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Synthesis of Silver Nanoparticles Using Methanolic Extract of Medicinal Plants (*Senna alata* and *Senna hirsuta*) and Analysis of Antibacterial Activity

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Abstract

Because of their environmental friendly nature and effectiveness cost wise, green synthesis of nanomaterial's from plant has become progressively popular. In this research, we used methanol extracts from fresh leaves of Senna alata and Senna hirsuta medicinal plants to synthesize silver (Ag) nanoparticles as bio reducing agents. This method allowed the synthesis of nanoparticles supported by spectrophotometry Ultraviolet-visible, FTIR, SEM, TEM and XRD research. UV-Vis spectra and visual observation showed that after treatment with Ag precursors, the color of the fresh *Senna alata* and *Senna hirsuta* leaf extracts turned into light greenish and brownish yellow, respectively. This screened antibacterial activity against five different bacteria, viz. *Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli* with the use of Disc Diffusion method. Silver nanoparticles extracted from *Senna alata* and *Senna hirsuta* leaf extracts, which inhibited *B.subtilis* (18 & 16), *E.faecalis* (19 & 14), *S.aureus* (20 & 14), *K.pneumoniae* (21& 16) and *E.coli* (17 and 15), size in mm. The higher Inhibition zone occurs in *K.pneumoniae* and *S.aureus* follows. It has been shown that inhibiting bacterial growth, the Methanol extracts of *Senna alata* leaves containing Ag nanoparticles is comparable with chloramphenicol.

Keywords: Silver nanoparticle synthesis, green synthesis, *Senna alata, Senna hirsuta,* Antibacterial activity.

Introduction

Nanotechnology has diversified applications like adsorption, optical sensor, and catalysis, treatment of water, drug delivery and nano medicine. Hence nanotechnology has begun as an enthralling area of study (Sadeghi and Gholamhoseinpoor, 2015). Nanoparticles exist as organic as well as inorganic materials, the synthesize of which could be by a variety of methods and size of particle varying from 1 to100 nm with area-to-volume ratio being large because of which it is extremely important (Iravani *et al.,* 2014). Application of Metallic nanoparticles (NPs) have been successful in various grounds because of their remarkable physicochemical things, inclusive of physical health, artificial biology, and cellular transportation (Burda *et al.,* 2005). AgNPs have received particular attention among various nanoparticles because of their unique morphology, stability, and regulated geometry (Grace and Pandian, 2007). AgNPs are largely used primarily in various electronic and sensing devices, coating materials, data processing, and molecular switches (Lee, 2010; Van der Molen *et al.,* 2008; Mackowski, 2010). Besides this, they were also utilized in the analysis and curing of different diseases (Khan *et al.,* 2014).

In particular, AgNPs have excellent antimicrobial activity against multiple microorganisms that are responsible for multiple dangerous illnesses. This has made successful usage in various equipment's in medical line like catheter coating materials for cerebrospinal fluid drainage (Galiano *et al.,* 2008), contact lenses (Weisbarth *et al.,* 2007), and others. Usage is also in bone cement (Alt *et al.,* 2004), surgical masks, impregnated silk fabrics, nanogels, nano-lotions (Li *et al.,* 2006), wound dressings, and so on (Atiyeh *et al.,* 2007). Indeed, most of the products developed on Ag-based have entered into the market and agreed by worldwide supervisory bodies. Extracts and parts of plants like leaves, stem bark, roots are in use at present to blend nanoparticles because of their green nature and can also serve as synthesis stabilizers as well as reducers (Dada *et al.,* 2018; Song and Kim, 2009).

