Abstract

Samanea saman (Mimosoideae –Leguminosae) is a tropical avenue tree distributed in Northern America and Asia. It has a wide range of pharmacological and antimicrobial potentials. In the present study, the leaf extracts of Samanea saman were prepared based on the polarity (Petroleum ether, Chloroform & Methanol). Antimycobacterial activity of these leaf extracts were screened against Mycobacterium tuberculosis (XDR-TB isolate) using radiospirometric BACTEC 460TB assay method (twofold dilution). It was found that Pet.ether and chloroform extracts of Samanea saman were devoid of activity. On contrary, alcoholic extract of Samanea saman exhibited strong activity against Mycobacterium tuberculosis at 50mg/mL dose level. Phytochemical investigation revealed the presence of phenols, glycosides, terpenoids, alkaloids, saponins, flavanoids and tannins. HPLC analysis indicate the presence of phytocomponents in the alcoholic extract of Samanea saman. Nutraceutical analysis was carried out to provide protocol of health benefits.

Key words: Samanea saman; Mycobacterium tuberculosis; BACTEC460 TB method; Nutraceutical standards; HPLC method.

Introduction

Tuberculosis is an ancient infection which plagued human throughout the archaeological history. According to the survey of the world Health Organization in 1990, there were approximately 8 million active cases, out of which
7.6 million new cases were reported in developing countries. Tuberculosis is a major cause of disease which is also accompanied by the HIV (Human Immuno Deficiency Virus). Tuberculosis appears to be as old as antiquity, skeletal remains of prehistoric human dating back to 8000 B.C. found in Germany shows a clear evidence of the disease. Egyptians skeletons which were dated from 2500 to 100 B.C. revealed evidence of Pot’s diseases of the spine. Ancient Hindu and Chinese writings have documented the presence of the disease. Tuberculosis bacilli can remain in viable form for many years in the tissues of healthy persons until they are immunocomprised yielding the disease. It forms a chronic and protected course which gives ample time for transmission to susceptible hosts. Many chemical derivatives were been synthesized to combat the Mycobacterium tuberculosis, but on contrary, the Mycobacterium species were been able to posses the resistance against them. Under such conditions, the Medicinal chemists were progressing a research in the field of herbal arena to explore its phytochemical perspectives associated with the potent pharmacological potentials. Evans gives a list of principal plant families and their genera of pharmaceutical interest. For Leguminosae, nearly 28 genera have been enlisted as important phytodrugs. The list does not include Pithecellobium and its related taxa. How ever, review of literature has lent support to the well established medicinal potential of Pithecellobium species. Since its erection as an independent taxon, its pharmacological and phytochemical perspectives remain untapped. There is uncontrolled sprouting of many human ailments at the global level, tuberculosis being one among them. To combat the menace, the herbalists are hectic in search of new plants that are sustainable in production. The plants that grow with least nursing care come to our rescue. Plants are renewable source of raw drugs. Samanea saman (Pithecellobium saman) is a common avenue tree with renewable and sustainable potentials similar to other member of Leguminosae. In a survey made for the present investigation, it was found that Samanea saman was abundantly available. In the present investigation, the habitual plant had been studied against Mycobacterium tuberculosis to explore its medicinal perspectives.
Materials and Methods

Collection of Plant specimen:

The plant specimen for the proposed study was collected from Tambaram, Chennai, Tamil Nadu. Care was taken to select healthy plants and for normal organs (i.e. Leaves, bark, fruits and seeds). The required samples of different organs were cut and removed from the plant and fixed in FAA (Formalin 5ml acetic acid 5ml 70% ethanol - 90 ml). After 24 hrs of fixing, the specimens were given by Sass \(^3\). Infiltration of the specimen was carried by gradual addition of paraffin- wax (M.P.58-60\(^0\)C) until TBA solution attained super saturation. The specimens were then cast in to paraffin blocks.

