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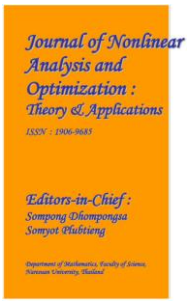

AN ANALYTICAL INVESTIGATION USING THE METHANOLIC EXTRACT OF TWO MEDICINAL PLANTS (SENNA ALATA AND SENNA HIRSUTA) TO PRODUCE SILVER NANOPARTICLES WITH ANTIBACTERIAL ACTIVITY

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Abstract
Plant-mediated green synthesis of nanomaterials has gained popularity due to its eco-friendliness and affordability. In this study, silver (Ag) nanoparticles were created as bio-reducing agents using methanol extracts from fresh leaves of the medicinal herbs Senna Alata and Senna Hirsuta. This technique made it possible to create nanoparticles that were supported by studies in spectrophotometry, including X-Ray Diffraction (XRD), Fourier Transform Infrared (FTIR), Scanning Electron Microscopy (SEM), and Ultraviolet-Visible (UV-Vis). Fresh Senna Alata and Senna Hirsuta leaf extracts changed from being light green to light greenish after being exposed to Ag precursors, as demonstrated by UV-Vis spectra and eye inspection. Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae, and Escherichia coli were the five microorganisms tested for antibacterial activity Using Disc Diffusion method. Silver nanoparticles extracted from Senna Alata and Senna Hirsuta leaf extracts, which inhibited B.subtilis (18 mm & 16 mm), E.faecalis (19 mm & 14 mm), S.aureus (20 mm & 14 mm), K.pneumoniae (21 mm & 16 mm) and E.coli (17 mm and 15 mm). The higher Inhibition zoneoccursin K.pneumoniae and S.aureus follows. It has been shown that inhibiting bacterial growth, the Methanlic extracts of Senna Alata leaves containing Ag nanoparticles is comparable withchloramphenicol.

Keywords:
Silver nanoparticle synthesis, green synthesis, Senna Alata, Senna Hirsuta,Antibacterial activity.

Introduction

Nanotechnology has become a fascinating subject of research as a result of its wide range of applications in several industries, including adsorption, optical sensors, catalysts, water treatment, drug delivery, and nanomedicine (Sadeghi and Gholamhoseinpoor, 2015). Organic or inorganic materials with particle sizes between 1 and 100 nm and a high area-to-volume ratio, known as nanoparticles, can be produced by a number of physical, chemical, and biological processes (Seil and Webster, 2012; Iravani et al., 2014). Due to their outstanding physicochemical characteristics, metallic nanoparticles (NPs) have been effectively used in a variety of sectors, including health care, synthetic biology, and cellular transportation (Burda et al., 2005). Because of their distinct shape, stability, and controlled geometry, AgNPs have attracted the most interest among different nanoparticles (Grace and Pandian, 2007). AgNPs have been 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 used in the diagnosis and treatment of different diseases (Khan etal., 2014). In particular, Ag NPs have excellent antimicrobial activity against multiple microorganisms known to be responsible for multiple infectious diseases. Because of this, they were used successfully in various medical products, such as catheter coating materials forcerebrospinal fluid drainage (Bayston et al.,

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2007; Galiano et al., 2008), contact lenses (Weisbarth et al., 2007), and other medical devices. They were also used in bone cement (Alt et al., 2004), surgical masks, impregnated silk fabrics, nanogels, nano-lotions (Furno et al., 2004; Ipet al., 2006; Leaper, 2006; Li et al., 2006), wound dressings, and so on (Atiyeh et al., 2007). Indeed, most of the products developed on Ag-based have been commercialized and approved byglobal regulatory bodies.

Various plant parts such as leaves, stem bark, roots and their extracts have recently beenused to synthesize nanoparticles because they are greener and can also serve as synthesisstabilizers and reducers (Dada et al., 2018; Song and Kim, 2009).

