iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 6 **Issue:** I **Month of publication:** January 2018

DOI: http://doi.org/10.22214/ijraset.2018.1075

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
Volume 6 Issue I, January 2018- Available at www.ijraset.com

Biogenic Silver Nanoparticles as Potential Agent against Mycobacterium Tuberculosis

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Abstract: Purpose: Numerous species of mycobacterium caused chronic contagious disease known as Tuberculosis. Owing to multi-drug resistant strains of mycobacterium and number of people infected with tuberculosis increasing worldwide and mostly acquired in patient who have acquired human immunodeficiency syndrome (AIDS). To overcome this situation and replace currently used medicine, there is an urgent need for new effective agents [1]. In present state, Nanoparticles have opened new opportunity in medicine, diagnosis, and therapeutics [2].

In sight of this, the present study was undertaken to synthesize biogenic silver nanoparticles using and to test their efficiency against the growth of Mycobacterium tuberculosis.

Methods: Silver nanoparticles synthesis using Sesbania grandiflora flower extracts. Characterized by UV-Vis spectroscopy, Zeta potential, DLS and TEM analysis. Anti mycobacterium activity determine by L.J Slope method against Mycobacterium tuberculosis H37 RV strain [3]. The strains were procured from Institute of Microbial Technology, Chandigarh.

Results: The UV–visible data reveal that an absorbance peak in between 400nm to 421 nm confirms formation of silver nanoparticles with zeta potential is -19.65 mV and they are spherical in shape with sizes between 20 nm and 56 nm.These nanoparticles control the growth of M. tuberculosis H37 RV strain at 12.5 ug/mL

Conclusion: synthesis of biogenic silver nanoparticles is green, eco friendly and inexpensive technology. In present study based on results we conclude that aqueous extracts of flower of can be used for synthesis of Silver nanoparticle and these nanoparticles shows antimycobacterial activity and this was confirmed by L.J Slop method. Thus, the potential of biogenic silver nanoparticles further investigate with other screening methods as well as on animal modelling for developed novel TB nanomedicine.

Keywords: Silver nanoparticles, Sesbania grandiflora, Tuberculosis

I. INTRODUCTION

Tuberculosis is most common a chronic contagious disease and is a key cause of morbidity and mortalityin developing countries. In majority cases people recover from primary TB infection without spread evidence of the disease, the infection may reside for years in dormant form and in more or less cases it can be reactive [4]. Conversely, multidrug-resistant (MDR)-TB and extensively drug-resistant (XDR)-TB needs second-line anti-TB drugs treatment, which are more expensive and have greater toxicity as well as side effects. Owing to this multi-drug resistant (MDR) and extensively-drug resistant (XDR) strains of *M. Tuber culosis* appeared in all over the world together with India[5]. As result there is development of resistance to conventional antibiotics threatens the growth made in TB control worldwide, emerging the need of alternative approaches, one of which may be nonmaterial.

Recently, green synthesis method like materialization of metal nanoparticles, especially silver, have drawn the attention because of wide-ranging application in drug designing as well as easy synthesis, controlled morphology, upscale production and reduced cost with increased sensitivity and specificity constitute an interesting alternative. As nanoparticles preparation by green synthesis are safe, less toxic and eco-friendly compared with chemically synthesised nanoparticles [6].

Sesbaniagrandiflorais an Indian medicinal plant commonly known as Agathi. S. Grandiflora flower contain oleanolic acid and its methyl ester and kaemfero l-3-rutinoside as active ingredients. S. grandiflorahas been known to have anti ulcerogenicactivit, antioxidant, anti cancer, anti microbial activity [7].

So, in recreation of developing alternate and novel antituberculant agents, we have focused our efforts to screen nanoparticles, synthesised through Sesbania grandiflora flower extracts for their feasibility as anti mycobacterial agents (Figure.1).

