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ANTIBACTERIAL ACTIVITY AND GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *STRYCHNOS POTATORUM* SEED AND BARK EXTRACT

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ABSTRACT

In this study, we describe a cost effective and environment friendly technique for green synthesis of silver nanoparticles from 0.1mM $AgNO_3$ solution through the extract of *Strychnos potatorum* seed and bark. Nanoparticles were characterized using UV-Vis absorption spectroscopy, FTIR analysis and phase contrast microscopes. Further these biologically synthesized nanoparticles were found to be highly toxic against different multi drug resistant human pathogens. The synthesized silver nanoparticle from *Strychnos potatorum* seed extract were tested against different pathogenic microorganisms such as *Bacillus sp.*, (14 mm), *E.coli* (19 mm), *Klebsiella sp.*, (16 mm), *Proteus vulgaris*, (22 mm), *Pseudomonas aeruginosa* (13 mm), *S. aureus* (22 mm), *S. epidermidis* (21 mm). Bark extract were tested against *Bacillus sp.*, (16 mm), *E.coli* (19 mm), *Klebsiella sp.*, (18 mm), *Proteus vulgaris*, (17 mm), *Pseudomonas aeruginosa* (16 mm), *S. aureus* (21 mm), *S. epidermidis* (17 mm). The maximum zone of inhibition observed in Proteus *sp.*, *S. aureus* and *S. epidermidis.*

KEYWORDS

Strychnos potatorum, Silver nanoparticles and Antibacterial activity.

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INTRODUCTION

The field of nanotechnology is one of the most active areas of research in modern materials science. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. New applications of nanoparticles are emerging rapidly[1]. Nanocrystalline silver particles have found tremendous applications in the field of high sensitivity biomolecular detection and diagnostics [2], antimicrobials and therapeutics catalysis and micro-

electronics. However, there is still need for economic, commercially viable as well environmentally clean synthesis route to synthesize silver nanoparticles[3].

The use of environmentally benign materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemicals for the synthesis protocol. Chemical synthesis methods lead to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications[4]. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process[5]. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds[6]. Further these biologically synthesized nanoparticles were found highly toxic against different multi drug resistant human pathogens.

Herbal medicines are popular as remedies for diseases by vast majority of world's population. *Strychnospotatorum*(Linn.) belongs to family Loganiaceae, is commonly known as Nirmali and the plant is native of India. According to Ayurveda, the seeds are acrid, alexipharmic, lithotriptic and cure strangury, urinary discharges andhead diseases[7]. S. *potatorum*is a medium sized deciduous tree growing to a height up to 12 meters. Bark is cracked and black. Trunk is irregularly fluted. Leaves are simple, opposite, elliptic acute, 15x6.25 cm, glabrous, shining, flowers are white fragrant, axillary cymes, fruits are ovoid orglobose, glabrous berries, and black when ripe. Roots cure Leucoderma whereas fruits are useful in eye diseases, thirst, poisoning and hallucinations[8]. The fruits are emetic, diaphoretic etc. According to Unani system of medicine, seeds are bitter, astringent to bowels, aphrodisiac, tonic, diuretic and good for liver, kidney complaints, gonorrhea, colic etc. Seeds are used to purify water. Seeds are rich source of polysaccharide gum suitable for paper and textile industries[9].

MATERIALS AND METHODS

Collection of plant material

Strychnos potatorum seeds and bark were collected from Velur near Viralimalai, Pudukottai district, Tamil Nadu India. The seed were identified by the Rapinat Herbarium, St. Josephs College, Tiruchirappalli, Tamilnadu, India. The seeds and bark were separated from the plant and dried under shade. After drying, it was powdered and used for our studies.

Preparation of the plant extract

The bark and seed were separated from the collected plant and dried under shade. After drying, it was powdered and used for our studies. 25 gram of powdered bark and seed were mixed in 100 ml of distilled water separately and boiled for 25 minutes. The extract obtained was filtered through whatman No.1 filter paper and the filtrate was collected and stored for further studies.

Photography of plant

Strychnospotatorum (Figure No.1).

Biosynthesis of silver nanoparticle

5 ml of the prepared extract was added to 10 ml of aqueous $AgNo_3$ (0.1 M solution) at room temperature. The mixture was stirred continuously for 5 - 10 minutes. The reduction was completed after 24 hours with the appearance of brownish - black colour which confirms the formation of silver nanaoparticles. The synthesized silver nanaoparticles were analyzed or UV - Visible spectroscopic studies after time duration of 24 hrs. It was observed that the absorbance peak was centered near nm, indicating the reduction of silver nitrate into silver nanoparticles.