Antimicrobial resistance is a global threat to drug development because of micro- organisms ability to adapt to the drugs intended to kill them, making them less efficient. Therefore, possible antimicrobial agents that have the power to end the danger of bacterial resistance need to be constantly discovered (WHO, 2015). Green synthesis using plant extracts has been shown to be an efficient method for preparing silver nanoparticles which could be useful in addressing the challenges surrounding antimicrobial resistance (Haase et al., 2015). Thenanoparticles release silver ions into bacterial cells, enhancing their bactericidal activity (Feng et al., 2000; Liau et al., 1997; Ahamed et al., 2010; Swain, 2014; Rai et al., 2009). Silver'santimicrobial activity was recorded in early history. The World Health Organization reports that 70 % of the population in many countries 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 between 30 and 60 cm long and consist of 8 to 20 pairs of leaflets. Leaflet is oblong or elliptic, with both ends rounded. The flowers in auxiliary racemes are small, about 20 to 50 cm long and 3 to 4 cm wide. The inflorescence appears like a yellow candle, and is used most often for 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; leaves 4-6 pairs, ovate elliptic, acuminate, rounded or cuneate at the base, 10 X 4 cm long. Terminal corimas in penicles; lanceolate bract, acuminate. The antheriferous stamens 6-7, 2 larger. Many 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 materials were washed under running tap water, air dried and then homogenized to fine powder, and placed in airtight refrigerated bottles. Extract of the raw sample was prepared using Soxhlet extraction method. Approximately 20 gm of powderedsample material was evenly packed into a thimble and extracted with 250ml of methanol solvent separately. The extraction process will continue for 24 hours, or until the siphon tube extractor solvent is colourless. Afterwards, the extract was taken in a beaker and placed on a hot plate, heating at 30-40°C until all the solvent was evaporated. Dried extract was placed in therefrigerator at 4°C for potential use.

Synthesis of Silver Nano-Particles

The 1 mM solution for the silver nitrate was prepared in a 100 ml flask. 1 ml of Senna Alata and Senna Hirsuta methanolic extract was combined with 9 ml of 1 mM silver nitrate. Throughout the experiment, methanolic leaf extracts from the Senna Alata and Senna Hirsuta leaf and silver nitrate solution were used as control (Smetana et al., 2005). 200 ml of final solution and centrifuged for 25 min at 18,000 rpm. The collected pellets were stored at 40°c. The supernatant was heated at 50°c to 95°c. A change in the colour of solution was observed duringthe heating process.

Analytical characterization

To confirm the formation of AgNPs in Senna Alata and Senna Hirsuta, absorption studies of developed AgNPs were performed on a Perklin-Elmer UV-visible spectrophotometer, Lamda 35, Germany) the

spectra were taken in different time intervals up to 24 hrs between 340 nm and 480 nm. An FTIR spectrometer (Perkin-Elmer LS-55-Luminescence spectrometer) was used to analyze the chemical composition of the synthesized silver nanoparticles. Fourier transforms infrared spectroscopy (FTIR) is only one of the superlative analytical tools which allow the identification of functional groups in the aqueous bark extract and generated SNs. The solutions were dried at 75^{o} C and pellet method was characterized by dried powders in the range 4000 – 400 cm^{-1} using KBr.
Powdered sample used for diffraction with X-rays. Using Scherer formula, the coherently diffracting Silver nano particle Crystallography domain size was determined from the width of the XRD peaks. Phillips PW 1830 instrument had XRD measurements of Ag-NPs cast into glass slides. The powdered Ag-NPs were spread evenly and platinum coated sputter in an ion coater for 120 seconds, then observed by SEM (JEOL-JSM 6360 MODEL, JAPAN). Scanning ElectronMicroscope (JSM-6480 LV) and Transmission electron microscopic analysis (TEM) investigatedthe synthesized extract. The SEM slides were prepared after 24 hrs of $AgNO_3$ addition bymaking a smear of the solutions on slides. The samples were coated with a thin layer of platinu to make them conductive. The samples were then characterized at a speeding voltage of 20 KVin the SEM. TEM tests were carried out on the Phillips model CM 20 instrument, powered at a 200kV accelerating voltage.