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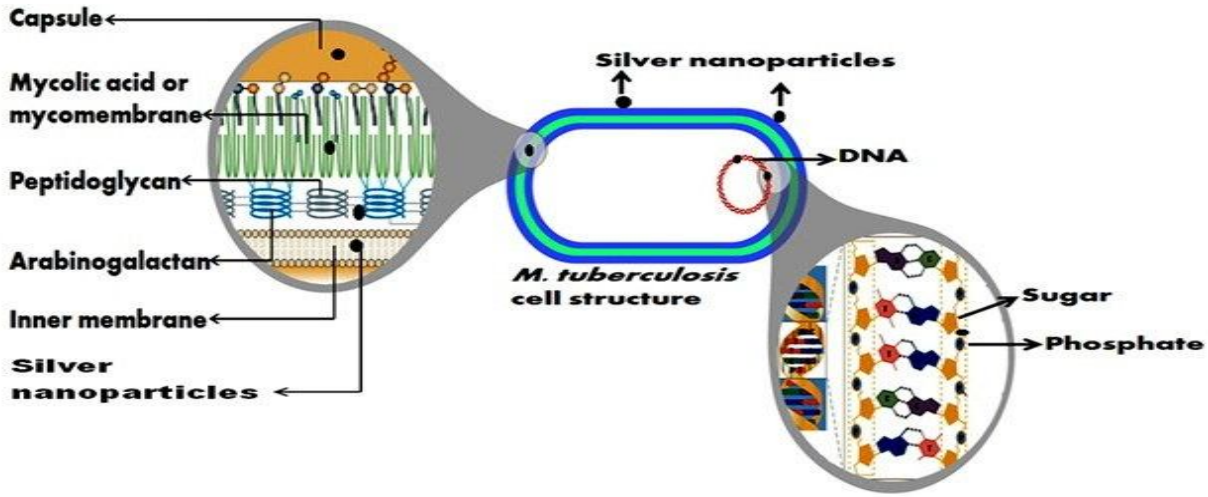

Fig.1 Schematic diagram depicting possible mechanism of activity of silver nanoparticles on *Mycobacterium Tuberculosis*[8]

II. MATERIAL AND METHODS

A. *Preparation of Plants extracts*

The fresh flower of Sesbaniagrandiflora was collected from Surat City. These flowers were washed several times to remove dust particles with de-ionised water and shade-dried for 5-6 week at room temperature (37°C) then powdered using kitchen blender. From that 5 gm of flower powder was weighed and mixed in 100 ml of double distilled water and the mixer was stirred and boiled at 60ºC for 10 to 20 minutes. Mixture was cooled and filtered through What-man No. 1 filter paper. The extract was refrigerated and used for further experimental procedures(Figure.2).

B. *Synthesis of Silver Nanoparticles Using Plants Extracts*

10 mL of flower extracts of S. grandiflora separately added to 250 mL conical flask containing 90 mL 5mM silver nitrate solution. AgNO3 solution was whitish in colour and the solution was stirred constantly for 30 to 60 min at room temperature (30° C) and colour change from whitish to brownish orange were observed, that visually confirms the formation of biogenic silver nanoparticles. Synthesized nanoparticles subjected to centrifugation for formation of pellet. Pellet of separate biogenic AgNPs were washed with distilled water and collected for further experimentation.

C. *Uv-visible Spectroscopy*

The formation of biogenic AgNPs and bio reduction of the Ag+ ions in solution was monitored using UV-Visible absorption Spectroscopy (Systronic 2203 UV–Vis) in the range of 400–500nm , its gives result of surface plasmon resonance formed for the metal. Absorption spectroscopy in the UV-Visible region has long been an important tool for the nanoparticle characterization. This surface plasmon resonance is caused by the coherent oscillation of the free conduction electrons induced by light [9].

