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Investigation of Anti-Tubercular Potential of *Dalbergia spinosa* Roxb., Root Extracts

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Abstract: ***Dalbergia spinosa Roxb. (Family: Papilionaceae), is a large shrub with a tendancy to climb. Root is having bitter taste, used to treat inflammations, urinary problems, pain and fever cases. Dalbergia spinosa have been reported for various pharmacological properties such as hypothermic, spermicidal, semen coagulant, hypoglycemic, cardio vascular, antimicrobial, diuretic and analgesic. The main objective of the present study is to evaluate anti-tubercular activity against Mycobacterium tuberculosis H37Rv using Dalbergia spinosa Roxb., root extracts in different doses, in order to combat the emergence of multi drug resistant tuberculosis caused by allopathic drugs. Phytochemical screening revealed the presence of Polyphenols, flavonoids and isoflavones, proteins. Dalbergia spinosa, Roxb., root extracts were exhibited significant anti-tubercular activity against M.tuberculosis H37RV at the MIC of 50µg/ml, by using Isoniazid as a standard, 0.025µg/ml.***

Keywords: *Dalbergia spinosa*, Phytoconstituents, *Mycobacterium tuberculosis H37RV*

1. Introduction

With the rising prevalence of microorganisms showing resistance to antibiotics, there is an urgent need to develop new antimicrobial compounds [1]. Man has used plants to treat common infectious diseases, and some of the traditional medicines are still part of the habitual treatment for various maladies [2]. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity [3].

One of the most serious infectious diseases that are currently being intensified by the existence of resistant strains is *tuberculosis* (TB), a disease caused by *Mycobacterium tuberculosis* [4].

Tuberculosis (TB) is a disease known since antiquity and evidence of spinal TB in the form of fossil bones dates back to around 8000 BC [5, 6]. TB occurred as an endemic disease among animals long before it affected humans [7]. The first confirmed instance of TB in humans was noted in the deformities of the skeletal and muscular remains of the Egyptian mummies of around 2400 BC [8].

Annually, 8 million people become ill with TB and two million people die from the disease world-wide [9]. India accounts for nearly one-third of the global burden of TB and approximately 2 million people acquire TB every year [10]. In view of this a need for the development of new TB drugs is felt to control the spread of multidrug resistant TB (multi-drug resistance [MDR]-TB) [11]. In spite of global research efforts, mechanisms underlying pathogenesis, virulence and persistence of *M. tuberculosis* infection remain poorly understood. The urgent need to find new drugs to reduce the global burden of TB is much discussed in the present biomedical research [12]. Natural products and their semi synthetic derivatives can play a vital role in curing TB [13]. There are several reasons that justify the need to search for new anti-tubercular drugs, such as shorten the treatment duration, to get efficient treatment for MDR-TB and to eradicate the latent infection [14, 15]. Plants have supplied the foundation for traditional medicine for millennia dating back to information inscribed on clay tablets from Mesopotamia in 2600 BC. Today, traditional remedies continue to play an important role in modern medicine with approximately 80% of the world's inhabitants relying on their primary health-care [16]. The Union of Traditional Knowledge and modern science may provide an innovative and valuable bio prospecting tool for affordable, safe, novel and effective therapies [17] and to determine their potency against *M. tuberculosis* which will help to develop new drugs against the infection from these traditional plant extracts.

Dalbergia spinosa Roxb (Papilionaceae), is a large shrub with a tendency to climb, known as Jantrikanta and Nechitanchedi in Tamil [18]. The plant is used for the treatment of inflammations, urinary excretion problems, pain and fever [19]. 50% ethanol root extracts were reported to possesses hypothermic, spermicidal, semen coagulant, hypoglycemic, cardio vascular effects, antimicrobial, diuretic, anti-inflammatory, analgesic effects [20-24] and seed oil is said to be used as cosmetic and for discharge from the ear, leaves and stem bark is used as febrifuge, emmenagogue, anthelmintic, antinociceptive, antioxidant and cytotoxicity activity [25]. A spoonful of powder in a tumblerful of water is said to be sufficient to destroy, the effects of alcohol, even in cases bordering on delirium tremens within less than half an hour [26].

