ISOLATION OF PITHECOLOBINE FROM THE LEAF EXTRACTS OF *Samanea saman* (Jacq.) Merr AND IT’S IN VITRO ANTITUBERCULAR SCREENING AND RELATED INFECTIONS

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Abstract

*Samanea saman* belongs to the family Leguminosae. It is a tropical avenue tree which is widely cultivated in Northern America and Asia. It possesses a wide range of biological potentials. In the present investigation, the bioactive alcoholic extract of *Samanea saman* had been prepared by extraction based on the polarity by subjecting to the preferable adsorbent to isolate and configure the bioactive compound using column chromatography. Based on this principle, the bioactive alcoholic extract (5%) was fractioned over a silica gel (100-200mesh) column by eluting with solvents of increasing polarity in the order of benzene, ethylacetate and methanol in various ratios. The fractions obtained were monitored by TLC analysis. Similarly TLC fractions were being combined and combined fractions were subjected to the antimycobacterial evaluation and its related secondary infections. It was observed that ethylacetate: methanol (80:20) showed remarkable antimycobacterial activity at the concentration of 20µg/mL by middle brook 7H9broth method. Further it is evident that the above fraction showed completely resistant against associated fungi and bacteria *Viz Aspergillus Niger* and *Pseudomonas aeruginosa*. The solvent of bioactive fraction ethyl acetate: methanol (80:20) is being evaporated under reduced pressure by utilizing a rota vap. The bioactive concentrated fraction is cooled in the ice bath. A brown oily liquid separates out (B.P 273°C) showing a single spot in TLC and positive reaction for alkaloids. The oily liquid is subjected to UV and IR spectral analysis to identify the absorbance and functional group respectively.

Keywords: *Samanea saman, Mycobacterium tuberculosis* Pithecolobine –I, *Aspergillus niger, Pseudomonas aeruginosa*

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INTRODUCTION

Samanea saman Jacq. (Merr) is a large umbraculiform tree growing over 20 meters height with a stout trunk about 1.5m in diameter and large spreading canopy providing shade. The leaves fold together on the approach of rain, hence named as rain tree. There are several folk remedies were prepared from the various parts of the rain tree. The boiled bark is applied as a poultice to cure constipation. The decoction of the fresh leaves were used for diarrhea [1]. The dried fallen leaves, flowers and stems were extracted with suitable solvents. The extracts possess anti oxidant properties [2]. Various antimicrobial perspectives had been explored in the leaf extracts of Samanea saman. The methanol and ethylacetate extracts of Samanea saman pods were investigated by well – diffusion method against pathogenic organisms such as Bacillus subtilis, Staphylococcus areus, E.coli and C.albicans. The methanolic extract possesses good inhibitory activity against these organisms [3]. Experiments were been conducted to explore the antifungal perspectives of the leaf extracts of the Samanea saman against Aspergillus species [4]. In the present investigation, we explore the bioactive compound which is responsible for the antitubercular activity.

MATERIALS AND METHODS

Plant Collection and Identification:

The plant specimen for the proposed study was collected from Turkapally – Village, Shamirpet – Mandal, Ranga Reddy district. Care was taken to select the healthy plants and for normal organs (i.e. leaves, bark, flowers, fruits and seeds). The required parts were cut and removed from the plant after proper identification and authentication has done by the National Institute of Herbal research, Chennai, Tamil Nadu (PARC/2014/858).

Extract Preparation

The air dried leaves of Samanea saman was thoroughly washed with water and then shade dried. The shade dried leaves were being pulverized in to coarse powder [5]. The coarse powder of the dried leaf parts of Samanea saman were extracted with methanol by cold maceration process at room temperature for 6 days. Then the methanolic extract was concentrated using a
rota vap. The obtained extract (1.6%) is stored in a air–tight dessicator and were utilized for the isolation of the bioactive compound

**Isolation of Pithecolobine –I**

The methanolic extract (1.6%) of *Samanea saman* was fractionated over a silica gel (100-200 mesh) column by eluting with solvents of increasing polarity. The fractions obtained were subjected to thin layer chromatographic analysis. Similar fractions were combined as monitored by TLC and these fractions were subjected to antimyobacterial screening. The solvents which were utilized for eluting these fractions were benzene, ethylacetate and methanol at different combination ratios. The obtained fractions were subjected to phytochemical analysis to explore their phytocconstituents. The solvent of the bioactive fraction (ethyl acetate: methanol 80:20) was removed by steam evaporation. The obtained brown oily liquids were subjected to spectral analysis for identification and elucidation of the structure.