D. *Particle Size And Zeta Potential Determination By Dynamic Light Scattering (Dls)*

The particle sizes (z-average), polydispersity index (PI) and the zeta potentials of silver nanoparticles were analyzed with a particle size/Zeta potential analyzer (Microtrac) at 25° C of synthesized biogenic AgNPs .Stability of synthesized AgNPs was also monitored using this analysis. [10].

E. *Electron Microscopy/ Energy Dispersive Spectroscopic Studies*

The shape and size of AgNPs was determined by transmission electron microscopy .The stable biogenic AgNPs were washed and diluted by distilled water to attain the absorbance range of 0.5. Then one drop of diluted AgNPs sample was placed on Copper grid with Ultrathin Copper on holey carbon disc and was allowed to dry in vacuo. After drying, the synthesized AgNPs were visualized using Tecnai G2 FEI High Resolution Transmission Electron Microscope operating at 200 kV of acceleration. The SAED pattern was also obtained[11].

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F. Determination of Minimum Inhibitory concentration of biogenic silver nanoparticles against Mycobacterium tuberculosis by L J Slop method

Anti mycobacterial activity determine by L J Slop method. L J slop is screening test used on the basis of principle of bacterial viability neutralization test for detecting anti mycobacterial activity using a rapid grower of atypical mycobacteria grown on Lowenstein Jensen (LJ) medium[12,13].

Growth was emulsified in 3 ml of sterile 0.9% saline and turbidity was adjusted to.0.5 on a densimeter. 0.5 ml each of this suspension was dispersed aseptically in two sterile tubes-marked as one “test” and the other “control”. Into the “test” tube 0.5 ml of biogenic silver nanoparticles dissolved in DMSO was added and into the control tubes 0.5 ml of DMSO was added. The tubes were vortexed on a cyclomixer and incubated at 37^0C. After 24 hours 0.5 ml from the control tube and “test” tube were inoculated on to LJ slopes each and incubated for 6 to 20 days and observed till sufficient growth was on the culture control LJ slope. This procedure followed for flower extracts and silver nitrate solution. In test standard antitubercular drug rifampicin and Isoniazide was used as a positive control[14].

III. RESULTS & DISCUSSION

A. Synthesis of biogenic Silver nanoparticles (AgNPs)

The green synthesis of silver nanoparticles through prepared flower extracts Sesbaniagrandiflora was carried out. The formation of brownish orange color confirmed the synthesis of AgNPs by using as flower extracts shown in (Figure.3) of S. grandiflora. The brownish orange colour is due to the reduction of Ag+ which indicates the formation of Ag nanoparticles.

Figure: 2 A= Sesbaniagrandiflora flowers& B=Sesbaniagrandifloraflower extracts

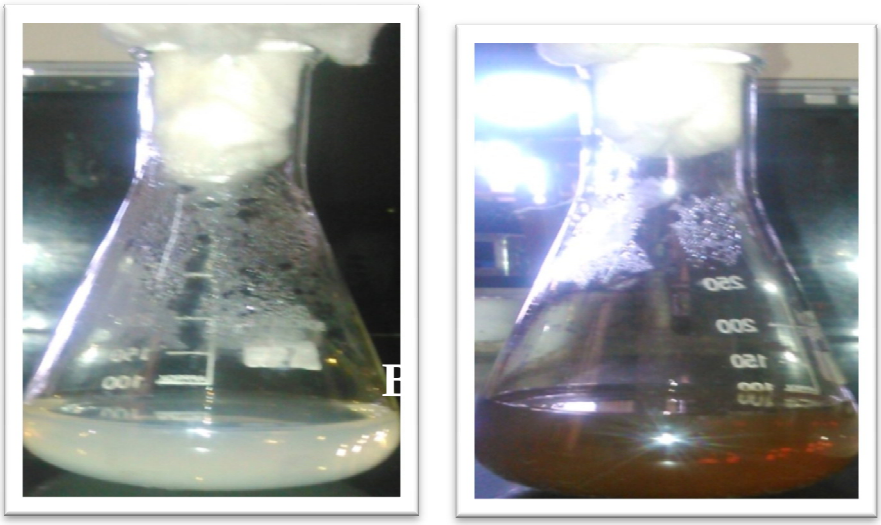

Figure: 3 Color Changeobserved after 60 minutes Reduction of AgNO3 to AgNPs using Sesbaniagrandiflora flower extracts. (A= $AgNO_3$,B=Color change 60 min)