Resistivity of anti-microbes is a universal threat found in the development of drugs because of micro-organisms ability to adjust to the drugs intended to destroy such ones, reducing their efficiency. Hence, discovery of possible antimicrobial agents which has the capability of

reducing the danger of bacterial resistance has to be carried out (WHO, 2015). Plant extracts, green synthesis has shown efficient in preparing silver nanoparticles that could be useful in addressing the challenges surrounding antimicrobial resistance (Haase *et al.,* 2015). Silver ions are released into bacterial cells by the nano particles, improving their activity (Ahamed *et al.,* 2010; Swain, 2014; Rai *et al.,* 2009). Silver's antimicrobial activity was recorded in early history. The World Health Organization reports that in most of the countries majority of the population use traditional medicine to treat various diseases (WHO, 1991). The woody annual herbs or undershrub herbs *Senna alata* L and *Senna hirsuta* are native to Africa as medicinal species with active functions and therapeutic agents (Ayo, 2010). *Senna alata* is a shrub typically between 1 and 5 meters in height, and has branches distributed horizontally. The par pinnate leaves measure from 30 to 60 cm length and found with 8 to 20 leaflet sets, which are oblong or oval, with rounded ends. The flowers in auxiliary racemes are small, with a length of 20 to 50 cm and with 3 to 4. The inflorescence appears like a yellow candle, and is used mostly in medicinal purposes (Farnsworth and Bunyapraphatsara, 1992). *Senna hirsuta* plant (Holm *et al.,* 1979; Irwin and Barneby, 1982) is commonly called hairy *senna* and stinking *senna*. The plant is softly tomentose. Branches grown, leaves with a gland at the base of the petiole 15-20 cm long; stipulations leaner with 4-6 pairs of leaves, ovate elliptic, acuminate, rounded or cuneate at the base, 10 X 4cm long. Terminal corimas in penicles; lanceolate bract, acuminate. The antheriferous stamens 6-7, 2 larger. Most of them were seeding 14x0.5 cm (Vellingiri *et al.,* 2011).

Materials and Methods

Collection and Extract preparation:

The yercaurd Hills fresh samples of *Senna alata* and *Senna hirsuta*, Tamil Nadu, were obtained at random. The sample was made into powder after washing in running tap water and air drying and then mixed well and ground well and placed in airtight refrigerated bottles. Extract of the raw sample was prepared using Soxhlet extraction method. Approximately 20 gm of powdered sample material was evenly filled into a thimble and extraction made with 250ml of methanol solvent separately. The process of extraction continues for a whole day, or until the siphon tube extractor solvent is colourless. Afterwards, extract taken in a beaker placed over the

hot plate, heating at 30-40°C until entire solvent evaporated and followed by placing in a refrigerator at 4°C for potential usage.

Synthesis of Silver Nano-Particles

In a 100ml flask, silver nitrate solution was prepared. 1 ml of *Senna alata* and methanolic extract of *Senna hirsuta* were combined with 9 ml of silver nitrate 1 mM. In the entire experiment, methanolic leaf extracts from the *Senna alata* and *Senna hirsuta* leaf and control as silver nitrate solution (Smetana *et al.,* 2005). Centrifuging continued for 25 min at 18000 rpm for 200 ml of final solution. Storing at 40°C was made for the collected pellets. Heating done for the supernatant at 50°c to 95°c. At the time of heating process, a change in the colour was observed.

Analytical characterization

Formation of SNs in *Senna alata* and *Senna hirsuta*, was confirmed by absorption studies of developed SNs, performed on a Perklin-Elmer LS-55 UV-visible spectrophotometer, Lamda 35, Germany) the spectra were taken in different time intervals up to 24 hrs between 340 nm and 480 nm. FTIR was used to analyze the chemical composition of the synthesized silver nanoparticles. Best analytical tool FTIR allows the identification of functional groups in the aqueous bark extract and generated SNs. By dried powders in the range 4000 – 400 cm^{-1} using KBr, The solutions were dried at 75° C and pellet method was characterized by dried powders.