Sectioning:

The paraffin embedded specimen was sectioned using a rotary microtome. Each section 10-12 µM thick, dewaxing of the sections was carried out by routine method. The sections were stained with toluidine blue is a polychromatic stain obtained. The dye rendered pink color to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained using safranin and fast green and Iodine solution (for starch). For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections were done according to Sass \(^3\). Glycerin mounted temporary preparation were made for macerated / cleared materials.

Photomicrographs:

Microscopic description of issue is supplemented with micrographs wherever necessary. Photographs of different magnifications were taken using Nikon lab photo 2- microscopic unit for a normal observations, bright field microscopy was used. For the study of crystals, starch grains and lignified cells, polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale bars.

Extraction of Plant material

The leaves were washed with water and cut in to small pieces and dried in the shade for 2-3 weeks dried was powdered and passed through the sieve of No.40. The powder thus obtained was used for extraction.
Preparation of the Pet. ether extract:
The shade dried coarsely powdered leaves *Samanea saman* (500gms) was extracted by cold maceration technique with hexane for 72 hours after the completion of extraction, it was filtered and the solvent was removed by rotavap under reduced pressure. The percentage yield of the Petroleum ether extract was found to be 0.8% w/w. the residue was stored in dessicator and green extract was obtained.

Preparation of chloroform extract:
The shade dry coarsely powdered leaves of *Samanea saman* (500gms) were extracted with chloroform by cold maceration technique. The extract has been recovered by rotavap under reduced pressure. The dark green color extract was obtained. The percentage yield was found to be 1.5% w/w. The residue was stored in the dessicator.

Preparation of Methanolic extract:
The shade dried coarsely powdered leaves of *Samanea saman* (500g) was extracted with Methanol by cold maceration technique. The extraction was continued for 72-76 hrs. The extract has been recovered by rotavap under reduced pressure. Dark green color extracts was obtained. The percentage yield was found to be 0.9% w/w.

Qualitative analysis of the leaf extracts of *Samanea saman*:
The extracts obtained from the powdered leaves of *Samanea saman* were subjected to qualitative anlaysis to explore the phytoconstituents such as Phytosterols, alkaloids, coumarins, terepenoids, flavanoids, tannins & glycosides.

Nutraceutical Perspectives:
According to food and drug administration (FDA) and dietary supplement health and education act of 1994 (DSHEA) a dietary supplement is a product that is intended to supplement the diet that bears or contains one or more of the following dietary ingredients: vitamins, minerals, amino acids and an herb to supplement daily intake or a concentrate, metabolite, constituent, extract or combinations of these ingredients labeled as a “dietary supplement”. The use of nutraceuticals as an attempt to accomplish desirable therapeutic outcomes with reduced
side effects as compared with other therapeutic agents. Thus it is a mandatory disclosure to identify the
neutraceutical profile of the plant. Hence nutraceutical studies were carried out to explore its nutraceutical
properties.

**Antimycobacterial screening:**

Anti-tubercular activity \(^5\) against the *Mycobacterium tuberculosis* (XDR-TB –Clinical isolate) was studied using
Radiospirometric BACTEC 460 TB system. The extracts (Pet.ether, chloroform and alcoholic) were taken in a
12B- Middle brook medium that is enriched with 7H9 broth. The whole process is carried out in a vial, which was
filled with 14-C substrate (fatty acid) about 1000 mci. The *Mycobacterium tuberculosis* species utilized the 14-C
labelled substrate present in the medium and release carbon-dioxide in the medium, which was detected by an ion-
detector. Specimens of *Mycobacterium tuberculosis* complex (*M.homensii, M.bovis, M. meracadii and
*M.africanam*) were collected from the infected sputum and CSF (cerebro spiral fluid) of the patients. The
specimens were sub cultured in the Lowenstein-Johnson Medium. Inoculation of the specimen has been performed
with in 2 hours of the addition of 12-Bmedium vial. The inoculated vials were incubated at 37\(^0\)C+/-1. These vials
tested on a BACTEC 460 TB instrument for the growth index (GI) of *Mycobacterium tuberculosis* complex.