Characterization of silver nanoparticle

Phase contrast analysis of silver nanoparticles

Sample is dispersed in double distilled water. A drop of thin dispersion is placed on a " Staining

mat and observed under phase contrast microscope (Nikon) at 20 x magnification".

FTIR Analysis and UV - Vis absorbance study

Perkin - Elmer spectrometer FTIR Spectrum one in the range 4000 – 400 cm^{-1} at a resolution of 4 cm^{-1} was used. The sample was mixed with KCl procured from Sigma. Thin sample disc was prepared by pressing with the disc preparing machine and placed in Fourier Transform Infra Red (FTIR) for the analysis of the nanoparticles.

UV - Vis absorbance study

The addition of bark and seed *Strychnos potatorum* extract to silver nitrate ($AgNo_3$) solution resulted in colour change of the solution from transparent to brown due to the production of silver nanoparticles. The colour changes arise from the excitation of surface plasmon vibrations with silver nanoparticles. The SPR of silver nanoparticles produced a peak centered near nm. UV - Vis absorbance of the reaction mixture was taken from 0 till 2 minutes.

Microorganisms

Pure culture of *Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumonia, Staphylococcus aureus, and Staphylococcus epidermidis* species of bacteria were procured from PG and Research Department of Microbiology, of Jamal Mohamed College, Trichy. The antibacterial activity carried out in the Department of Microbiology.

Antibacterial activity test

The antibacterial activities of silver nanoparticles were carried out by disc diffusion method. Muller Hinton Agar medium plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates. The sterile discs were dipped in silver nanoparticles solution and placed in the Muller Hinton Agar plates and kept for incubation at 37° C for 24 hours. After incubation the zones of inhibition were measured.

RESULTS

The present study the reduction was completed after 24 hours with the appearance of brownish - black colour which confirms the formation of silver nanoparticles. The synthesized silver nanoparticles were analyzed for UV - Visible spectroscopic studies after the time duration of 24 hours. The staining of silver nanoparticles were observed under phase contrast microscope at 20 x magnification. The addition of seed and bark *Strychnos potatorum* extract to silver nitrate ($AgNO_3$) solution resulted in colour change of the solution from transparent brown due to the production of silver nanoparticles. The colour changes arise from the excitation of surface Plasmon vibrations with the silver nanoparticles.

The appearances of brown colour in the reaction vessels suggest the formation of silver nanoparticles in seed and bark extract (Figure No.2 and Figure No.3). After silver nanoparticles synthesizing we observed the different views of photos in Phase Contrast Microscope (Figure No.4 and Figure No.5).

DISCUSSION

In previous study, the proteins isolated from this seed powder were used in an attempt to understand the role of proteins in Cd(II) adsorption from the aqueous media. As the proteins isolated did not shown any glycoprotein nature or hemagglutinating activity, they might possibly represent the storage proteins in the seed. The present study provided evidence for immobilized proteins as effective biosorbents for adsorption of Cd (II) from aqueous solution. The results show that the optimum conditions for Cd(II) adsorption are almost same for the three proteins used in the study. The Cd(II) removal is pH, contact time, initial metal concentration and temperature dependent. The maximum removal was at pH 5.0, which was achieved at 360 minutes. Investigations are in progress to test the prepared nanoparticles of these proteins and to enhance the ability of cadmium removal[10].

In earlier study the effect of process variables like reductant concentrations, reaction pH, mixing ratio of the reactants and interaction time on the morphology and size of silver nanoparticles synthesized using aqueous extract of Azadirachtaindica(Neem) leaves. The formation of crystalline silver nanoparticles was confirmed using X-ray diffraction analysis. By means of UV spectroscopy, Scanning and Transmission Electron Microscopy techniques, it was observed that the morphology and size of the nanoparticles were strongly dependent on the process parameters. Within 4 h interaction period, nanoparticles below 20-nm size with nearly spherical shape were produced. On increasing interaction time (ageing) to 66 days, both aggregation and shape anisotropy (ellipsoidal, polyhedral and capsular) of the particles increased. In alkaline pH range, the stability of cluster distribution increased with a declined tendency for aggregation of the particles. It can be inferred from the study that fine tuning the bioprocess parameters will enhance possibilities of desired nano-product tailor made for particular applications[11].

In previous study, green synthesis of silver nanoparticles from 1mM AgNO3 solution through the extract of papaya fruit as reducing as well as capping agent. Nanoparticles were characterized using UV–Vis absorption spectroscopy, FTIR, XRD and SEM. X-ray diffraction and SEM analysis showed the average particle size of 15 nm as well as revealed their cubic structure. Further these biologically synthesized nanoparticles were found to be highly toxic against different multi drug resistant human pathogens. This is for the first time that any plant fruit extract was used for the synthesis of nanoparticles[12].