B. Characterization Methods For Biogenic Silver Nanoparticles (Agnps)

1) Uv-Vis Spectroscopic Studies: The reduction of silver ion to silver nanoparticles was reflected in spectral range of 400-500 nm under visible region. In the present work, the biogenic AgNPs are rapidly formed (at pH 7) after the addition of Sesbaniagrandiflora flower extract obvious from the colour change of brownish orange at 421 nm which is the characteristic wavelength of AgNPs with increase in absorbance at regular time intervals as depicted in (Figure.4a, b)

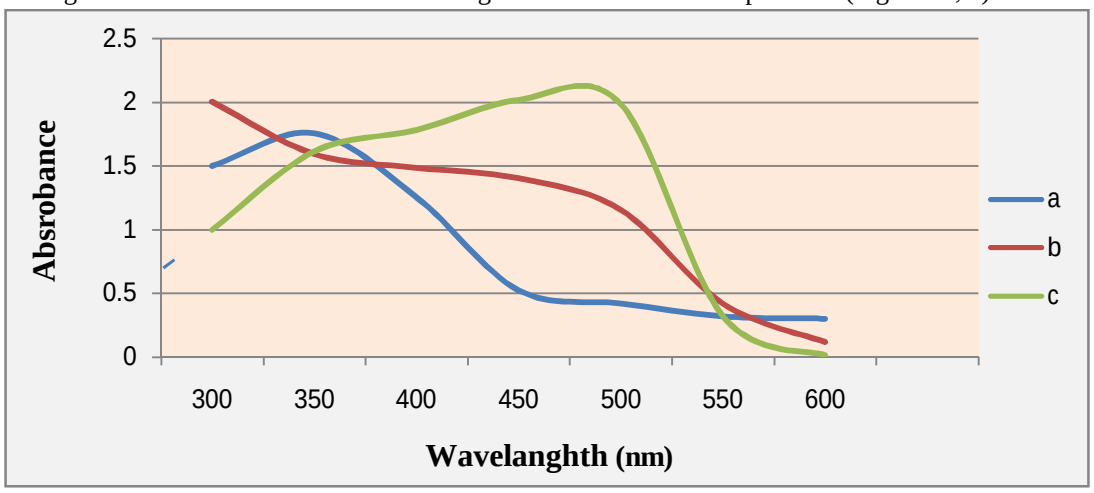

Figure: 4 (a) Synthesized biogenic silver nanoparticles showed strong peak in the visible region of 400 - 450 nm in UV spectroscopy
(a: Flower extract b:Silver nitrate c: Biogenic AgNPs)

