Green synthesis of silver nanoparticle using leaf extract of ***Samanea saman*** and their antitubercular perspectives

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ABSTRACT

Background:

Samanea saman species are endemic to the Indian subcontinent. It is a large tree as high as sixty meters tall. It has a wide variety of medicinal properties especially in the treatment of gastrointestinal disorders, liver disorders, and respiratory tract problems. The present research work was aimed to carry out the green synthesis of silver nano particle of ***Samanea saman*** leaf extract and to investigate the antitubercular perspectives. The extraction was carried out by using eco-friendly solvent such as water. It was further characterized by UV and IR spectroscopic studies. The antitubercular activity was screened by using Alamar blue assay method and the opportunistic pathogens were screened by Disc diffusion method. The UV absorption data analysis reveals the Surface Plasmon Resonance around 430nm. The antitubercular activity by Alamar blue assay results explored that the silver nano particle synthesized by using leaf extract of ***Samanea saman*** exhibit minimum inhibitory concentration of 1.6 µg/ml. The Silver nano particle of the extract has also shown activity against opportunistic pathogens which are associated with tuberculosis.

Key words: Green synthesis; Silver nano particle; ***Samanea saman***; Alamar blue assay

INTRODUCTION

Nature has given numerous resources to mankind for the betterment of life free from different disease ailments. It plays an important role in maintaining the homeostatis.From the ancient arena, humans were dependent on plants and other natural resources for living day to day life. Food is the medicine quoted by **Hippocrates;** It is a magical text for leading a healthy life. Folklore medicine aims in curing of various unsung diseases from ancient period.Root decoctions, leaf extracts, volatile oils, seeds, fruits of medicinal plants were used in the treatment of snake poisoning, fever, commom cold and other related diseases.Ancient Egyptians used garlic, onions, radish, honey and lint & lard for the treatment of infections and the dressing of wounds. Indian palm scripts denotes the various traditional system of medicine which has given the access to treat life –threatening chronic diseases.The unsung values of herbal wealth were still under exploration.

In the Modern era of civilization, eventhough many synthetic analogues were been utilised in the treatment of ailments, due to the prevailing drug resistance, medicinal chemist switch over to the exploration of indigenous system of medicine. Hence the demand for rationale drug discovery flourished in order to combat the prevailing vulnerable diseases like tuberculosis. India has the highest burden of Tuberculosis in the world with over two million incident cases amounting to more than one fifth of global burden. Tuberculosis has been known to have devastating effects on the socioeconomic development especially in the developing countries due to its association with dreaded diseases like AIDS, COVID and malnutrition in the poorest of the poor. Drug resistance, diabetes, smoking and other associated factors complicate TB scenario further making it difficult to control.

Green synthesis has gained its own importance in the recent years because of its ecofriendly nature, reliability and its sustainability for synthesizing nanomaterials and its wide range application in the field of medicine. For synthesizing of nanoparticles mediated by leaf extract, parameters such as nature of phytochemicals, concentration of phytochemicals, Concentration of metal salt, pH, and temperature control their rate of formation and stability.

The phytochemical present in the leaf extract is having the potency to reduce metal ions compared to bacteria and fungi which needs longer incubation time. The phytochemicals present in plants are glycosides, flavones, terpenoids, ketones, aldehydes, carboxylic acids, and amides, which are responsible for bio reduction of nanoparticles[1].

There is a lot of pharmaceuticals and medicinal important plant family given by Evans. Nearly twenty-eight genera and forty genera have been given under the family of Leguminosae and Umbelliferae respectively. ***Pithecellobium*** (Albizia) and related genera were not listed. However, a review of literature lent abutment for the plant ***Samanea saman*** to explore the untapped biological potentials.

Earlier works revealed that cytotoxic effect was shown on mitotic chromosomes of **Drimia indica (Roxb.)** with the biosynthesis of silver nanoparticles by leaf extract of ***Albizia saman (Jacq.) Merr***. (Azharuddin Daphedar & Tarikeri C. Taranath., 2018). Green synthesis of silver nanoparticles of plant extract containing flavonoid content has shown enhance antibacterial activity. (Siddhant Jain & Mohan Singh Mehata., 2017). With the foregoing evidences, the present work was aimed to perform green synthesis of silver nano particle by using plant extract of ***Samanea saman*** and evaluate its anti-tubercular, and other opportunistic pathogens activity associated with tuberculosis.