In our study the aqueous seed and bark extract of *Strychnos potatorum* showed the capable of synthesizing silver nanoparticle. Colour changes occurs due to Surface Plasmon Resonance (SPR) during the reaction with the presence of active ingredients in the *Strychnos potatorum* seed and bark extract resulting in the formation of silver nanoparticles, which was confirmed by FTIR , UV - Visible Spectroscopy, and Phase contrast microscopy. The antibacterial activity of synthesized silver nanoparticles was evaluated against *Bacillus sp., E. coli, Klebsiella sp., Proteus vulgaris, Pseudomonas aeruginosa, S. aureus, S. epidermidis*. The maximum zone of inhibition observed in 22mm.

Thin sample disc was prepared by pressing with the disc preparing machine and placed in Fourier Transform infraRed (FTIR) for the analysis of the nanoparticles (Table No.1 and Table No.2) (Figure No.6 and Figure No. 8). The SPR of silver nanoparticles produced a peak centered near 480 nm. UV - Vis absorbance of the reaction mixture was taken from 0 till 2 min. It was observed that the absorbance peak was centered near 480 nm, indicating the reduction of silver nitrate in to silver nanoparticles. It was also observed that the reduction of silver ions into silver nanoparticles started at the start of reaction and reduction was completed at almost 2 min temprature, indicating rapid bisynthesis of silver nanoparticles (Figure No.7 and Figure No.9).

The synthesized silver nanoparticle from *Strychnos potatorum* seed extract were tested against different pathogenic microorganisms such as *Bacillus sp.,* (14 mm), *E.coli* (19 mm), *Klebsiella sp.,* (16 mm), *Proteus vulgaris,* (22 mm), *Pseudomonas aeruginosa* (13 mm), *S. aureus* (22 mm), S. *epidermidis* (21 mm) (Table No.3 and Figure No.10).

The synthesized silver nanoparticle from *Strychnos potatorum* bark extract were tested against different pathogenic microorganisms such as *Bacillus sp.,* (16 mm), *E.coli* (19 mm), *Klebsiella sp.,* (18 mm), *Proteus vulgaris*, (17 mm), *Pseudomonas aeruginosa* (16 mm), *S. aureus* (21 mm), S. *epidermidis* (17 mm) (Table No.4 and Figure No.10).

Table No.1: Infrared spectrum analysis by *Strychnos potatorum* seed extract with $AgNo_3$

S.No	Peak value	Stretching	Interpretation
1	428.20	N-O Stretching	Amine
2	457.13	N-O Stretching	Amine
3	486.06	N-O Stretching	Amine
4	530.42	C-Br Stretching	Halogen
5	578.64	C-Br Stretching	Halogen
6	609.51	C-Cl Stretching	Halogen
7	707.88	C-H Def	Hydrocarbons
8	763.81	C-H Def	Hydrocarbons
9	860.25	C-H Def	Hydrocarbons
10	1020.34	C-O Stretching	Ethers
11	1080.14	C-O Stretching	Ethers
12	1155.36	C-O Stretching	Tertiary alcohols
13	1203.58	C-O Stretching	Ethers
14	1244.09	C-O Stretching	Ethers
15	1381.03	C-H Def	Alkanes
16	1529.55	N=O Stretching	Nitro compounds
17	1562.34	C-H Stretching	Aldehydes
18	1653.00	N=O Stretching	Nitro compounds
19	1822.52	C=O Stretching	Phenol
20	1855.52	C=O Stretching	Anhydrides
21	2374.37	P-H Stretching	Phosphines
22	2924.09	C-H Stretching	Cyclo alkanes
23	3352.28	O-H Stretching	Alcohols
24	3772.76	-N-H Rocking	Amines

Table No.2: Infrared spectrum analysis by *Strychnos potatorum* bark extract with AgNo3

S.No	Peak value	Stretching	Interpretation
1	534.28	C-Br Stretching	Halogen
2	777.31	C-H Def	Hydrocarbons
3	1028.06	C-O Stretching	Ethers
4	1097.50	C-O Stretching	Ethers
5	1143.79	C-O Stretching	Ethers
6	1381.03	C-H Def	Alkanes
7	1593.20	C-H Stretching	Aldehydes
8	1815.02	C=O Stretching	Acid Anhydrides
9	1874.81	C=O Stretching	Ester
10	2376.30	P-H Stretching	Phosphines
11	2850.79	C-H Stretching	Alkanes
12	2918.30	C-H Stretching	Cyclo alkanes
13	3280.92	O-H Stretching	Alcohols
14	3770.84	-N-H Rocking	Amines

Table No.3: Antibacterial activity of silver nanoparticle from *Strychnos potatorum* seed extract