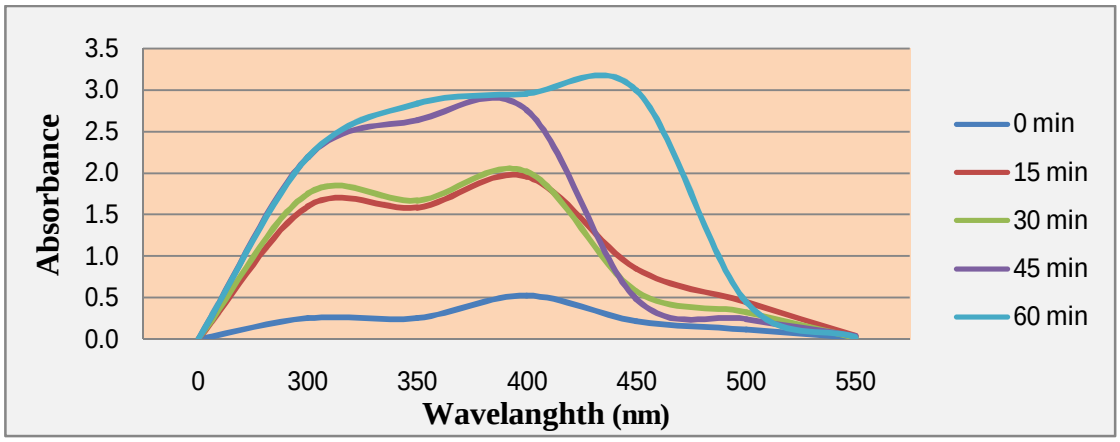

Figure: 4 (b) UV spectrum of biogenic silver nanoparticles formed at characteristic wavelength at 421 nm with increase in wavelength at regular time intervals

2) Dynamic Light Scattering Analysis And Zeta-Potential Measurements: Dynamic light scattering (DLS) is a technique used to measure the size, size distribution profile and poly dispersity index of particles in a colloidal suspension and the surface potential of the silver nanoparticles study by a zeta potential. Both are important characterizations of the silver nanoparticles because they direct the other characterizations, such as saturation solubility and suspension velocity, physical stability, or even biological performances

a) Particle size measurements: The size of colloidal silver nanoparticles, their granulometric distribution measured by the particles number and their occupied volume [15]. The average particle size (z-average) is 192.0 nm and there polydispersity indices PDI value is 0.353. It is presented in the (Figure.5).

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b) Zeta Potential Measurement: A minimum of +30mV zeta potential is required for the indication of stable silver nanoparticles. For the obtained nanoparticles, zeta values were measured is -19.65 mV with a peak area of 100% intensity. These values present full stabilization of the nanoparticles, which may be the main reason in producing particle sizes with a narrow size distribution index (table 1) [16].

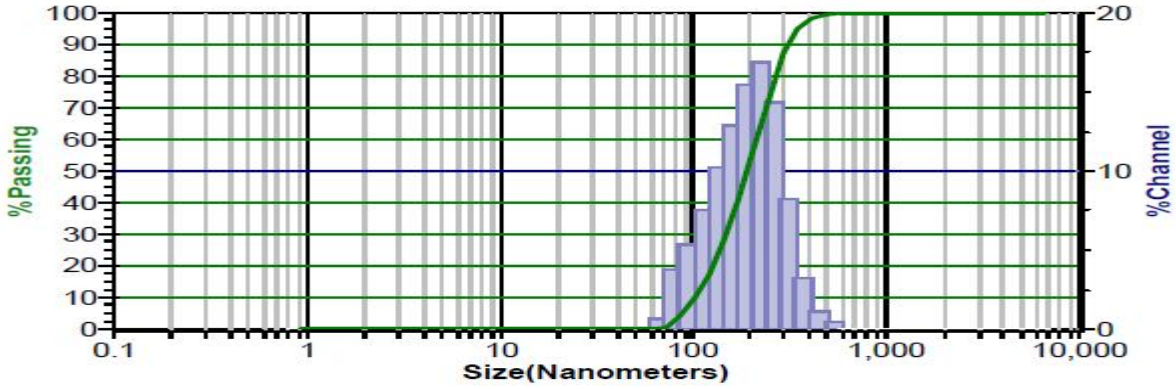

Figure: 5 Particle size of Sesbaniagrandiflora flower extract mediated Silver Nanoparticle

Table: 1 Result of Zeta- potential analysis and particle size analysis

Plant sample	Average size	Zeta Potential	PDI	Polarity
*Sesbaniagrandiflor a*AgNPs	192.0nm	-19.65 mV	0.353	Negative

3) Electron Microscopy/ Energy Dispersive Spectroscopic studies: TEM studies electrons were carried out to determine and view the size of the synthesized biogenic silver nanoparticles. The HR-TEM image of synthesized AgNPs depicted in (Figure.6 a) give clear information regarding size, shape and size distribution of nanoparticles. The size of the synthesized biogenic AgNPs was found to be 10-50nm. The SAED pattern of AgNPs reveals its crystalline nature (Figure.6 b). From TEM images (Figure.7), it can be observed that the AgNPs are capped with phytoconstituents of flower extracts of Sesbaniagrandiflora.