Materials and Methods

The plant specimen for proposed study was collected from Venkatramapuram Village, Tirupati. A.P. Care was taken to select the healthy plants and for normal organs (i.e., leaves, Bark, Flowers). The required samples of different organs (leaves) were hand plucked and removed from the plant. The plant was authenticated by Prof.P. Jayaraman, Ph.D., National Institute of Herbal Science, Plant Anatomy Research Centre: PARC, Chennai. (Reg no: PARC/858 ***Samanea saman***). The equipment used for research work was U.V Visible Spectrophotometer (Analytical Ltd) and IR Spectrophotometer ((Analytical Ltd). The extract solvent Milli Q water was used for research work.

The leaves of the plant ***Samanea saman*** were washed with water cut into small pieces and shade dried for 2-3 weeks then they were coarse powdered by passed through sieve no. 40. The powder thus obtained was used for extraction.

Preparation of Aqueous extract: -

The shade dried coarsely powdered leaves of the plant was extracted. About 10 g powder was add into 250-ml beaker containing 100 ml of MilliQ water and heated for 15–20 minutes at 60 °C. The extract obtained was filtered with Whatman No.1 filter paper to remove the particulate matter and then filtered extract was stored in refrigerator for further use.

Synthesis of Silver Nanoparticle[14]: -

To 95 ml of freshly prepared 1millimoles of silver nitrate solution, 5 ml of aqueous extract is added in a flask. Silver ions gets reduced to silver nanoparticles was clearly observed with the colour change from colourless to brown within 15 minutes indicates the formation of silver nanoparticles.

Qualitative analysis of different extracts[16]

The Aqueous extract of ***Samanea saman*** were subjected to qualitative tests for the identification of various plant constituents in their respective extracts.

Characterization of silver nanoparticles

The biosynthesized nanoparticles were subjected to characterization by UV and IR spectrometric analysis. The bio reduction was observed by measuring UV Visible spectra at

300-800 nm and the involvement of phytochemical compounds was characterized by IR spectroscopy studies.

The silver nano particle powder was mixed with KBr to obtain a pellet, and the IR spectrum was recorded in the range of 400–4000 cm^{-1}. The results were tabulated.

in-vitro screening of synthesized nanoparticles of *Samanea saman against Mycobacterium tuberculosis* by Alamar blue Assay method[17]

The antitubercular activity of compounds was assessed against ***M. tuberculosis*** using micro plate Alamar Blue assay (MABA). This method uses a thermally stable reagent and a non-toxic method. 200µl of sterile deionized water was added to all wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate added with 100 µl of the Middle brook 7H9 broth and serial dilutions of test compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for 5 days. After that, 25µl of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. A blue colour in the well was interpreted as no bacterial growth, and pink colour was scored as growth.

The Minimum inhibitory concentration was calculated as the lowest drug concentration which prevented the colour change from blue to pink. The standard drugs used are streptomycin 6.25 µg/ml, Ciprofloxacin 3.125 µg/ml and Pyrazinamide3.125 µg/ml. The Standard Strain used: ***Mycobacteria tuberculosis*** (Vaccine strain, H37 RV strain): ATCC No- 27294. The results were tabulated.

invitro screening of synthesized nanoparticles of *Samanea saman* against *opportunistic* pathogens by Disc Diffusion method[18]

The most common pathogens which were associated with tuberculosis was selected for our study. The protocol involved as follows. 9 dilutions of each drug have to be done with Brain Heart Infusion broth (BHI) for Minimum inhibitory concentration. In the initial tube 20 µl of test sample was added into the 380 µl of BHI broth. For dilutions 200 µl of Brain Heart Infusion broth was added into the next nine tubes separately. Then from the initial tube 200 µl was transferred to the first tube containing 200 µl of BHI broth. This was considered as 10-1 dilution. From 10-1 diluted tube, 200 µl was transferred to second tube to make 10-2 dilution. The serial dilution was repeated up to 10-9 dilution for each sample. From the maintained stock cultures of required organisms, 5 µl was taken and added into 2 ml of BHI (brain heart infusion) broth. In each serially diluted tube 200 µl of above culture suspension was added. The tubes were incubated for 24 hours and observed for Zone of Inhibition. The results were tabulated.

Results and Discussion

An attempt was made to prepare eco-friendly green synthesis of silver nano particle using Samanea saman leaf extract. The leaves extract of the medicinal plants ***Samanea saman*** by using water was prepared and the extractive value was found to be 0.8% W/V. The Qualitative analysis of plant extract helps in identifying the phytoconstituent present in the medicinal plants.