Powdered sample used for diffraction with X-rays. Using Scherer formula, the coherently diffracting Silver nano particle domain size of Crystallography was determined from the width of the XRD peaks. Phillips PW 1830 instrument had XRD measurements of Ag-NPs cast into glass slides. Even spread of the powdered Ag-NPs and platinum coated sputter in an ion coater for 120 seconds was made evenly then observed by SEM (JEOL-JSM 6360 MODEL, JAPAN). Scanning Electron Microscope (JSM-6480 LV) and Transmission electron microscopic analysis (TEM) investigated the synthesized extract. The SEM slides were prepared after 24 hrs of $AgNO_3$ addition by making a smudge of the solutions on slides. To make them conductive, coating of thin layer of platinum was made on the samples. At a speeding voltage of 20 KV in the SEM, the samples were characterised. TEM tests were carried out on the Phillips model CM 20 instrument, powered at a 200kV accelerating voltage.

Antibacterial studies

Testing of antimicrobial activity was done by the process of disc diffusion (Bauer *et al.,* 1966). *In-vitro* antimicrobial activity was screened with the use of Hi-media (Mumbai) obtained from Muller Hinton Agar (MHA). MHA plates were prepared by pouring 15 ml of molten media onto sterile petri dishes. The plates were allowed to solidify for 5 minutes, and 0.1 percent inoculum suspension was uniformly swabbed and 5 minutes allowed the inoculum to dry out. Extract concentration is 40 mg / disk placed on 6 mm sterile disk. The loaded disk was placed on the medium surface and for 5 minutes the extract was allowed to diffuse, and for incubation, with the plates held at 37ºC for 24 hours. Inhibition zones formed around the disc were measured in millimeter with transparent ruler at the end of incubation.

Result and Discussion

Synthesis of Silver Nanoparticles

This study identifies the bioactive chemical constituents that are present in the Senna alat a and Senna hirsuta extract and are responsible for biosynthesis AgNPs. Reduction of Ag nano partciles could be detected during the exposure to the extract, by the color shift. Colour shift from pale yellow to brown was observed in 5 minutes, and dark brown in 30 minutes. This might be due to the addition of aqueous $AgNO_3$ to the SC bark extract. Ag+ ions were attracted by the-O-group of biomolecules to form a silver complex after free electrons formed during the reduction process reduced it to zero valence of silver (Ago). Carboxyl (-COO-), hydroxyl (OH-) groups present in the extract of methanol generate and stabilize the SNs. This effect is the product of AgNPs vibrations in surface plasmon. This is because of the presence of free electrons at AgNPs. This SNs plasmon surface vibrations produced a peak of 420 nm, suggesting the reduction of $AgNO_3$ into SNs. It is a best known fact that the metal nanoparticles ' optical properties depend shape and size strongly (Man *et al.,* 2007).

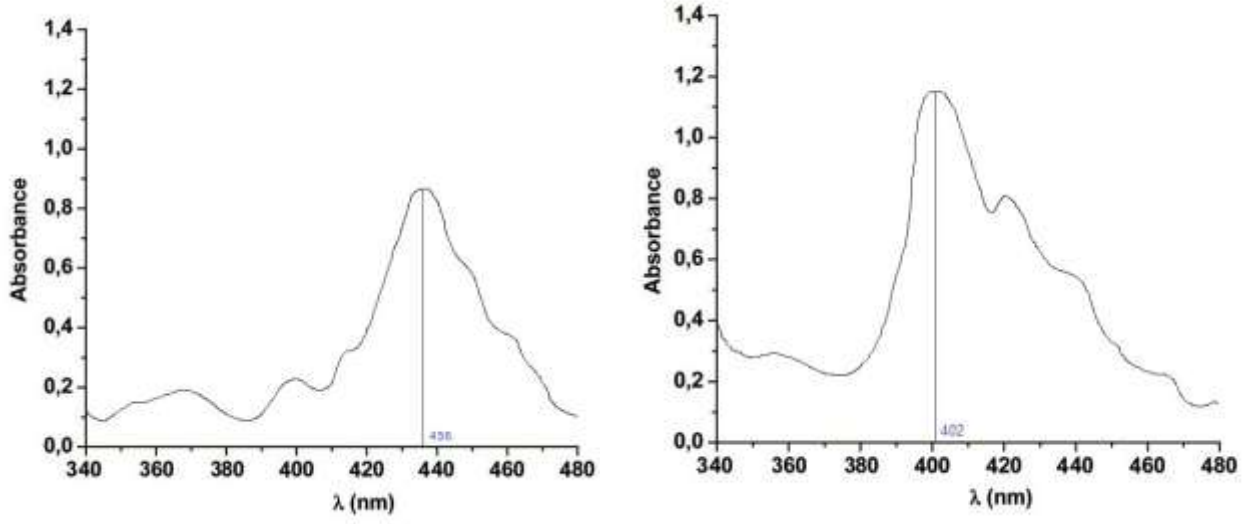
Figure 1: Synthesis of Silver Nanoparticles of *Senna alata* and *Senna hirsuta*

