PRODUCTION OF $AgNO_3$ FROM X-RAY SHEETS AND PLANT MEDIATED SYNTHESIS OF SILVER NANOPARTICLES

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Abstract: A chemical processing scheme is adopted to recover silver metal from waste radiographic films. The aqueous silver nitrate (1mM) obtained is synthesised to silver nanoparticles by green synthesis. The radiographic films were dissolved in 1N nitric acid (HNO_3) solution for approximately 1 hour at 90°C to convert silver into silver nitrate $AgNO_3$(aq). The aqueous silver ions were exposed to leaf broth of Psidium guajava as reductant which resulted the silver nanoparticles. The bio reduced silver nanoparticle are characterized by UV is spectrophotometer, Scanning electron microscope (SEM) and Transmission electron microscope (TEM). The observed peaks in UV a broad spectrum at 390 nm-450 nm wave length. Size of silver nanoparticles range 30 nm - 60 nm observed by TEM. The antibacterial activity of the synthesized silver nanoparticles were tested against E. coli and observed the average zone of inhibition of 20-30 mm.

Key words: **radiographic films, silver nanoparticles, green technology, Psidium guajava, silver recovery**

1. INTRODUCTION

Nanotechnology is emerging as a rapidly growing field with its application in science and technology for the purpose of manufacturing new materials at the nanoscale level. Nanoparticles are clusters of atoms in the size range of 1-100 nm. Nanoscale materials exhibits novel and/or improved properties over either atoms/molecules or bulk state which is resulted from limited size of their constituent components. The properties of nanoparticles entirely differ from conventional macroscopic materials. These unique characteristic properties of nanoparticles arise from the high surface to volume ratio. One of the most important criteria's of nanotechnology is that of the development of clean, nontoxic and environmentally acceptable "green chemistry" procedures, involving organisms ranging from bacteria to fungi and even plants.

Among the metal nanoparticles, silver nanoparticles (AgNPs) are become the focus of extensive research due to their wide range of applications. AgNPs have several characteristics that make them useful in many different areas of science which include medicine, agriculture and catalysis. Silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms.

For production of silver nanoparticles the major precursor used is silver nitrate solution, which is prepared by reacting silver, such as silver bullion or silver foil, with nitric acid, resulting in silver nitrate, water and oxides of nitrogen. Reaction of by products depends upon the concentration of nitric acid used.

$3Ag + 4\ HNO_3 \rightarrow 3\ AgNO_3 + 2\ H_2O + NO$

$Ag + 4\ HNO_3 \rightarrow AgNO_3 + H_2O + NO_2$

1.1 Sources of silver recovery

It is reported that 25% of the world's silver needs are supplied by recycling and that 75% is obtained from photographic waste. In terms of the environment and economy, it is important to recover silver from photographic waste. Compared to other films, waste X-ray photographic films contain an appreciable amount of silver that is 0.7 to 2% silver by weight left behind in the emulsion on the polyester film base, even after the development and fixing processes. These waste films are a good source of silver, which is reused for a variety of purposes, including light sensitive materials.
Silver is recovery methods from waste radiographic films can be classified as a) burning the films directly, b) oxidation of the metallic silver followed by electrolysis and c) stripping the gelatine-silver layer using different solutions. The second and third methods are used more extensively than the first method. In general, the methods reported for the recovery of silver from films involve two steps; stripping the silver from the film followed by recovery of the stripped silver by smelting or electrolysis. The first step is commonly leaching, which may be either microbiological or chemical. For synthesis of silver nanoparticles chemical reagents are used which are harmful to the environment, so in this present study green synthesis method is adopted for the synthesis. In green synthesis plant sources and microbial sources are used. Plants offer a better option for synthesis of

nanoparticles as the protocols involving plant sources are free from toxic chemicals; moreover, natural capping agents are readily supplied by the plants. Plants have a broad variety of metabolites that can aid in the reduction of the silver ions, and are quicker than microbes in the synthesis. Most of the studies available on the synthesis of silver nanoparticles use broths resulting from boiling fresh plant leaves. The various research works related to this study is given in the next section.