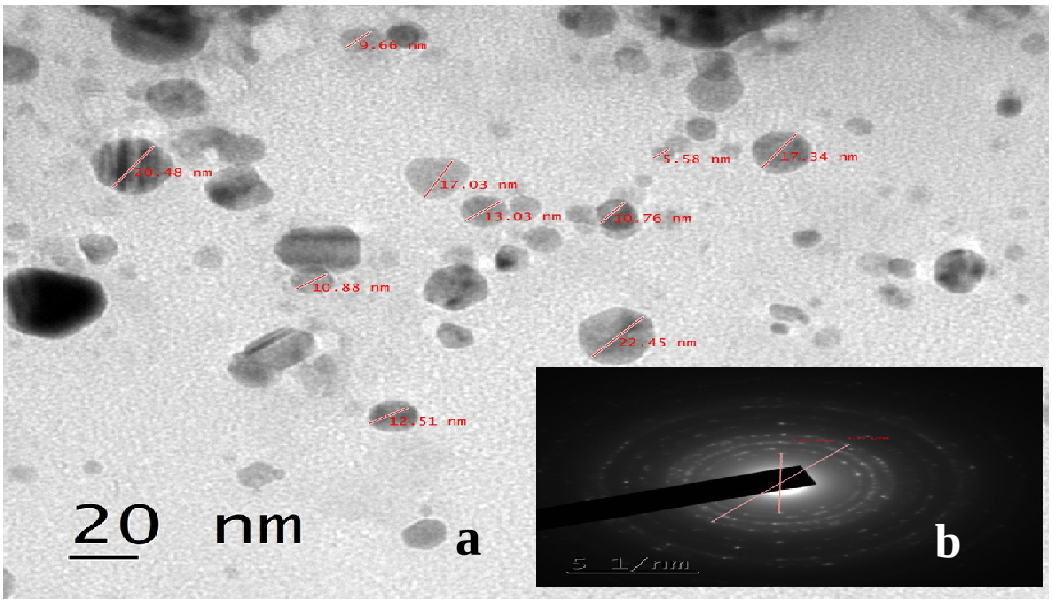

Figure: 6 (a) High Resolution Transmission Electron Microscopy studies of biogenic silver nanoparticles (b) SAED pattern of biogenic silver Nanoparticles

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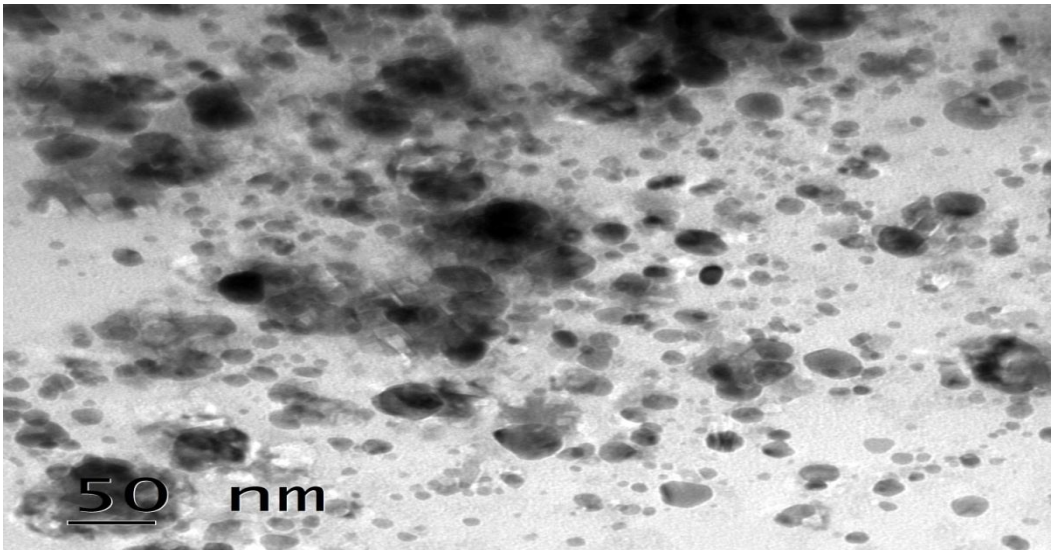