Jain *et al.,* (2009) also observed transformation into reddish brown, when the leaf extracts were mixed with the aqueous solution of the silver ion complex, it was transformed into reddish brown due to excitation of surface plasmon vibrations, indicating that Ag nanoparticles were formed. It was well known that Silver nanoparticles in aqueous solution exhibits greenish brown color by the avoidance of plasma in silver nanoparticles on vibrations.

UV Analysis

UV Vis spectrophotometer detected the synthesized Silver nanoparticle using the extracts from *Senna alata* and *Senna hirsuta* plants (Fig.2). The *Senna alata* and *Senna hirsuta* UV-Vis colloidal solution spectrum of Silver nanoparticles has a maximum absorbance value at 436 nm and 402 nm, which is proven to be the synthesis of silver nanoparticles in the colloidal solution. The location and form of the plasmon absorption depends on the surrounding medium's particle size, shape and dielectric constant. The particle displayed gradual decline from 340-480 nm.

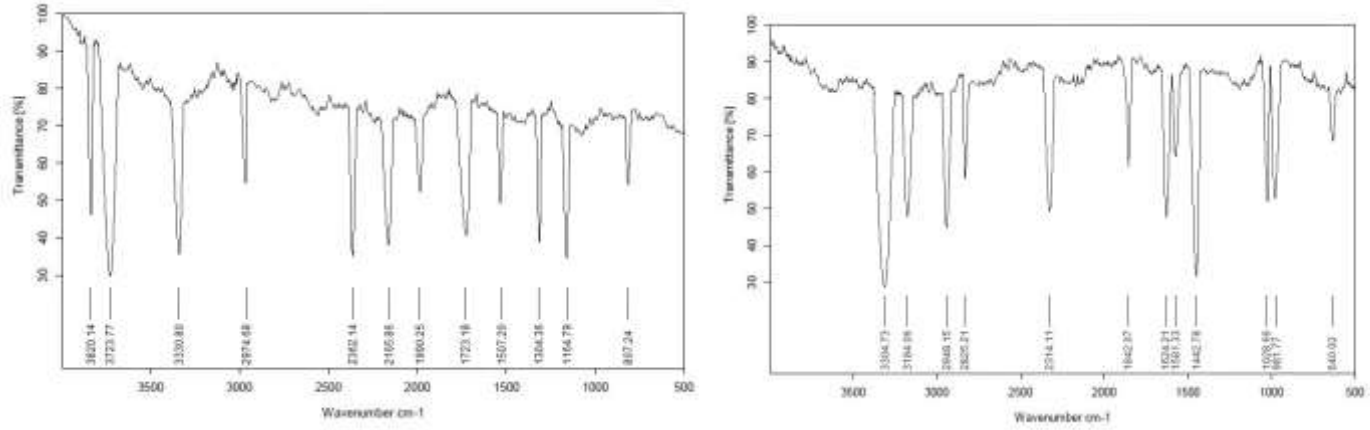
Figure 2: UV Analysis for Synthesis of Silver Nanoparticles of *Senna alata* & *Senna hirsuta*



FTIR Analysis

With the use of FTIR analysis, the characterization of the extract and the resulting nanoparticle was done (Fig. 3). FTIR absorption spectra of soluble extract by reduction of Ag ions observed in the absorbance bands in the region of 500-3500 cm^{-1}.

