration through 0.2 µm Whatman membrane filter. For test sample, standard (2 µg/ml) and three drug concentration (50, 100 and 200µg/ml) were prepared, and incubated for 72 h at 37°C. The mixture was incubated for another 4 h at 37^{o}C by adding 50 µl of the high-titre phage phAE129 and 40 µl of 0.1 M calcium chloride. After incubation from each tube, 100 ml of the mixture was transferred into a star tube, and an equal amount of working D-luciferin (0.05 M sodium citrate buffer in 0.3mM of pH 4.5) was added. After 10s of integration in the luminometer (Monolight 2010), relative light unit (RLU) was measured and its percentage reduction was calculated for the test sample by comparing with the standard. A reduction of 50% in relative light units indicates antimycobacterial activity of test sample by comparing with control (Carriere *et al.,* 1997).

RESULTS AND DISCUSSION

There is a need to find new compounds from herbal medicines due to the emergence of MDR (multi drug resistant), extreme drug resistant and

Table 1: Percentage reduction in RLU by methanol extract against standard strain and clinical isolates of M. tuberculosis

Strain	% Reduction in RLU			
	50 µg/ml	100 µg/ml	200 µg/ml	Rifampicin 2 µg/ml
M. tuberculosis H37Rv (standard strain)	53.26	82.87	94.56	98.35
Clinical isolate: SHRE Resistant	55.30	65.08	88.87	**0**
Clinical isolate: SHRE Sensitive	60.24	69.58	92.46	**0**

total drug resistant strains of *M. tuberculosis* (Pandya *et al.,* 2012). The methanol extract of the stem bark of *Alangium salvifolium* demonstrated to have potent antimycobacterial activity against *Mycobacterium tuberculosis* (MDR and H37Rv strains). The methanol extract of *Alangium salvifolium* showed good activity against H37Rv *M. tuberculosis* strain in the present study.

Figure 1: TLC of Methanol extract of Alangium salvifolium

The percentage of inhibition of *Alangium salvifolium* at higher concentration was 94.56% and 88.87% against *M. tuberculosis* H37Rv and MDR strain. Antimycobacterial activity of *Alangium salvifolium* extract against MDR strain of *M. tuberculosis* is reported for the first time. The bioactive constituents present in the plant extract were glycosides, alkaloids, terpenoids, flavonoids and tannins. The presence of these phytoconstituents may be responsible for the antimycobacterial activity of plant extract (McCarthy and Mahony, 2011; Arya, 2011).

CONCLUSION

Based on the findings of the present study, the stem bark of *Alangium salvifolium* exhibited promising antimycobacterial activity against multi drug resistant of *Mycobacterium tuberculosis* strain which could serve as an alternative treatment. Extension of work needed isolation of bioactive compounds exhibiting antitubercular activity from the stem bark of *Alangium salvifolium.*

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