2. LITERATURE REVIEW

Liquid and solid wastes containing silver metal emanate from the electronics, telecommunication, computer, plating, and photographic industries. The consumption of silver in photographic materials alone comes to 978 ton in Japan, 1555 ton in USA, and 466 ton in Canada (Serdar and Onuralp, 2010). They reported different hydrometallurgical methods to recover silver from waste radiographic films. The recovered silver is synthesized by green synthesis like plant leaf extract (Begum et al., 2009, Krishnaraj et al., 2010, Geethalakshmi and Sarada 2010, Dipankar and Murugan 2012, Lalitha et al., 2013,Arun et al., 2013), bacteria (Bakeret.al 2005, Le et al., 2010), fungi (Bhainsa and D'Souza 2006, Kim et.al, 2009,Dattu et al., 2014), and enzymes which offers numerous benefits of eco- friendliness and compatibility for pharmaceutical and other biochemical applications as they do not use toxic chemicals for synthesis protocol.

Leela and Vivekandan (2008) focused on tapping the unexploited resources for the synthesis of silver nanoparticles. The bio reduction behaviour of various plant leaf extracts such as *Helianthus cannus* (Asteraceae), *Basella alba* (Basellaceae), *oryza sativa, Saccharam officinarum, Sorghum bicolour andZea mays* (Poaceae). Among them *H.annus* has shown strong potential for rapid reduction of silver nanoparticles. After exposing the silver ins to *E. officinalis* leaves extract, rapid reduction of silver ions is observed leading to formation of silver nanoparticles showed peak absorbance at 400-420 nm with size of range 139-595nm. Elumlai et al., 2010 used aqueous extract of shade dried leaves of *Euphorbia hiria* resulted silver nanoparticles with size ranging from 40nm to 50 nm. Biosynthesis of silver nanoparticles are studied (Sasikala et al., 2009) using calotropis gigantean leaf extract and observed the formation of silver nanoparticles with different time intervals. The nanoparticles showed absorption peak at 420nm and observed nanoparticles with the size ranging from 83.7nm, 15.9nm and 11.8nm. Kaneria et al. (2012) used an aqueous solution of $AgNO_3$ with black pepper (*Piper nigrum*) corn extract. It reduced most of the silver ions into silver nanoparticles with a reaction time of 120s with aid of microwave. Spherical silver nanoparticles are detected with size ranging of 5 to 50 nm. Sohail et al., 2013 the biosynthesis of silver nanoparticles has studied by bamboo leaves extract. Lalitha et at., 2013 studied the anti bacterial and anti oxidant property of silver nanoparticles produced from leaf extract of Azhadiracta which acts as reducing as well as caping agent. The synthesized nanoparticles showed the peak at 351nm and identified the nanoparticles of size 21.07nm. The significant parameters in the optimization of bio synthesis of silver nanoparticles (Lutterodt 1989) studied from *Psidim guajava* leaf extract and evaluation of the anti microbial activity against human pathogenic bacteria. The various optimization parameters are concentration of the silver precursors reducing agent, pH, temperature and time of synthesis. The strong absorption is observed at 440nm and ranged in size from 5-50nm. Dattu et al., 2014 conducted experiments using healthy leaves of *Curcuma longai (*tumeric*)* subjected to extra celluar biosynthesis of silver nanoparticles. The antimicrobial activity against *E.coli and S. Aureus is studied.* Parametric optimization showed maximum absorbance of 420-425 nm at pH-7, 25ºC with 1mM $AgNO_3$ concentration of wet biomass. The size of silver nanoparticles is ranging between 25 to 30 nm. Even though various researchers are studied the recovery of silver from waste radiographic sheets and green synthesis of silver nanoparticles separately. An interest is felt to synthesize the silver which is recovered from waste radiographic films.