Figure 3: FTIR Analysis for Synthesis of Silver Nanoparticles of *Senna alata* & *Senna hirsuta*



In *senna alata* the absorbance bands are 3820.14 cm^{-1} and 3723.77 cm^{-1} are Intermolecular hydrogen bonded OH (Strong), 3330.80 cm^{-1} Secondary amines (Weak), 2974.68 cm^{-1} Vinyl Terminal (Medium), 2362.14 cm^{-1} Acids (Medium), 2165.86 cm^{-1} Aromatic Methane (Medium), 1990.25 cm^{-1}Aromatic Methane (Weak), 1723.18 cm^{-1} Aromatic Methane (Weak), 1507.20 cm^{-1} Quaternary compounds (Strong), 1304.36 cm^{-1} Aromatic esters (Very strong), 1164.79 cm^{-1} Aromatic esters (Very strong), 807.24 cm^{-1} Meta Di substituted (Very strong). In *Senna hirsuta* the absorbance bands are 3304.73 cm^{-1} Intermolecular hydrogen bonded OH (Strong), 3184.06 cm^{-1} Quaternary compounds (Strong), 2948.15 cm^{-1} Acids (Medium), 2825.21 cm^{-1}Methoxy (Medium), 2314.11 cm^{-1} Acids (Medium), 1842.07 cm^{-1}Acid peroxide (Very strong), 1624.21 cm^{-1} Aromatic Methane (Weak), 1581.33 cm^{-1} Secondary amines (Weak), 1442.78 cm^{-1} Allyl (Medium), 1028.66 cm^{-1} Cyclo alkanes (Strong), 981.77 cm^{-1} Cyclo alkanes (Strong), 640.02 cm^{-1} Aromatic Methane (Strong).

SEM and TEM Analysis

Scanning electron microscope is used to measure silver nanoparticles size. In this study the size of silver nanoparticles with different magnifications was between 1µm-0.5µm (Fig. 3). Nanoparticle of a fairly spherical shape shaped with a diameter of 1 µm was shown by the scanning electron microscopy (SEM). Observations were made by the SEM image recording from drop coated films of the Ag nanoparticles synthesized with *Senna alata* and *Senna hirsuta* leaf extract. For the determination of morphology, size and crystalline nature of the synthesized

Ag Nano-particles, TEM analysis was done. Nanoparticles of about 80 nm was shown by the TEM image and size distributions of Ag-NPs.

Figure 4: SEM Analysis for Synthesis of Silver Nanoparticles of *Senna alata* & *Senna hirsuta*