**Results:**

**Pharmacognostical studies:**

*Samanea saman* (Jacq.)Merr.

Common name: Rain tree

Synonyms: *Mimosa samanea* Jacq.

The plant is a densely foliaceous tree withan extensive hemispherical crown branches. The tree is about 20m tall;
young branches are yellowish tomentose. The leaves are **bipinnate and compound** in nature; the pinnae are 4 or 5
pairs with 3-7 pairs of leaf lets. The basal pair of leaf lets are **oblong elliptic** and upper leaflets are **obovate** and
are in equilateral measuring **1-3.5µm** long and 0.7-2cm wide. The leaflets are glaberous above and Pubescent
below. Extrafloral, nectariferous glands are present opposite in all pinnae.
Flowers are borne on a terminal or axillary corymbose cyme. Flowers are pentamerous, actinomorphic, bisexual with numerous monadelphous stamens. The petals are purplish. The pods are woody, twisted, indeliquescent and dark colored (Fig. 1).

**Leaflets:** The leaflets are distinctly dorsiventral with prominent midrib and thick lamina (Fig. 1).

**Fig. 1:** Morphology of the *Samanea saman* Leaves with the inflorescence.

**Midrib:** it is a planoconvex with flat adaxial side and thick semicircular abaxial midrib. The midrib is wavy with shallow less prominent ridges and furrows (Fig. 2.1). The midrib is 380μm thick and 300μm wide. The epidermal layer of the midrib consists of small squarish or papillate cells. The ground tissue consists of three or four layers of angular compact cells. The vascular strand is large and occupies the entire midrib. It comprises 3-5, short vertical files of narrow thick walled xylem elements and wide and deep arch of about eight small nests of discrete phloem elements enclosing the xylem strands (Fig. 2.1). The xylem and phloem strands are enclosed with in a thick sclerenchyma covering. The covering is much thicker than on the adaxial part than other parts of the cylinder. The vascular cylinder is 200μm wide and 300μm thick in vertical plane.
The lateral veins have similar structure as the midrib. The lateral veins do not project on the abaxial side (Fig. 2.2.3). The lateral veins consist of small group of xylem elements and one or two phloem strands. The vascular strand is surrounded by thick bundle sheath fibres with adaxial bundle sheath extension.

**Lamina (Fig 2.1, 2.2, 2.3):** The lamina is dorsiventral and hypostomatic (having stomata on the abaxial side only). The adaxial epidermis comprises fairly wide rectangular thin walled cells with thick cuticle. The abaxial epidermis consists of small squarish, thin walled cells with tubular finger like papillate outgrowths. The adaxial epidermis is 10µm thick; the abaxial papillate epidermis with the papillae 20µm thick. The mesophyll tissue is differentiated into abaxial thick zone of palisade cells and abaxial zone of cylindrical spongy parenchyma cells. The palisade cells are single layered, less compact and 50µm in height.

**Fig. 2: Midrib and Lamina of the Leaf of Samanea saman.**

![Image of leaf structure](image-url)
**Rachis:** T S of rachis at different levels were prepared and studied.

a. **Structure of the secondary rachis basal part:** (Fig 3.1): the basal part of secondary rachis is roughly circular with shallow adaxial depression, abaxial–lateral wide ridges and abaxial lower short ridges. It is 700µm thick. It consists of a thin epidermal layer, parenchymatous ground tissue and abaxial and adaxial arcs of vascular strands. The xylem elements of the two strands are wide and occur in many short compact parallel rows. The vascular arcs are surrounded by a closed cylinder of sclerenchyma cells.

b. **Secondary rachis–Terminal part (Fig. 3.2):** The terminal part of the secondary rachis is similar to the basal part except that the terminal part has two adaxial ridges on the lateral part. The adaxial and abaxial vascular strands are surrounded by sclerenchyma covering. The outline of this rachis is wavy with short ridges and shallow furrows.