Dalspinin I, dalspinosin II, prunetin-4-O-β-D-galactoside, dalspinosin-7-O-β-glucopyranoside and a new isoflavone apioglucoside were isolated from the root and stem bark [27-30]. Objective of the present study is to the anti-tubercular potential and preliminary phytochemical screening of *Dalbergia spinosa*, Roxb., roots.

2. Materials and Methods

2.1 Plant material

The roots of *Dalbergia spinosa* were collected during the month of September from the mangrove forests in Thandavarayan Solanganpettai, Chidambaram, Tamilnadu, India. The plant species were identified and authenticated by plant taxonomist of Raphinat Herbarium, St. Josephs College, Tiruchirappalli with the help of herbarium (Plate No. 381, RHT 12844) and a voucher specimen was deposited in the Department of Pharmacognosy, Periyar College of Pharmaceutical Sciences, Tiruchirappalli, for further reference. The roots were separated from other aerial parts and washed completely with water and then shade dried further studies.

2.2 Reagents and Chemicals

All the reagents and chemicals used for analyzing various parameters were obtained from E. Merck Pvt. Limited, Mumbai, India, of analytical grade.

The roots were shade dried at room temperature in a clean environment to avoid contamination. A total of 200 g roots plant were powdered and extracted by continuous hot percolation using soxhlet apparatus with petroleum ether, benzene and ethanol and distilled water (0.25% $CHCl_3$ in water) by cold maceration method. All the extracts were concentrated under low pressure to dryness at 30°C using vacuum concentrator. The root extracts were air-dried and then packed in 1mL vials with proper labeling for future references. The extracts were kept refrigerated and away from the sunlight (wrapping with aluminum foil) prior to further processing. Stock solutions of the extracts were prepared in Di-Methyl Sulfoxide (DMSO) at a concentration of 5 mg/ml and stored at -20°C until use. All the crude extracts of were tested for anti-tubercular activity [31, 32].

Phytochemical analysis of extracts
The plant extracts were screened for phytochemicals using standard procedures for the detection of the saponins, tannins, terpenoids, alkaloids, steroids, flavonoids, anthraquinones and phloba tannins [31, 32].

Evaluation of anti-tubercular activity
Anti-tubercular testing against standard strain *of M. tuberculosis H37RV.*

Preparation of test extracts of *Dalbergia spinosa* Roxb
Prepared the stock solutions of each different extract as 5mg/ml and from the stock solution, the different concentrations i.e. 50, 100, 150 and 200 µg/ml of each extract sample were prepared by using dimethyl sulphoxide according to their solubility. Standard drug rifampicin (40 µg/ml) was also prepared.

Anti-tubercular activity against *M. tuberculosis* H37Rv American Type Culture Collection (ATCC 27294) by proportion assay

This assay measures the capability of the test compound to kill (or inhibit) the multiplication of pathogenic *M. tuberculosis* H37Rv (27294), which was procured from the ATCC, USA. The extracts of *Dalbergia spinosa,* Roxb., was dissolved in DMSO separately to make stocks (5 mg/ml). Serial dilutions from stocks were also made in DMSO. Each test extract of 0.1 ml or DMSO (negative control) or Rifampicin (positive control) were added in to 1.9 ml Middle Brook (MB 7H10) agar medium (Experiment was conducted in Commercial (MB 7H10) medium purchased from Becton Dickinson Company (Difco™) supplemented with Oleic Albumin Dextrose Catalyse. The contents were mixed and allowed to solidify as slants. 3-week old culture of *M. tuberculosis* H37Rv (from L-J medium slant) was harvested and its suspension (0.1 mg/ml, equivalent to approx. 10^7 bacilli/ml) was made in normal saline containing 0.05% Tween-80. 10 µl of this suspension (~10^5 bacilli) was inoculated on to each tube and incubated at 37°C for 4 weeks. The lowest concentration of an extract up to which there was no visible growth of bacilli was its Minimum Inhibitory Concentration (MIC) [33, 34].