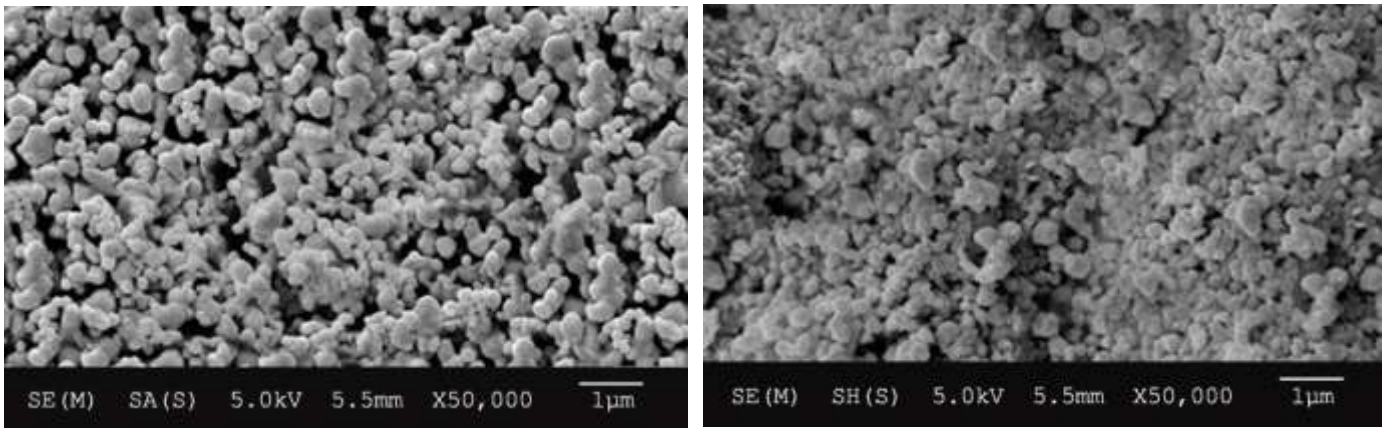
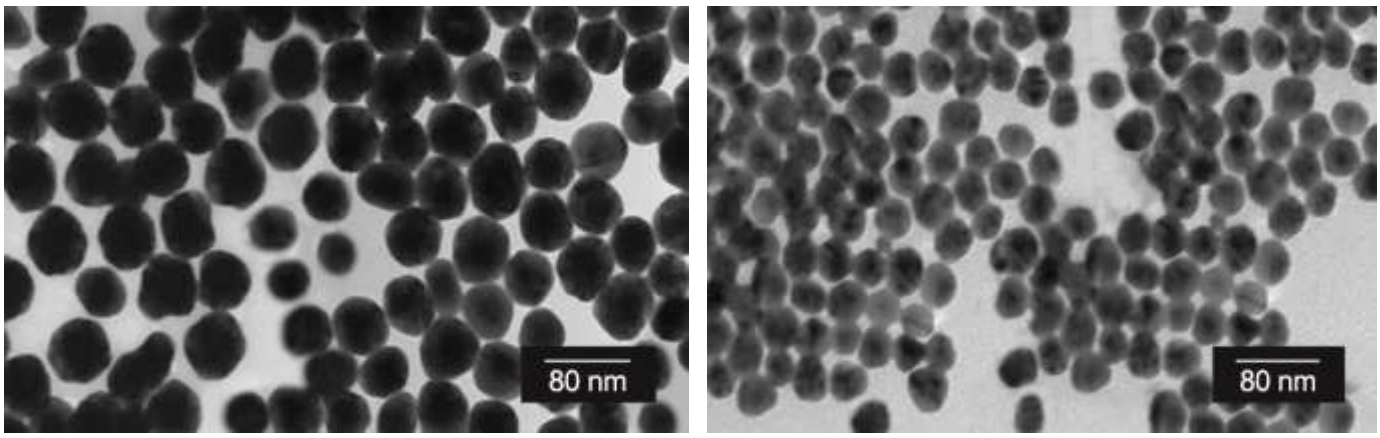


Figure 5: TEM Analysis for Synthesis of Silver Nanoparticles of *Senna alata* & *Senna hirsuta*