S.No	Bacterial strains used	*Strychnos potatorum* Seed extract with silver nitrate (zone of inhibition mm)
1	*Bacillus sp.,*	14
2	*E. coli*	19
3	*Klebsiella sp.,*	16
4	*P. vulgaris*	22
5	*P. aeruginosa*	13
6	*S. aureus*	22
7	*S. epidermidis*	21

Table No.4: Antibacterial activity of silver nanoparticles from *Strychnos potatorum* seed extract

S.No	Bacterial strains used	*Strychnos potatorum* bark extract with silver nitrate (zone of inhibition mm)
1	*Bacillus sp.,*	16
2	*E. coli*	16
3	*Klebsiella sp.,*	18
4	*P. vulgaris*	17
5	*P. aeruginosa*	16
6	*S. aureus*	21
7	*S. epidermidis*	17

Figure No.1: *Strychnos potatorum* L.f plant

a b c d

Figure No.2: The colour change of plant seed extracts after addition of silver nitrate synthesizing various hours of incubation(a – Silver nitrate, b, c, d - colour changes gradually increased)

a b c d

Figure No.3: The colour change of plantbark extracts after addition of silver nitrate synthesizing various hours of incubation (a silver nitrate b, c, d -colour changes gradually increased in hour basis)

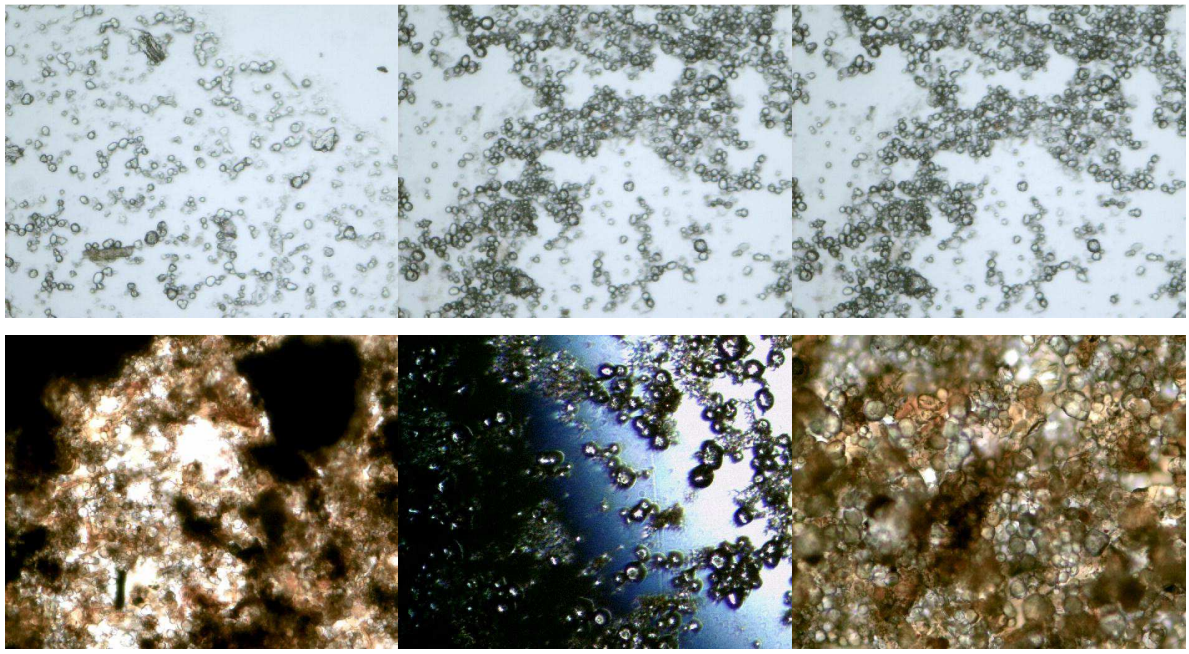

Figure No.4: After silver nanoparticles synthesizing different views of photos observed in Phase contrast microscope (1 to 3 Seed crude extract), (4 to 6 seed extract added with $AgNo_3$)