**Antitubercular screening by Middle Brook 7H9 Broth**

The Middle brook 7H9 broth comprises of sodium hypophosphate, potassium hypophosphate, monosodium glutamate, ammonium sulphate, sodium citrate, magnesium sulphate, ferric ammonium citrate, copper sulphate, pyridoxine, zinc sulphate, biotin, calcium chloride, glycerol.

The above components were added to distilled water and the volume was brought up to 5 mL and mixed thoroughly. About 0.47%w/w of 7H9 broth was mixed with the other components and the volume was made up to 5 mL with deionized water adjusting the final pH 6.8 and sterilized by autoclaving at 15lbs pressure (121°C) for 10 minutes. Cool to 45°C or below aspetically and add the contents in the vial of Middle brook ADC growth supplement [6]. The *Mycobacterium tuberculosis* culture has been procured from Microbial type culture and collection (MTCC 300), chandigarah and incorporated in to the Middle brook ADC growth tube with indicator. The bioactive fraction (Ethyl acetate: Methanol 80:20) has been dissolved in the few mL of dimethyl formamide and the resultant dilution was made at the concentration of 20, 40&60 µg/mL. The contents were been incubated at 37°C +/- 1°C utilizing the BOD incubator.
In vitro screening for tuberculosis related infections by the disc diffusion assay method

*Pseudomonas aeruginosa* (MTCC109) & *Aspergillus niger* (MTCC 2425) were procured from microbial type culture and collection, chandigrah. They were freshly subcultured on a suitable slant and incubated for 48 hrs. The inocula were prepared according to National committee for clinical laboratory standard method (NCCLS M-27A). The inocula suspension was prepared by picking five colonies of 1mm square and dissolved in 5mL of sterile 0.85% sodium chloride. The inocula were diluted with working media in the ration of 1:2000 which obtained about 0.5x10³ cells/mL. The bioactive fraction (Ethylacetate : Methanol 80:20) were prepared in the concentration of 20,40 & 60mg/mL using DMF as solvent. The discs were prepared and they were immersed in the fraction at various concentration levels (20, 40 & 60 µg/mL). Obtain a plate culture of one of the organism to be tested. Using a sterile loop, emulsify a colony from the plate in the sterile saline solution. Mix thoroughly, making sure that no solid material from the colony is visible. Dip the swab in to the broth culture of the organism. Squeeze the swab gently to remove excess fluid. Use the swab to streak a nutrient agar plate (*Pseudomonas aeruginosa – MTCC 109*) and SD agar plate (*Aspergillus niger – MTCC 2425*) for a lawn of growth which can be accomplished by streaking the plate in one direction and then streaking at right angles to the first streaking and finally streaking diagonally. Allow the plates to dry for about 5 minutes. The drug concentrated discs and antibiotic discs can be placed on the surface of the agar using a dispenser at a correct distance apart. Invert the plates and incubate for 24 hrs at 37°C. Using a metric ruler measure the diameter of the zone of inhibition for each drug and antibiotic used [7].