3. Results and Discussions

TB has been a major health problem in developing countries including India. Due to increase in MDR and Extensive Drug Resistance strains of *M. tuberculosis*, there is an urgent need of finding newer anti-mycobacterial agents without side-effects to combat this problem. Plants are a valuable source of new anti-mycobacterial compounds. A number of studies have explored a wide range of natural products with strong activity against *M. tuberculosis* [35]. There is a growing interest in identifying the compounds responsible for anti-mycobacterial activity of traditional medicine and developing them as potential TB drugs [32]. In the present scenario, drug resistance has become important medical crisis in many countries. In order to find a solution for the abatement of TB, the discovery of new anti-tubercular drugs is significantly important [36]. The plant extracts are considered as inactive if they could not prevent the growth of *M. tuberculosis* up to concentration of 200 µg/ml [37, 38]. TB is one of the leading killers of humans world-wide. Its agent, *M. tuberculosis* causes more death than any other single infectious disease on earth. Search for new anti-TB drugs become obvious due to the shortage and expensive nature of TB drugs. Herbal remedies become the readily alternative in the search for new antimycobacterial compounds from A*nogeissu sleiocarpus, Terminalia avicenniodes, Combretum spp*. and *Combretum brassii* have been reported as remedies for the management of TB [39]. Moreover, a clear discrimination of activity profile among different extracts could also be possible with the tested concentrations.

Table 1: Antimycobacterial activity of *Dalbergia spinosa*, Roxb., extracts

S. No	Extract	Concentration (µg/ml)	
		50	100
1	Benzene Extract	Active	Active
2	70% Ethanol Extract	Active	Active
3	Aqueous Extract	Not active	Active

The phytochemical screening carried out on these medicinal plants showed that it contained major classes of natural products. The activity shown by the crude extracts may be a

synergistic action of complex phytochemicals. Investigation of wild medicinal plants is an efficient way of searching for new candidate chemotherapeutic drugs. Plant extracts are attractive and effective sources of new drugs [40]. The use of herbs for the treatment of TB is increasing due to increased incidence of resistance to the available antibiotics. Natural products play a significant role in drug discovery and development of highly active anti-mycobacterial metabolites [41, 42]. Our investigation indicate the presence of saponins, steroids, terpenes, anthraquinones, flavones, tannins, phlobatannins and these phytochemicals are responsible for the anti-tubercular activity. Similar observations have been made in plants employed for traditional medicines, which were known to contain the said mentioned bioactive components [43]. Literature survey indicated that these compounds have bioactivity.

Figure 1: Anti-tubercular activity of Benzene extract

Figure 2: Anti-tubercular activity of Ethanol extract

Figure 3: Anti-tubercular activity of Aqueous extract

Anti-mycobacterial herbal medicine where some metabolites such as terpenes, steroids and alkaloids are found to be abundant in the plant extracts and possess potential structural skeletons that could provide useful scaffolds or templates for the development of new anti-mycobacterial drugs has been revived [44]. The bioactivity demonstrated by the extracts is attributable to the presence of secondary metabolites. There are reports about the potent activity of tannins against *M. tuberculosis* [45]. Alkaloids from medicinal plants have been reported for anti-mycobacterial activity [46]. This present study is the first report of anti-mycobacterial activity on these selected medicinal plant *Dalbergia spinosa*, Roxb., was collected from the Mangroove forests of Chidambaram, TamilNadu against *M. tuberculosis* by using agar based proportion assay. The preliminary results presented in this study showed the potential of extracts from these medicinal plants to be used against *M.tuberculosis*. However, further *in-vitro* studies are needed to understand the mechanism of action of crude drugs.

4. Conclusion

The present study exhibited various antituberculosis effects of various extracts of the *Dalbergia spinosa* Roxb., The inhibitory effects of some extracts justify their medicinal use. The present investigation provides important baseline information for further research. The results obtained conclude that ethanol extract of *Dalbergia spinosa* Roxb., has potent anti-tubercular activity which may be probably be due to the phytoconstituents present in the plant or could be a function of either the individual or the additive effects of the phytoconstituents. All these findings justified the claim made in the indigenous system of medicine *Dalbergia spinosa* Roxb., for the treatment of tuberculosis. Pharmacological and toxicological studies of the active plants and investigation of the antimicrobial mechanisms of action are underway and will be soon been reported.

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