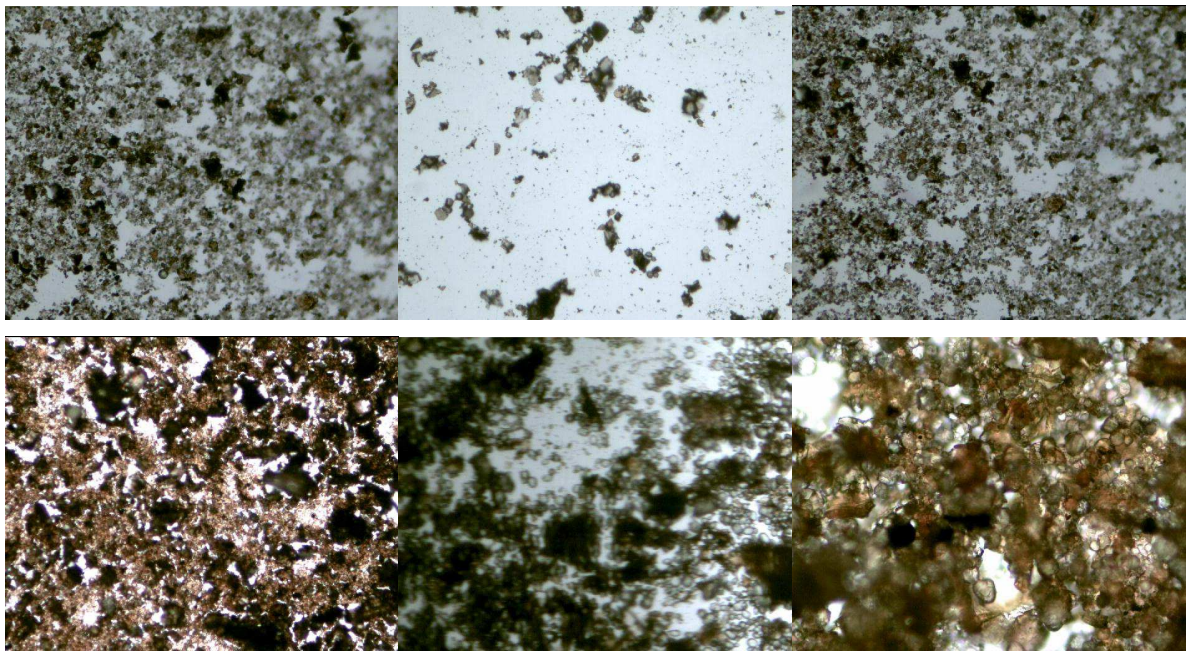

Figure No.5: After silver nanoparticles synthesizing different views of photos observed in Phase contrast microscope (1 to 3 bark crude extract), (4 to 6 bark extract added with $AgNo_3$)

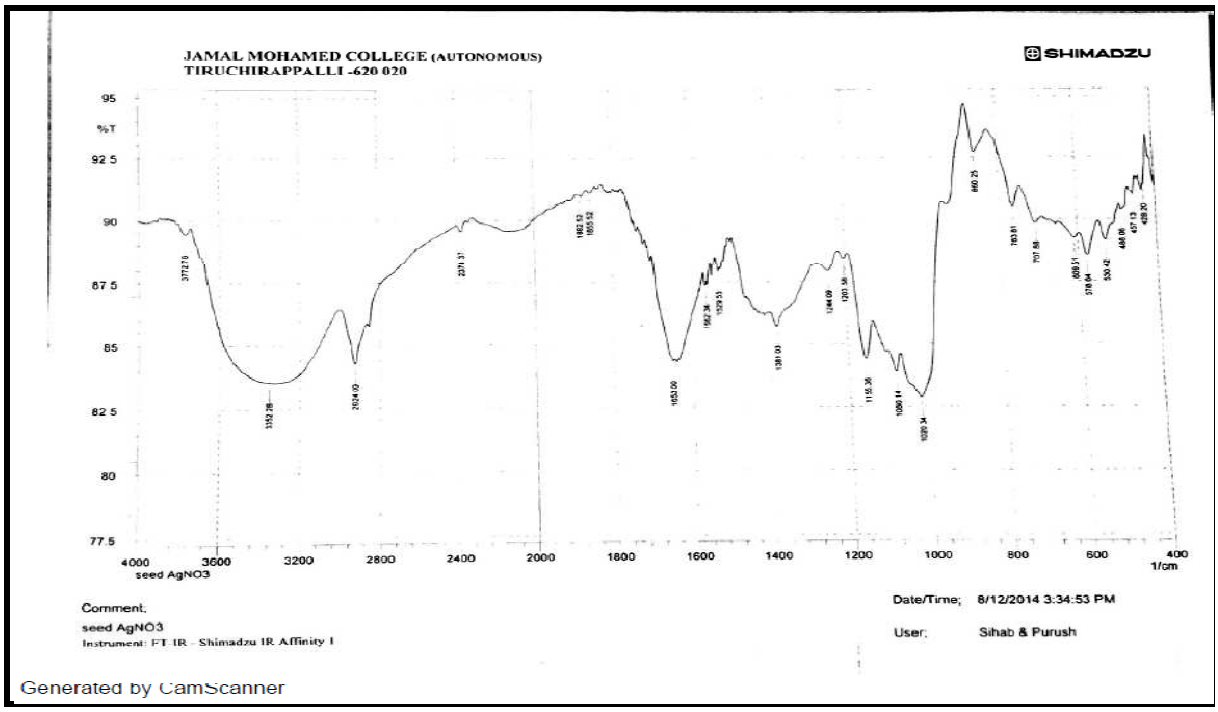

Figure No.6: FT-IR Spectrum of *Strychnos potatorum* seed extract with silver nitrate