Figure: 7 Transmission Electron Microscope image of biogenic silver Nanoparticles capped with phyto constituents of flowers extracts of Sesbaniagrandiflora.

C. Determination Of Minimum Inhibitory Concentration Of Biogenic Agnps Against Mycobacterium Tuberculosis By L J Slop Method

The anti-tuberculosis activities of biogenic silver nanoparticles have been investigated against Mycobacterium tuberculosis H37 RV strain on L J medium. The activity of flower extract and silver nitrate (Table 2) shows the inhibitory effect on M. tuberculosis at 100 µg/ml and 25 g/ml respectively. The standard antibiotics viz. Isoniazide and Rifampicin have shown the inhibition at 0.2 and 40µg/ml, respectively, similarly biogenic silver nanoparticles shows the inhibition at 12.5 µg/ml and it is more effective in comparison to flower extract, silver nitrate and Rifampicin.MIC test results of synthesised biogenic silver nanoparticles against M. tuberculosis H37 RV strain given in table.2

Table: 1 Result of MIC of biogenic AgNPs against Mycobacterium tuberculosis by L J Slop method

Sample	MIC µg/ml
SesbaniagrandifloraAgNPs	12.5
Silver Nitrate	25
Sesbaniagrandiflora	100
Isoniazide	0.2
Rifampicin	40

IV. CONCLUSION

Biogenic synthesis of silver nanoparticles is green, eco friendly and inexpensive technology. In present study based on results we conclude that aqueous extracts of flower of can be used for synthesis of Silver nanoparticles. The synthesis process was completed within 2 to 6 hours of silver ions coming contact with flower extracts. The synthesis process was quite fast and the maximum absorbance was observed at 421 nm. The zeta potential of biogenic AgNPs was The TEM image suggests that the particles are monodispersed and spherical in shape. The size ranges from 3 to 20 nm.

In present study we concluded that biogenic silver nanoparticles shows antimycobacterial activity and confirmed using L J slop

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assay for determination of MIC. From results biogenic silver nanoparticles confirmed the greater efficiency of in terms of mycobacterial inhibition. Thus, the potential of biogenic silver nanoparticles may be harnessed as a novel medicine ingredient to combat the tuberculosis disease. For that the biogenic silver nanoparticles further investigate with other screening methods as well as on animal modelling for developed novel TB nanomedicine

Biogenic synthesis of silver nanoparticles is green, ecofriendly and inexpensive technology. In present study based on results we conclude that aqueous extracts of flower of can be used for synthesis of Silver nanoparticles. These nanoparticles shows antimycobacterial activity and this was confirmed by L.J Slop method. Thus, the potential of biogenic silver nanoparticles further investigate with other screening methods as well as on animal modelling for developed novel TB nanomedicine.

V. ACKNOWLEDGMENT

The authors thank Department of Pharmaceutical Sciences (Saurashtra University, Rajkot) for providing particle size and zeta potential analysis facilities ,STIC (Cochin University of Science and Technology

Cochin, Kerala) for providing TEM analysis facilities and Dr Dhanji P Rajani (Microcarelaboratory ,Surat) for providing Mycobacterium tuberculosis facilities for study.