The exploration study in the phytoconstituents present in the leaf extracts of ***Samanea saman*** revealed the presence of alkaloids, phenolic compounds, glycosides, and tannins present in aqueous extract of ***Samanea saman.*** The results were tabulated in the table no:2

Table no1: Extractive Value

S.NO	**Type of extract**	**Extractive Value (% W/V)**
1	***Samanea saman* Aqueous**	0.8

Table no: 02 Qualitative analysis of leaf extract of ***Samanea saman***

S.no	**Chemical Constituent**	**Aqueous Extract**
1	Alkaloids	+
2	Glycosides	+
3	Tannins	+
4	Flavonoids	-
5	Steroids	-
6	Carbohydrates	+
7	Terpenoids	+
8	Glycosides	+

The nanoparticles obtained from the leaf extract of ***Samanea saman*** using 1mM aqueous silver nitrate solution which is confirmed by the formation of nanoparticles by a change in colour of the reaction mixture from pale yellow to dark brown at pH 10. This is mainly due to the excitation of the plasmon vibrations (Kumar and Mamidyal., 2012). The formation of the silver nano particles during the reduction process is indicated by change in

the colour of the reaction solution from colourless to dark brown (Chaudhari et al.,2012; Yamal et al., 2013) which can be visually observed. Metal nanoparticles have free electrons, which yield a surface plasmon resonance (SPR) absorption band, due to the mutual vibration of electrons of metal nanoparticles in resonance with light wave. The appearances of the peaks show the characteristics of surface plasmon resonance of silver nanoparticles. UV–visible spectrum of the aqueous medium containing silver nano particles showed absorption peak at around 425 nm (Thu et al., 2013) (Figure no:1)

Figure No 1: UV spectra of silver nano particle synthesized using leaf extract of ***Samanea saman***

Fourier transform infrared spectroscopy has become an imperative tool to identify biomolecules present in leaf extract of Samanea Saman which are responsible for the reduction, capping, and stabilization of silver nanoparticles (Saha et al., 2010). The FTIR spectrum showed bands at 3440, 2923, 2853, 1620, 1383, 1265, 1124, and 1030 cm-1. The broad absorption band at 1620 cm−1 corresponds to amide band 1 of proteins. (Sharma et al., 2012). The absorption peak at 3440 cm−1 corresponds O-H stretch, H-bonded of the alcohols, and phenols. The absorption peak at 2923 and 2853 cm−1 C-H stretching of aliphatic amines. The absorption band at 1383 cm−1 corresponds C-N stretching vibrations of aromatic amines.