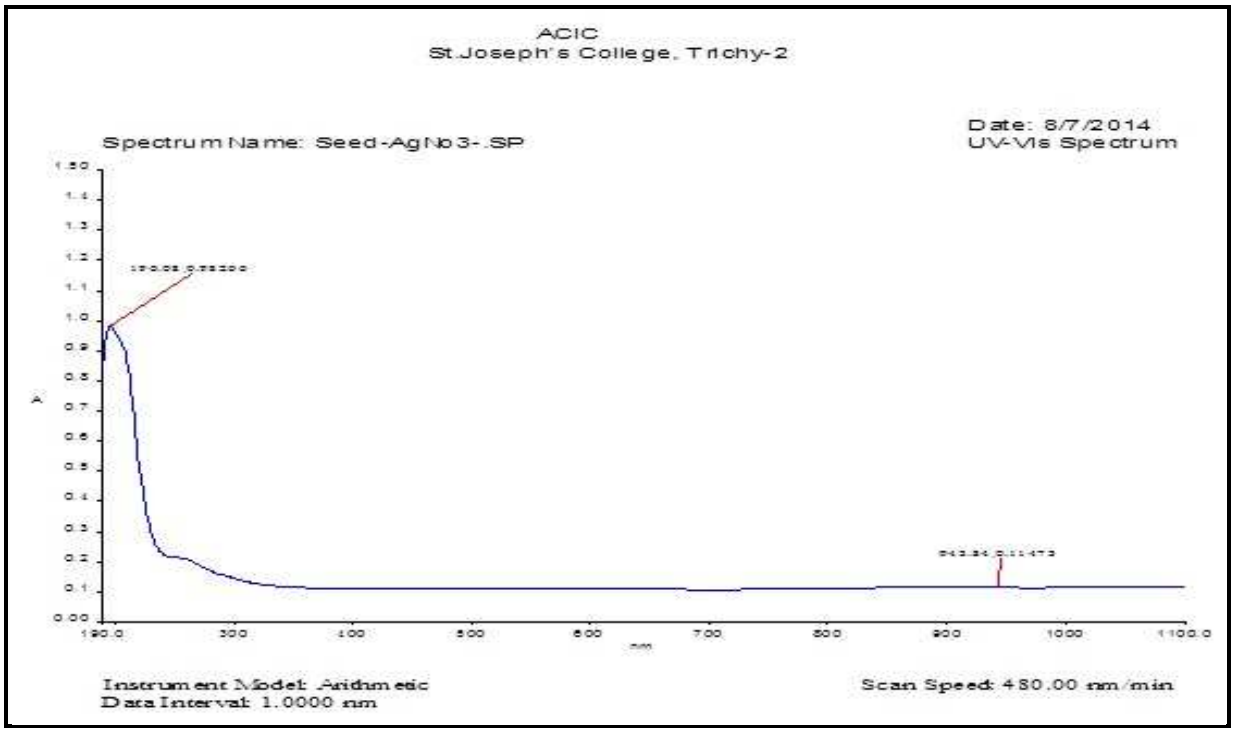

Figure No.7: UV Visible Spectrum of 0.1 M aqueous solution of silver nitrate with *Strychnos potatorum* seed extract