3. EXPERIMENTATION

3.1 Production of silver nitrate solution

Waste radiographic films containing 1.5% silver are supplied from a hospital in Visakhapatnam. The waste X-ray films with dimensions of 297×420 mm are cut manually into small pieces with dimensions of 50×50 mm. Fifteen grams of waste films are dissolved in 250mL of 1 M HNO_3. Excessive water content is subsequently evaporated and optimal dissolution parameters are established. These are heated for 1 hour at 80°C-90°C at an agitation rate of 300 rpm in a temperature controlled water bath. The experiments are carried out in a fume cupboard. Nitrogen oxide gas, which formed during the dissolution reaction, is successfully neutralized with the help of the washing unit of the fume cupboard and subsequently released to the atmosphere. This is due to the leaching time is so long because the films are so voluminous that they became pasted to each other and hence made interaction with the acid difficult. After the silver is dissolved in the nitric acid solution, the concentration of the solution is measured by titrating with 0.001M NaCl solution. The obtained solution contains 0.001M $AgNO_3$ along with some excess HNO_3. The HNO_3 is neutralized using 1M NaOH or Na_2CO_3 solutions. The obtained 0.001M silver nitrate solution is stored in sterile amber colour bottle and kept at room temperature. The obtained $AgNo_3$ can be tested by titrating with sodium chloride solution to get white precipitate. The schematic of silver recovery from the waste X-ray sheets is shown in Fig- 1.

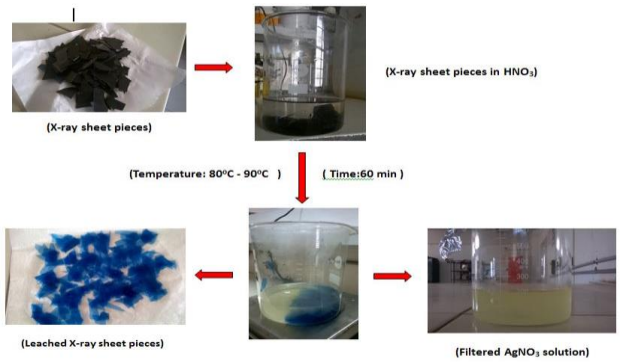

Fig -1: Flow chart for the production of silver nitrate solution

3.2 Production of silver nitrate solution

The matured *Psidium guajava* leaves are collected from the college campus. The fresh leaves are thoroughly cleaned under running tap water followed by distilled water. The leaves are dried at room temperature and 30grams of the dried leaves are sliced into small pieces. The sliced leaves are boiled in 300ml of distilled water at 50ºC-60ºC in Erlenmeyer flask for 20-30 minutes. The obtained liquid is filtered and the plant extract stored at 4ºC for further use. Preparation of plant extract is shown in Fig-2.

Fig- 2: Preparation and filtration of plant extract

3.3 Synthesis of silver nanoparticles (AgNP)

The leaf extract of various concentrations is mixed with $AgNO_3$ to get different ratios of aqueous solution of in 250ml Erlenmeyer flask and incubated at different temperature. A control setup is also maintained without leaf extract. A change in colour from light yellow to brown indicated the synthesis of nanoparticles as shown in Fig- 3. The brown liquid which is obtained is centrifuged at 6000-15000 RPM to separate the silver nanoparticles from the supernatant liquid. The supernatant liquid is again subjected to centrifugation for separating remaining silver nanoparticles. The obtained nanoparticles are washed with ethanol and dried in the hot air oven. The obtained Silver nanoparticles are stored for further study.

Fig- 3: Nanoparticles formation

Series of experiments are conducted by changing parameters to know the effect of process parameters.

3.4 Effects of process variables

Ratio of leaf extract concentration/ silver nitrate: The effect of concentration of leaf extract and silver nitrate is observed with the increasing concentration of leaf extract in 1ml of silver nitrate. The absorbance of the resulting solutions is measured with UV-VIS spectroscopy.

pH: The effect of pH on the reaction is observed by varying pH from 1, 3, 5, 7, 9, 11 and 14. It is maintained with help of 0.1N HNO_3 and 0.1N NaOH. The absorbance of the resulting solutions is measured with UV-VIS spectroscopy.

Time The effect of time on the reaction is observed by using the different time intervals. The reaction time is monitored from 0 min to 120 min. The absorbance of the resulting solutions is measured with UV-VIS spectroscopy.

Temperature The effect of temperature on the reaction is observed by incubating at 30ºC, 40ºC and 50ºC. The absorbance of the resulting solution is measured.

Stirring The effect of stirring is observed by incubating the reaction mixture with and without stirring. The absorbance of the resulting solutions was measured with UV-VIS spectroscopy.

3.5 Antimicrobial activity

The antimicrobial activity of synthesized silver nanoparticles is determined using the agar well diffusion method against pathogenic bacteria. All the test cultures are procured from the National Collection of Industrial Microorganisms (NCIM) pune, India. The antimicrobial activity of biosynthesized silver nanoparticles is tested against bacteria, *E.coli*. To test antimicrobial activity many methods are described in the literature among those Agar well diffusion or disk diffusion methods is selected for present study.