**Primary rachis:**

a. **Terminal part (Fig.3.3):** The terminal part of the primary rachis is circular with flat adaxial side and median depression. It consists of thin epidermal layer and wide parenchymatous ground tissue. These are two bands of vascular tissues – one shallow, wide abaxial arc of vascular strand and an adaxial and abaxial strands have sclerenchyma bands adjoining the phloem tissue. The xylem elements are wide and occurs in short, compact vertical rows. The rachis is 1.5mm thick. The adaxial vascular strand is 1mm wide and 300µm thick. The abaxial strand is 1mm wide and 500µm thick.

b. **Basal Part (Fig.3.4):** The basal part of primary rachis is 2mm thick. It is circular in sectional view. It consists of a thin epidermal layer and parenchymatous tissue where tannin containing cells are sparely seen. The vascular system consists of a wide, thick arc of xylem – phloem tissues. The sclerenchyma sheath is thin and less prominent.
Crystals: Calcium oxalate crystals are abundant in the leaflets, especially in the midrib and lateral veins. In the mid rib the crystals are seen associated with the bundle sheath sclerenchyma (Fig.4.1). In the lateral veins, they occur along in periphery of the sclerenchyma bundle sheath (Fig.4.2). The crystals are also seen in the ground tissue of the mid rib. The crystals located in the sclerencyma cells are Prismatic type; those in the ground parenchyma druses.
Fig.4: Crystals identified in the Leaf of *Samanea saman*.

Qualitative analysis:

The leaf extracts of *Samanea saman* indicates the presence of Phytosterols, Phenols in the Petroleum ether extract; Glycosides is present both in the chloroform & alcoholic extract; While alkaloids,flavanoids and glycosides were present in the Methanolic extract (Table.1).
Table-1: Qualitative analysis of the Leaf extracts of *Samanea saman*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Chemical Test</th>
<th>PetEther extract</th>
<th>Chloroform Extract</th>
<th>Alcohol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Phytosterols</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Phenols</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Flavanoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Furanoid</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ : Present  
- : Absent

Nutraceutical Values:

The leaf powder of *Samanea saman* were subjected to nutraceutical analysis to explore its secondary nutrients. The result of the analysis revealed the presence of carbohydrates, proteins, minerals like sodium, potassium, calcium, sodium and zinc, iron and phosphorus. Vitamins such as thiamine and riboflavin (Table.2).

Table-2: Nutraceutical Values of the Leaf extracts of *Samanea saman*.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Description</th>
<th>Values obtained</th>
<th>Normal Values</th>
</tr>
</thead>
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<tr>
<td>1.</td>
<td>Carbohydrates</td>
<td>4.56g</td>
<td>3.00-5.00g/100g</td>
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<tr>
<td>2.</td>
<td>Protien</td>
<td>7.67g</td>
<td>5.00-8.00g/100g</td>
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Antimycobacterial activity:

The extracts of *Samanea saman* were subjected to *in vitro* antimycobacterial screening on the *Mycobacterium tuberculosis* (XDR-Clinical isolate) by BACTEC 460TB method. The results revealed that Δ GI indicates all the primary drugs were compactable to the solvent control. They show positive growth of the *Mycobacterium tuberculosis* representing the drugs were sensitive to the organism. Further, the leaf extracts of *Samanea saman* represents the activity at the concentration of 50mg/mL (Methanolic extract – Δ GI – 8). On contrary, Petroleum ether and chloroform extracts showed devoid of activity (Table.3; Fig.5).
Table-3: Antimycobacterial activity of the *Samanea saman* leaf extracts –XDR-TB.