**RESULTS AND DISCUSSION**

In the present investigation, the fractions obtained from the alcoholic leaf extracts of *Samanea saman* were subjected to qualitative analysis revealed the presence of various bioactive components like alkaloids, flavanoids, steroids and tannins. All the obtained fractions were subjected to antitubercular screening and its related infections. The bioactive fraction (Ethyl acetate: Methanol 80:20) concentrated in the rota vap and cooled in an ice bath. A brown oily liquid separated out. The B.P of the oily liquid is 273°C. The oily liquid answered for the test for alkaloids (Dragendroff test and Mayer’s test). The UV spectrum of the oily liquid showed peaks at 310 nm (band –II) and 355 nm (band-I) (Fig.1). The bathochromic shift of band –II to band –I (Δλ = 5nm). This bathochromic shift indicates the presence of amino group. The Infra –red (IR)
spectrum of oily liquid shows a U-shaped stretching at 3441.01 cm$^{-1}$ indicating the presence of – OH (hydroxyl) stretching. A sharp peak is observed at 1764.87 cm$^{-1}$ revealed the presence of the unsaturated carbonyl (C=O) stretching. An absorption band was observed at 1643.35 cm$^{-1}$ showing the presence of the (CO –NH) stretching. The presence of C-H stretching aliphatic was observed in 1242.16 cm$^{-1}$ (Fig.2). The above all data revealed the presence of the active compound Pithecolobine –I (Fig. 3)
**Figure 3: Structure of the Pithecolobine -I**

**In vitro antimycobacterial screening of the Pithecolobine**

The fraction of Ethylacetate : Methanol ( Pithecolobine) were subjected to *in vitro* antimycobacterial screening for the *Mycobacterium tuberculosis* (MTCC 300) by modified middle brook 7H9 broth method at the concentration of 20, 40 & 60 µg/mL and rifampicin at the concentration of 10mg/mL. The results revealed that there is no growth of *Mycobacterium tuberculosis*. Since the indicator vial does not appear to magenta color. This implies that the EM (Ethyl acetate: Methanol – 80:20) is more active against mycobacterial growth. The data is interpreted in the table 1. (Fig. 4 & Fig.5)

**Table 1: In vitro Antimycobacterial screening of pithecolobine –I obtained from Samanea saman**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the Compound - Fraction</th>
<th>Solvent control</th>
<th>Rifampicin 10µg/mL</th>
<th>Rifampicin 20µg/mL</th>
<th>Rifampicin 40µg/mL</th>
<th>Rifampicin 60µg/mL</th>
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</thead>
<tbody>
<tr>
<td>01.</td>
<td>Ethyl acetate : Methanol fraction ( 80:20) – Pithecolobine - I</td>
<td>Growth – appearance of pink color</td>
<td>No growth – No appearance of Pink color</td>
<td>No growth – No appearance of Pink color</td>
<td>No growth – No appearance of Pink color</td>
<td>No growth – No appearance of Pink color</td>
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Figure 4: Antimycobacterial screening of the Pithecolobine -I

Figure 5: Scanning electron micrograph of MTCC -300

In vitro screening of bioactive fraction (Pithecolobine –I) against tuberculosis related infections

The EM (Ethyl acetate: Methanol) fraction were subjected to an in vitro screening of bacteria (Pseudomonas aeruginosa) and fungi (Aspergillus niger) which are closely related to tuberculosis. They cause secondary infections. The EM fraction has been screened against these organisms by disc –diffusion assay at the concentration of 20, 40 & 60µg/mL. The results revealed that EM fraction is completely resistant to Aspergillus niger & Pseudomonas aeruginosa. The data interpreted in table 2 (Fig.5&6).
Table 2: In vitro screening of tuberculosis related infections

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the compound</th>
<th>Zone of inhibition ($\mu$g/mL)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Aspergillus niger</td>
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<tr>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>1.</td>
<td>Ethyl acetate : Methanol fraction (80:20)</td>
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- Resistant

CONCLUSION

The present study reveals that *Samanea saman* is a tropical avenue medicinal potential plant. Even though the literature pertains the biological perspectives of the alcoholic extract of the *Samanea saman*, most of the extensive pharmacological studies remained untapped. In the present investigation, a bioactive compound named as pithecolobine has been isolated from the ethylacetate: methanol fraction (80:20) and the compound is screened against antitubercular perspectives and its related infections. The bioactive compound (Pitheceolobine –I) possess remarkable antitubercular activity. On contrary, the compound showed resistant with the related infections (tuberculosis associated bacteria and fungi). Hence, this study implicates the additional of new herbal plant in the Indian systems of medicinal plant armory.

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REFERENCES