Agar well diffusion or disk diffusion method

To test the antimicrobial activity of biosynthesized silver nanoparticles medium is prepared by dissolving 28g of the commercially available Nutrient Agar Medium and 5g of Agar powder in 1000 ml of distilled water. The dissolved medium is autoclaved at 15 lbs pressure at 120ºC for 15 minutes. The autoclaved medium is mixed well and poured onto 100 mm petriplates (25-30ml/plate), when it is still molten stage. Petriplates containing 20ml Nutrient Agar medium are seeded with culture of microbial strains for 24 hour and biosynthesized silver nanoparticles (20µl) are added by making wells. The discs are placed in the Agar plate seeded with the culture and then incubated at 37ºC for 24 hours. The antimicrobial activity is assessed by measuring the diameter of the inhibition zone formed around the discs. The diameter of zone of inhibition can be measured in millimetres.

4. RESULTS AND DISCUSSION

4.1 Synthesis and characterization of silver nanoparticles

The silver nanoparticles are synthesized using different ratios of *Psidium guajava* (guava) leaf extract with silver nitrate solution and the reduction of pure silver ions is monitored by using optical densities by the UV spectrometer. The change in colour occurred due to excitation of surface plasma on vibrations. Fig-3 shows the presence of silver nanoparticles. The formation of silver nanoparticles is manifested by brown colour formation of the reaction medium. The obtained reaction mixture containing silver nanoparticles is tested in UV-visible spectroscopy. From Fig-4 it shows that the maximum absorption peak for this reaction mixture is observed at 390nm. It is compared with the standard graphs of the commercial silver nanoparticles and the size of nanoparticles is identified as 60-70nm.

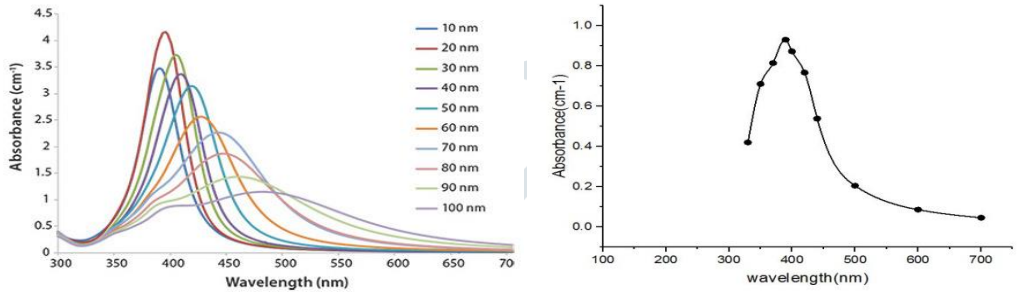

Fig-4 Comparison between absorbance of standard and synthesized silver nanoparticles

4.2 Characterization of synthesized silver nanoparticles

Synthesized nanoparticles with *Psidium guajava* (guava) leaf extract is characterized with standard characterisation techniques Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) to know the size and shape of synthesized particles. The images of SEM analysis for biologically synthesized silver nanoparticles are shown in Fig- 5. This shows that the structure of biologically synthesized nanoparticles is like small flakes and also clustering of the powder is observed. This kind of nature is observed in silver nanoparticles only. This gives strong evidence that synthesized silver particles are in nanoscale only.

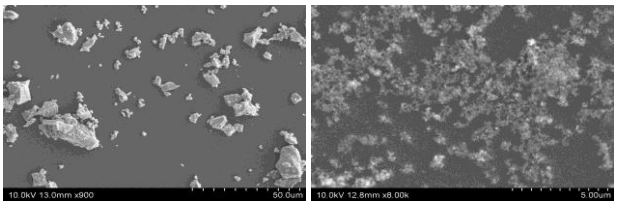

Fig- 5: SEM images of synthesized silver nanoparticles.

TEM analysis provided information about the size of the particles synthesized. TEM of rapid biologically synthesized silver nanoparticles is shown in Fig- 6, which gives exact shape of the nanoparticles. The size observed from the TEM analysis is in line with the size observed from absorbance study. From TEM analysis the ovsetved size of the particles lies in between 30-60nm.