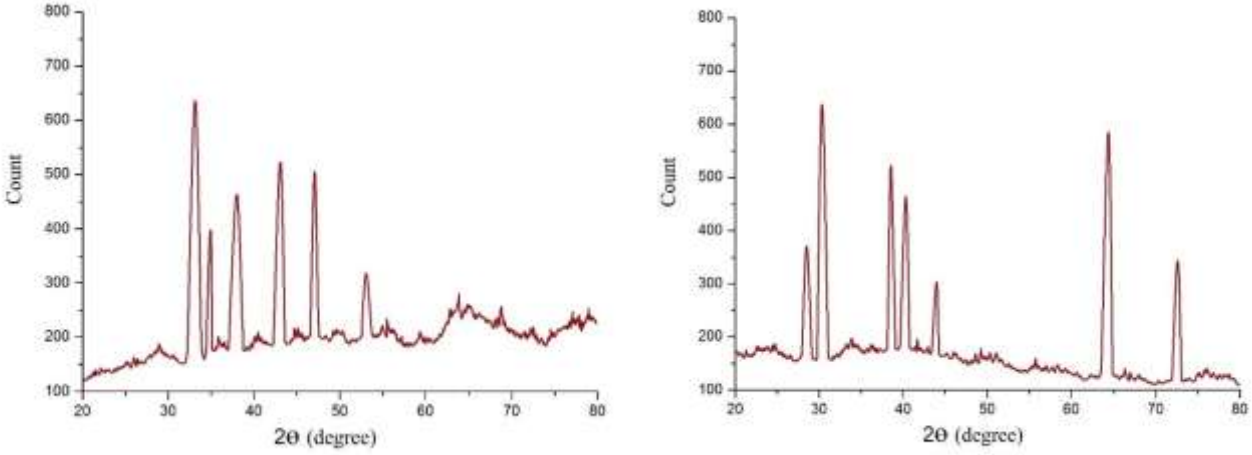


XRD Analysis

By the use of XRD technique, the phase identification and characterization of the nanoparticles ' crystal structure can be done (Sun *et al*., 2000). X-rays penetrate deep into the nanomaterial and the resulting diffraction pattern is compared with structural information gathering standards (Strasser *et al*., 2010). Senna alata 2ɵ value and hkl value are 32.18 (002), 34.16 (100), 37.22 (101), 42.15 (102), 46.17 (110) and 52.34 (103). The 2ɵ value and hkl value

for Senna hirsute are 28.41 (211), 30.13 (220), 38.55 (311), 40.10 (222), 43.95 (400), 64.22 (440) and 79.60 (533).

Figure 6: XRD Analysis for Synthesis of Silver Nanoparticles of *Senna alata* & *Senna hirsuta*



Antibacterial Activity

In the fields of biological systems and medicine, Silver (Ag) displays potential applications among the noble metals (e.g., Ag, Pt, Au and Pd) (Mulvaney *et al*., 1996). Biosynthesis of Plant extracts with metal ions has produced metallic nanoparticipants; surrounded by proteins and metabolites of various functional groups of amines, alcohols, aldehydes and carboxylic acids (Dhermendra *et al*., 2008). The antibacterial activity was tested for *Senna alata* and *Senna hirsuta* synthesized sample against five different bacterial organisms (*Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae* and *Escherichia coli*). The sample was loaded in two different concentrations 30 and 60. In two synthesized sample *senna alata* shows better activity when compared to *Senna hirsuta*. *B. subtilis* (18mm, 16mm), *E.faecalis* (19mm, 14mm), *S.aureus* (20mm, 14mm), *K.pneumoniae* (21mm, 16mm) and *E.coli* (17mm, 15mm). *Klebsiella pneumoniae* shows highest activity in both the samples.