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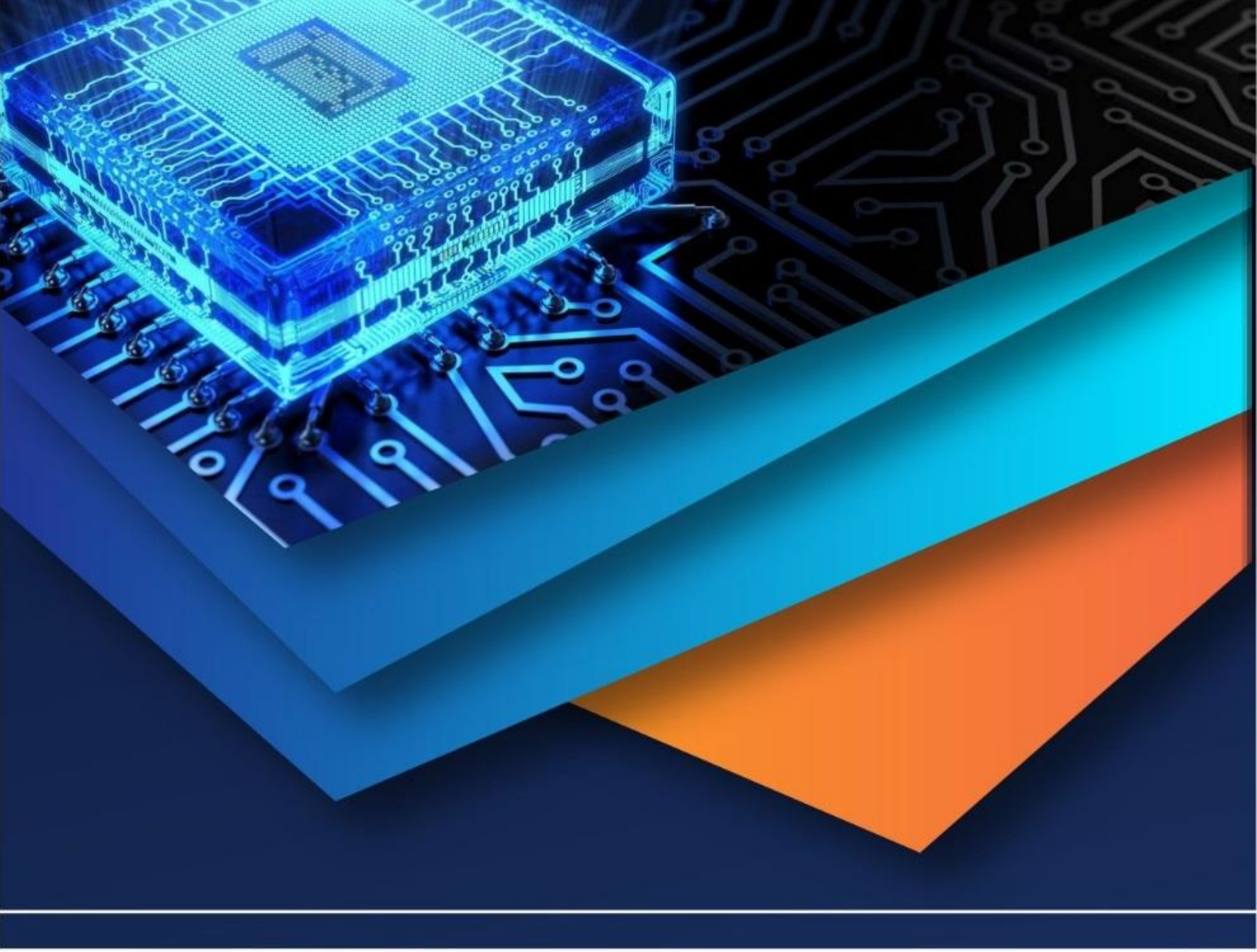

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