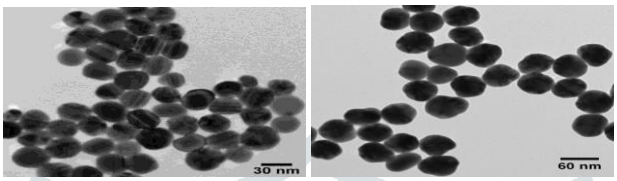

Fig-6: TEM images of synthesized silver nanoparticles.

4.3 Effects of Different Process Variables:

The effect of different process parameters are studied by varying ratio of leaf extract concentration to silver nitrate concentration, pH, time, temperature, and Stirring for bio synthesis of silver nanoparticles. Different concentration ratio of leaf extracts and silver nitrate solution are characterized for silver nanoparticles by UV-Visible spectroscopy. The absorbance curve for various leaf extract is shown in Fig-7. It shows that with increase in plant extract concentration the absorbance values are also increasing. At a ratio of 5:1 observed the characteristic absorption peak 400nm. It stands for the nanoparticles of size from 60-70nm.

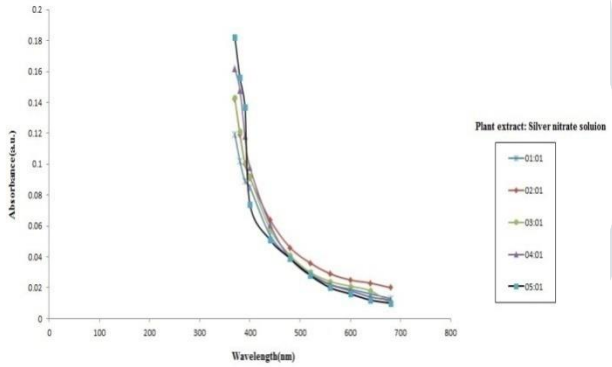

Fig-7 Effect of ratio of leaf extract concentration/ silver nitrate solution for synthesis of silver nanoparticles

The effect of pH on synthesis of silver nanoparticles is shown in Fig-8. It is the key factor that plays a significant role in formation nanoparticles by biosynthesis. Figure 8 shows major influence of the reaction pH is its ability to change the electrical charges of bio molecules which affects their capping and stabilizing abilities and subsequently the growth of the nanoparticles. The particle size is expected to be larger in acidic medium than in basic medium. From the figure it is observed that there no peak at lower pH ranges i.e at pH 1, 3, and 5. It clearly represents that acidic medium is not favourable for the formation of silver nanoparticles. The peak increases at alkaline pH of 9, 14, and 11 due to the increased formation of AgNPs.

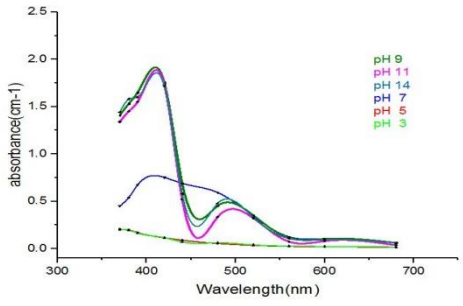

Fig-8 Effect of pH on synthesis of silver nanoparticles

The time required for the completion of reaction is studied by changing time of the reaction which is shown in Fig-9. Increase of absorption is found as a function of reaction time and observed highest peak at 24 hrs of incubation. The UV-Vis spectra recorded after 24hrs showed that there was no increase in the absorption, which confirmed that the reaction was completed within 24 hrs.

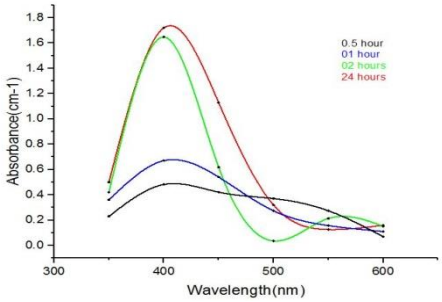

Fig-9 Effect of time on the formation of silver nanoparticles

Temperature:

For selecting optimum temperature for the reaction set of experiments are conducted at various temperatures. The effect of temperature on the formation of silver nanoparticles is shown in Fig-10. As the temperature increased, the rate of silver nanoparticles formation also increased. Increasing the temperature beyond a point (30ºC) decreased the absorption.