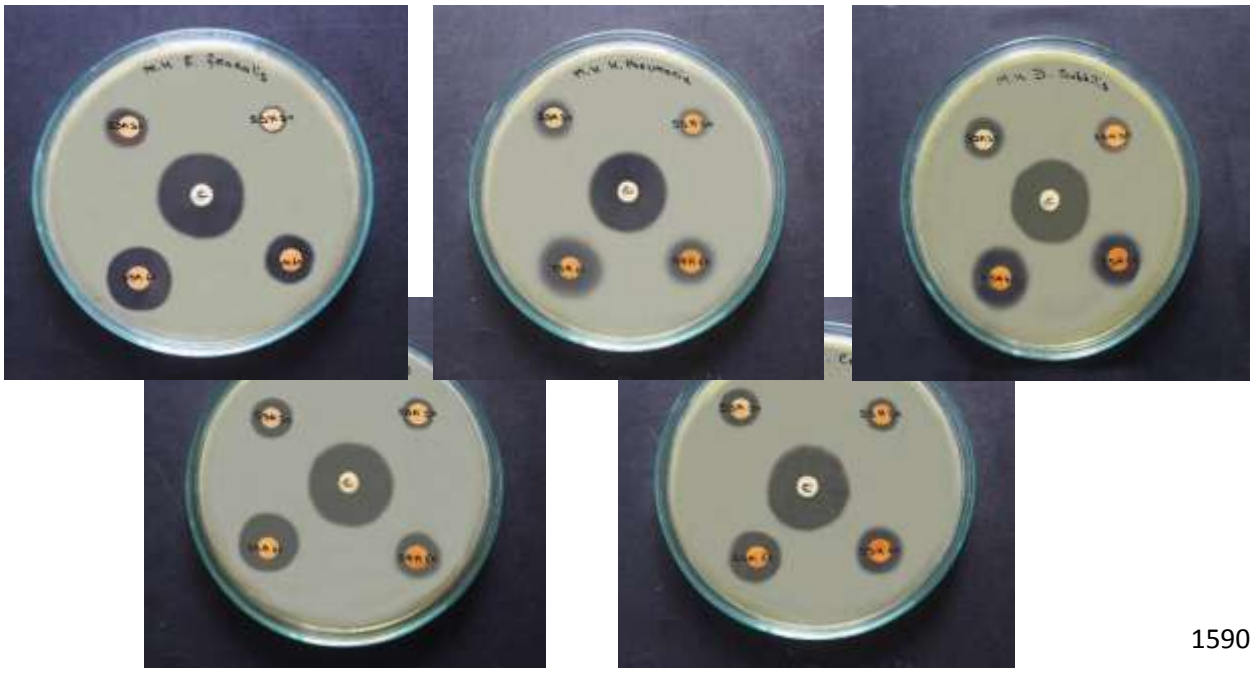
Nanoparticle's shapes decide the antimicrobial activity of nanoparticles. This attribute can be further verified by using various formed nanoparticles to research the inhibition of bacterial growth (Mahitha *et al.*, 2013). According to (Pal *et al.*, 2007), the smaller nanoparticles

displayed bacterial inhibition with concentration of silver ion 1 as large as 10^{-3} M. This means that the SNs with different shapes influence bacterial cells differently.

Table 1: Antibacterial Activity for Synthesized Methanolic extracts

Organisms	Control	SSA30	SSA60	SSH30	SSH60
Bacillus subtilis	23	14	18	11	16
Enterococcus faecalis	26	13	19	8	14
Staphylococcus aureus	27	13	20	10	14
Klebsiella pneumoniae	26	12	21	9	16
Escherichia coli	24	12	17	10	15

Figure 7: Antibacterial Activity for Synthesized Methanolic extracts



Conclusion

The present study uses the leaf extracts of *Senna alata* and *Senna hirsuta* to explore bio-reductive synthesis of nano sized silver particles. The methanolic extracts of *Senna alata* tend to be naturally pleasant, hence this technique could be utilised for quick silver nanoparticles growth. The green approach testified in this study with the use of methanolic extracts from *Senna alata* was synthesized with silver nanoparticles. The *Senna alata* extract was able to reduce the Ag ions and also to regulate scale. Therefore, the UV-Visible, FTIR, SEM, TEM and XRD-conforming SNs. In days to come, choice of such medicinal plants could create a new stage for biomedical applications to recognize the probable of natural medicines in nanoscience. AgNPs suspension antibacterial activity demonstrated an improved activity against *Klebsiella pneumoniae* and *Staphylococcus aureus*. Therefore, the method constructed has numerous benefits such as regulating the size of metal nanoparticles being environmentally approachable and at minimized cost.

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