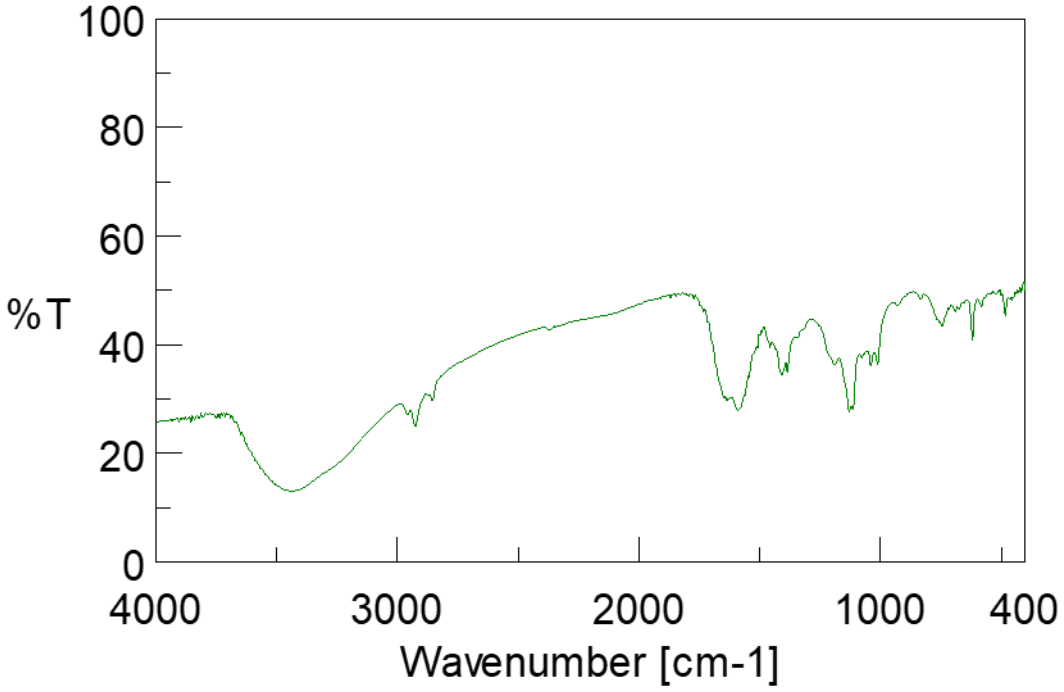


Figure No 2: IR spectra of silver nano particle synthesized using leaf extract of ***Samanea saman***

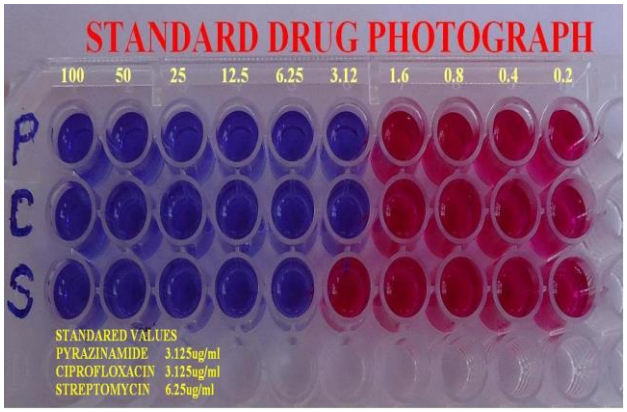
The absorption band at 1265 and 1124 cm−1 corresponds to C-O stretching of aromatic amines, and the band at 1030 cm−1 corresponds to C-O stretching of aliphatic amines. The bands obtained indicate that the carboxyl, amide groups, phenolic, as well as aromatic compounds are present in the leaf extract and in turn participate in the synthesis and stabilization of silver nanoparticles.

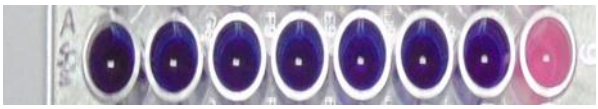
The synthesized silver nano particles were screened against ***Mycobacterium tuberculosis*** by Alamar blue assay method. The study on antimycobacterial perspectives of reveals that the silver nano particle synthesized by using leaf extract of ***Samanea saman*** has minimum inhibitory concentration of 1.6 µg/ml compared with standard drugs. The nanoparticle shown its efficacy which is comparatively more effective against the tuberculosis. The antitubercular efficacy tests of the extracts tested by Alamar blue assay are found to be in good agreement with the existed results of the literature. It was also found that the silver nanoparticles of leaf extract possess potent inhibitory action compared to non-silver nanoparticle leaf extract of ***samanea saman.*** (Satheesh Kumar G and S.D. Shanmuga Kumaran., 2019)

Table No :3 Screening of ***Samanea Saman*** Silver nano particle extract against tuberculosis

S.No	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	ASS	S	S	S	S	S	S	S	R

S - Sensitive
R - Resistant ; ASS- A= Ag(Silver), SS=*Samanea saman.*
Strain used: *M.tuberculosis (H37 RV strain) : ATCC No- 27294.*
Here are the *standard values for the Anti-Tb test which was performed.*
Pyrazinamide- 3.125µg/ml
Streptomycin- 6.25µg/ml
Ciprofloxacin-3.125µg/ml





Tested Sample photograph

Figure No:3: Screening of ***Samanea Saman*** Silver nano particle extract against tuberculosis

Sl. No.	Name of the organism	75µl/ml	50µl/ml	25µl/ml	10µl/ml	5µl/ml
		Zone of inhibition (mm)				
1	*Staphylococcus aureus*	22	17	15	R	R
2	*Streptococcus mutants*	23	20	18	R	R
3	*Escherichia coli*	38	35	28	20	10
4	*Candida albicans*	27	25	23	13	R

Standard Values

Ciprofloxacin- *Staph.aureus*- 26mm; *S.mutans*- 26mm; E.coli- 32mm; Flucanazole- *Candida albicans*- 24mm. Media Used: - Brain Heart Infusion agar R= Resistant

Table No :4 screening of synthesized silver nanoparticles of leaf extract of ***Samanea saman*** against opportunistic infections