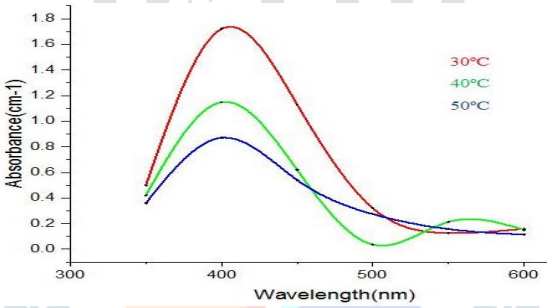

Fig-10 Effect of temperature on the formation of silver nanoparticles.

Stirring

It is well known that mixing place a vital role in the completion of the reaction. To know the effect stirring experiments are conducted with and without stirring of the reaction mixture. The obtained results are presented in graphical form in Fig-11. It shows that when the reaction mixture is stagnant the formation of silver nanoparticles is less. Increase absorption is found as the reaction mixture is subjected to the stirring. Stirring increases formation of silver nanoparticles vigorous mixing.

Antimicrobial activity:

The synthesized silver nanoparticles showed significant antimicrobial activity against bacterial pathogens *E.coli* using agar well diffusion method. The Fig-12 shows antimicrobial activity of the biosynthesized silver nanoparticles. The biosynthesized silver nanoparticles showed highest antimicrobial activity against gram positive bacteria *E.coli* with average zone of inhibition of 20-30 mm. The silver nanoparticles would cause an increase in cell membrane permeability and result finally in cell death (Bakeret.al 2005).

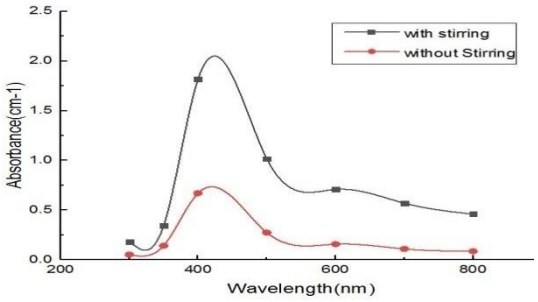

Fig-11 Effect of stirring on the formation of silver nanoparticles.

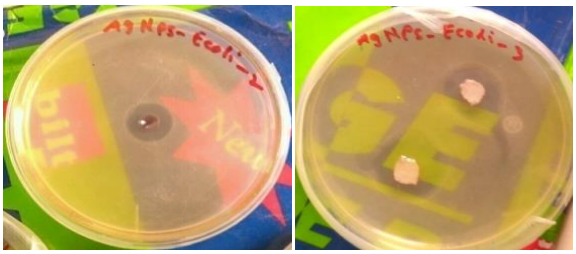

Fig- 12: Antimicrobial activity of the biosynthesized silver nanoparticles

5. CONCLUSIONS

The present study gives a promising route to synthesize silver nanoparticles by using precursors like *Psidium guajava* leaf extract. The $AgNO_3$ is extracted from used radiographic sheets (X-ray sheets) with HNO_3. The precursor solution is prepared from *Psidium guajava* leaf extract. The precursor and $AgNO_3$ are mixed in the required proportions and kept for reaction to complete. After the completion of reaction, mixture is sent to ultra centrifuge to separate silver nanoparticles. The separated silver nanoparticles are washed with ethanol and dried. The dried silver nanoparticles are stored at room temperature indeppendorf tube. An optimum condition for the synthesis of nanoparticles is studied by varying process parameters such as ratio of leaf extract and silver nitrate, pH, time, temperature and stirring. High amount of silver nanoparticles are produced when ratio of leaf extract and silver nitrate is maintained high in basic medium (pH 8-14) at a temperature of 30ºC along with stirring. The produced silver nanoparctiles from used radio graphic sheets with *Psidium guajava* leaf extract is characterized by UV-visible spectroscopy, SEM and TEM. The peaks of absorbance is observed at 390-430nm in UV-visible spectroscopy. The morphology of the silver nanoparticles observed from SEM is same as that of commercial silver nanoparticles and size obtained from TEM ranging from 30-60 nm. The anti bacterial activity of synthesized silver nanoparticles is tested against bacterial pathogens *E. coli* using agar well diffusion method and identified the average zone of inhibition of 20-30 mm.

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