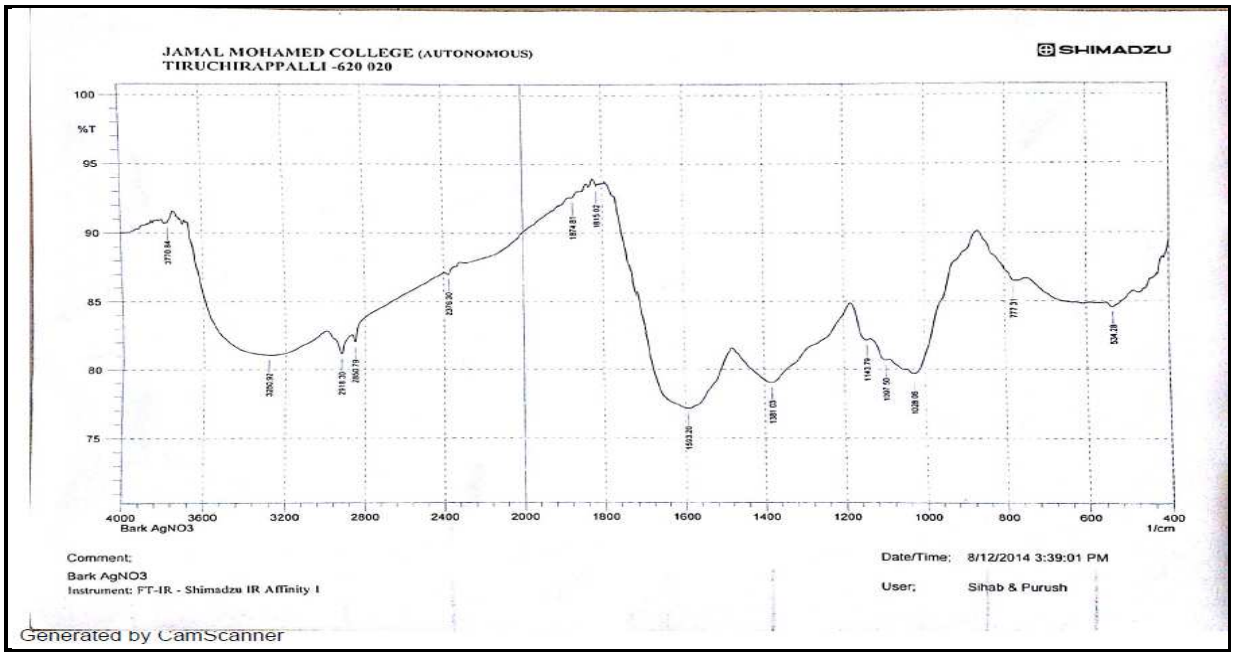

Figure No.8: FT-IR Spectrum of *Strychnos potatorum* bark extract with silver nitrate

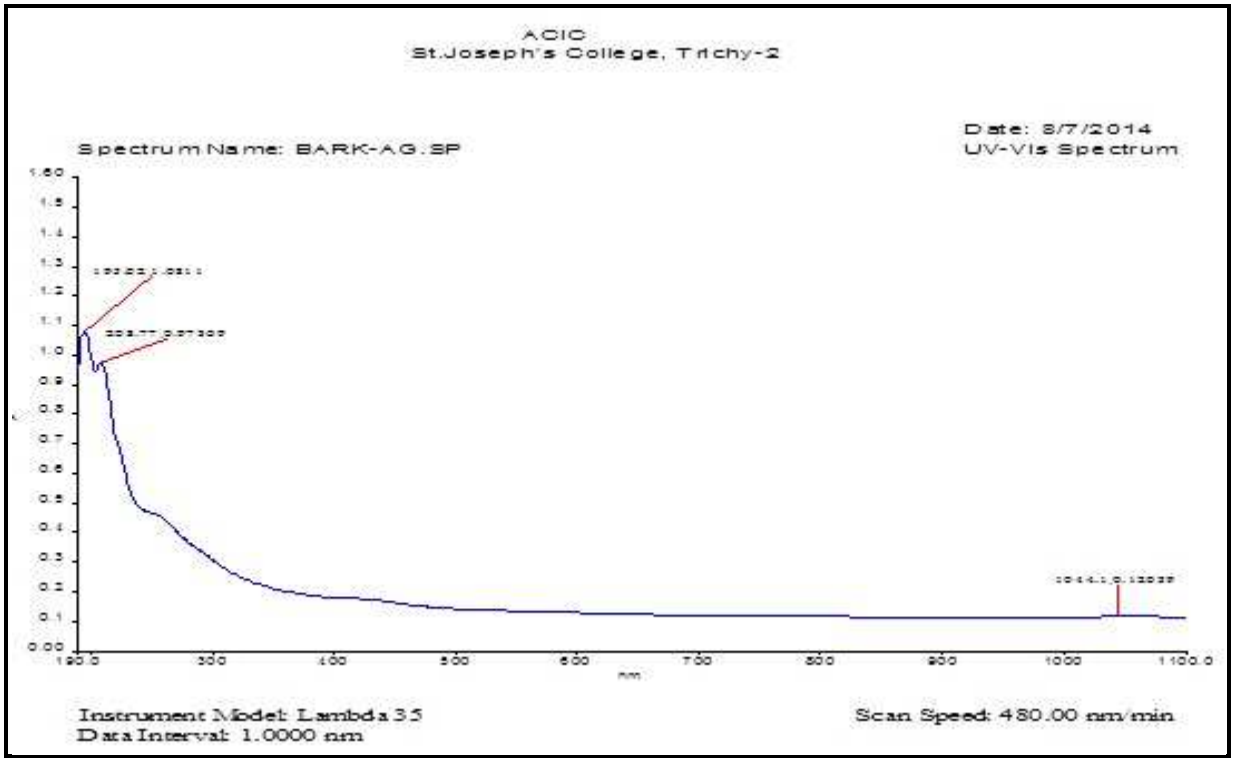

Figure No.9: UV Visible Spectrum of 0.1M aqueous solution of silver nitrate with *Strychnos potatorum* seed extract