<table>
<thead>
<tr>
<th>S.No</th>
<th>No: of days</th>
<th>SC</th>
<th>RM</th>
<th>INH</th>
<th>EM</th>
<th>PZ</th>
<th>PET-EXT</th>
<th>CH-EXT</th>
<th>ME-EXT</th>
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<tr>
<td>Drug Concentrations</td>
<td>0.2mcg/mL</td>
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<td>0.5mg/mL</td>
<td>0.5mg/mL</td>
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<td>267</td>
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<td>299</td>
</tr>
<tr>
<td>Δ GI</td>
<td></td>
<td>+129</td>
<td>+43</td>
<td>+52</td>
<td>+40</td>
<td>+42</td>
<td>+48</td>
<td>+49</td>
<td>+74</td>
</tr>
</tbody>
</table>

**R= Resistant**

**S= Sensitive**

SC- Solvent control  
RM – Rifampicin – 0.2mcg/mL; INH – Isoniazid – 2.0mcg/mL; EM – Ethambutol – 0.5mcg/mL  
PZ- Pyrazinamide – 0.5mcg/mL; PET-EXT – petroleum ether extract; CH-EXT – Chloroform extract; ME-EXT – Methanol extract.
Fig. 5: Pie representation of the Antimycobacterial activity (XDR-TB) of *Samanea saman* leaf extracts.

Δ GI : +129 – Solvent control
Δ GI : + 43 – Rifampicin
Δ GI : + 52 – Isoniazid
Δ GI : + 40 – Ethambutol
Δ GI : + 42 – Pyrazinamide
Δ GI : +59 – Chloroform extract -100mg/mL
Δ GI : +35 – Chloroform extract -25mg/mL
Δ GI : +34 – Chloroform extract -50mg/mL
Δ GI : +49 – Pet.ether extract -50mg/mL
Δ GI : +74 – Pet.ether extract -100mg/mL
Δ GI : +48 – Pet.ether extract -25mg/mL
Δ GI : -8 – Methanolic extract – 50mg/mL
Δ GI : -17 – Methanolic extract – 100mg/mL

Fig. 6: AFB staining of *Mycobacterium tuberculosis* –XDR-TB.
Discussion:

Tuberculosis is an infective disease which had victimized many innocent peoples including the leaders, poets. Many synthetic analogues were been popularly utilized against these microorganism, they possess a prevailing resistance against these drug molecules. Hence, the medicinal chemists were trapping an alternative medicine therapy to combat the tuberculosis. According to the *Materia medica*, there 160 plants of leguminosae were possessing an wide range of phytochemical perspectives. The *Samanea saman* is a plant which belongs to the family leguminosae which is found in the common tropical regions such as south asian regions. The folklore claims that the plant had various pharmacological potentials which remain untapped. In the present study, we had investigated the antimycobacterial potency of the leaf extracts of *Samanea saman* against extreme drug resistant tuberculosis, the culture is obtained from the clinical specimen infected with the XDR-TB. The study was carried out in a BACTEC460TB method compared with the standard drugs such as Rifampicin, Isoniazid, Ethambutol and Pyrazinamide. The investigation concluded that the methanolic extract of *Samanea saman* possess activity against XDR-TB (*in vitro*) at the concentration of 50mg/mL. Further, The investigation on Nutraceutical profiles explore the nutrient properties of the plant such as minerals, carbohydrates, proteins and lipids which indicate that the leaf of *Samanea saman* would be utilized as an alternative natural nutrient products. To identify and authentify the plant, Pharmacognostical studies were carried out.

Conclusion:

In the present study reveals that the *Samanea saman* is a tropical avenue medicinal potential plant. Even though the literature is pertaining to the biological perspectives of the extracts of the *Samanea saman*, most of the extensive pharmacological studies remain untapped. In this investigation, the antimycobacterial activity of the plant had been explored. They combat against the *Mycobacterium tuberculosis* (XDR-TB) at the concentration of 50mg/mL (Methanolic extract). Further, the plant resembles as a nutraceutical potential which would aid in the antioxidant properties. Hence, the study indicates the additional of a new herbal plant in the Indian systems of alternative medica armory.
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References:


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