The nanoparticles of the leaf extract shown good inhibitory activity (zone of inhibition) against the associated pathogens such as ***Escherichia coli*** (10 mm at 5µl/ml)**,** ***Staphylococcus aureus*** (15 mm at 25 µl/ml)**,** ***Streptococcus mutans*** (18 mm at 25 µl/ml) and ***Candia albicans*** (13 mm at 10 µl/ml). (Table no: 4)

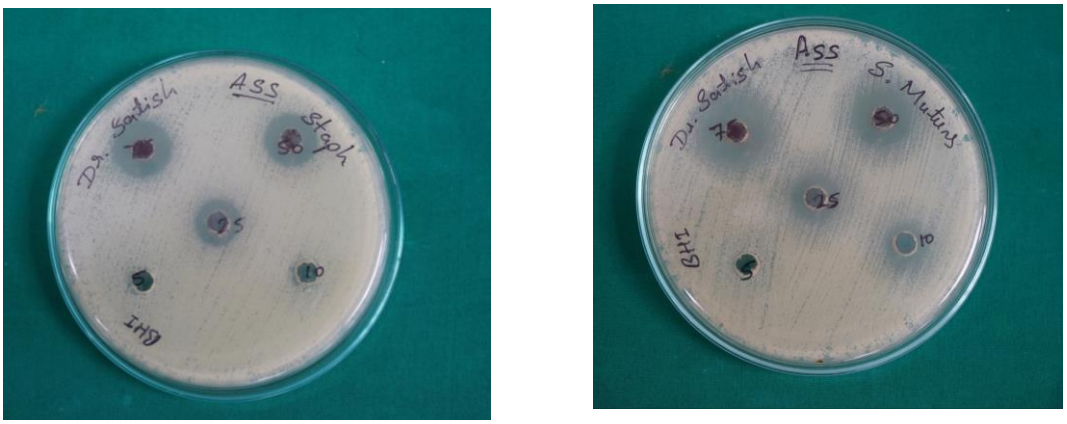


Figure No: 4 Silver nano particle activity on ***Staphylococcus aureus*** and ***Streptococcus mutans***

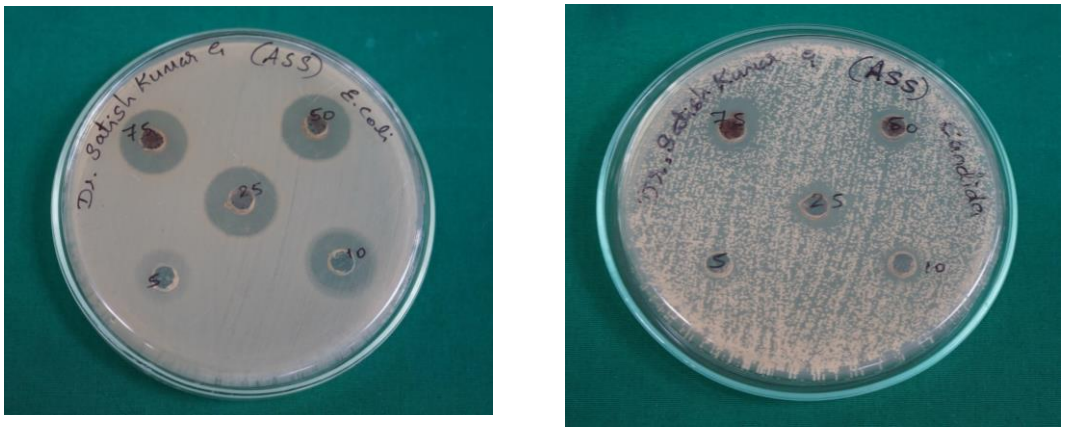


Figure No: 5 Silver nano particle activity on ***Escherichia coli*** and ***Candida albicans***

Conclusion

This study has provided a scientific validation of a common tropical tree ***Samanea saman*** and the application of nanotechnology in the synthesis of silver nanoparticles of the extract aided a promising tool in development of potent drugs in the field of medicine. This research work emphasizes the medicinal value in the Indian system of medical armory and it provides a zest to develop targeted drugs by using nanotechnology and green synthesis.

The research work explored the green synthesis and the potential values of the plant in the medical arena in the investigation of resistant pathogens.

Acknowledgement

The author sincerely thanks the management of Seven Hills College of Pharmacy, Tirupati for their support and encouragement. The author extend the thanks to Maratha Mandal Central Research Laboratory, Belgaum, Karnataka for their support in research work.

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