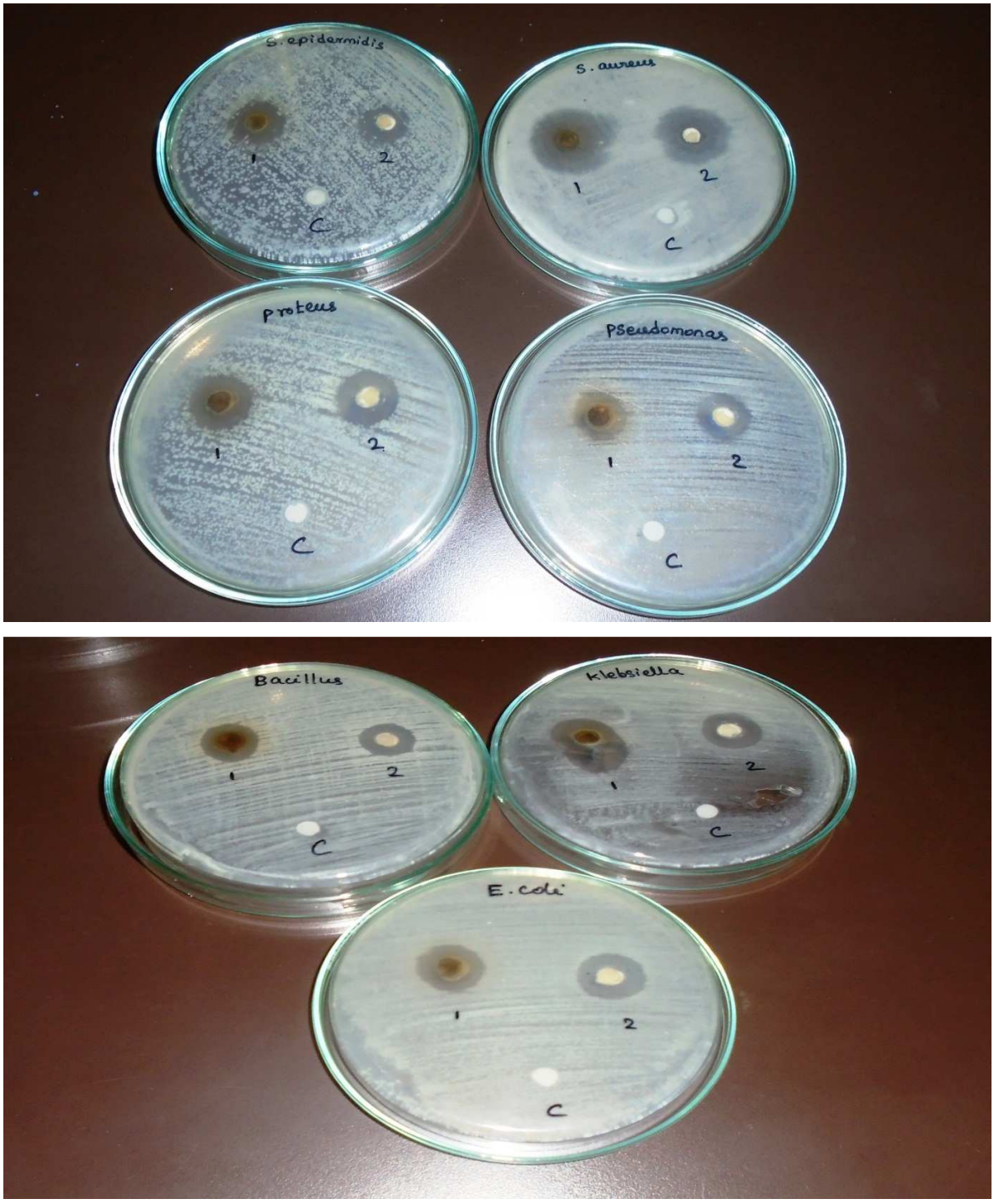

Figure No.10: Antibacterial activity of seed and bark silver nanoparticle (1 – Seed AgNO3 and 2 – Bark AgNO3 C - Control)

CONCLUSION

The present study is regarding the green synthesis of silver nanoparticles from the extract of *Strychnos potatorum* seed and bark capable of producing silver nanoparticles extracellular and are quite stable in solution and their antimicrobial activity against the pathogenic bacteria *i.e. Bacillus sp., Escherichia coli, Klebsiella sp., Proteus vulgaris, Pseudomonas aeruginosa, S. aureus, S. epidermidis.* It is confirmed that silver nanoparticles are capable of rendering high antibacterial efficacy and hence has a great potential in the field of medicine.

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