2. Antibacterial studies

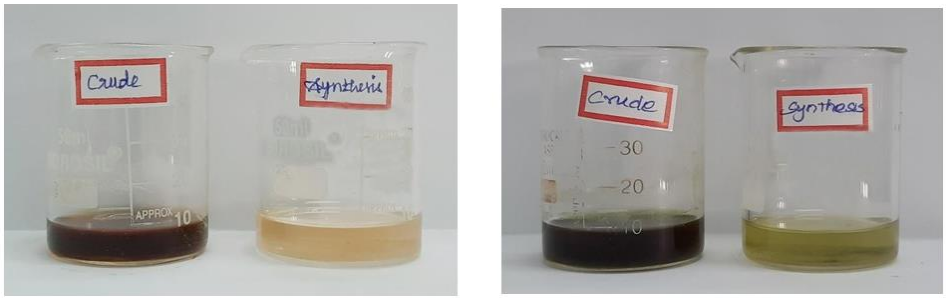
The antimicrobial activity was tested using the process of disc diffusion (Bauer et al., 1966) In vitro antimicrobial activity was screened with the use of Hi-media (Mumbai) obtained from Muller Hinton Agar (MHA). They prepared the MHA plates by pouring 15 ml of molten media onto sterile petri dishes. The plates were allowed to solidify for 5 minutes, and 0.1 percentinoculum 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 onthe medium surface and the extract was allowed to diffuse for 5 minutes, while the plates were held at 37ºC for 24 hours for incubation. Inhibition zones formed around the disc were measured in millimeter with transparent ruler at the end of incubation.

3. Result and Discussion

Synthesis of Silver Nanoparticles

This study identifies the bioactive chemical constituents that are present in the Senna Alata and SennaHirsuta extract and are responsible for biosynthesis AgNPs. The color shift coul d detect reduction of Ag nanoparticles during exposure to the extract. A change in color from pale yellow to brown was observed within 5 minutes, and within 30 minutes for dark brown. It may be due to the addition of aqueous $AgNO_3$ solution to the SC bark extract that the 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 methanolic extract generates and stabilize the SNs. This effect is the product of AgNPs vibrations in surface plasmon. This is because of the free electrons present at AgNPs. This AgNPs plasmon surface vibrations produced a peak of420 nm, suggesting the reduction of $AgNO_3$ into SNs. It is a well-known fact that the metal nanoparticles ' optical properties depend strongly on their shape and size (Man et al., 2007).

Figure 1: Synthesis of Silver Nanoparticles of Senna Alata and Senna Hirsuta



Jain et al., (2009) also observed that leaf extracts were mixed with the aqueous solutionof the silver ion complex, it was transformed into reddish brown due to excitation of surface plasmon

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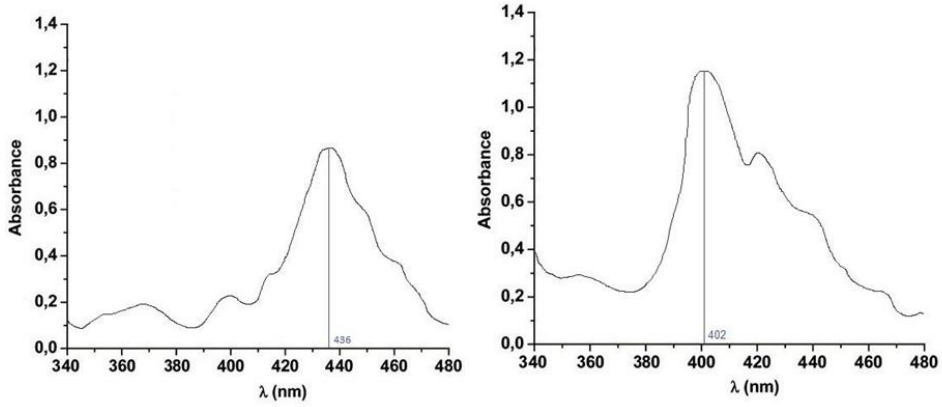
vibrations, indicating that Ag nanoparticles were formed. It was well known that Silver nanoparticles in aqueous solution exhibits greenish brown color due to the avoidance of plasma on vibrations in silver nanoparticles.

UV Analysis

UV Vis spectrophotometer detected the synthesized Silver nanoparticle using the extractsfrom 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

FTIR analysis was used for the characterization of the extract and the resulting nanoparticle (Fig.3). FTIR absorption spectra of soluble extract reduction of Ag ions. Absorbance bands in the region of 500-3500 cm^{-1}.

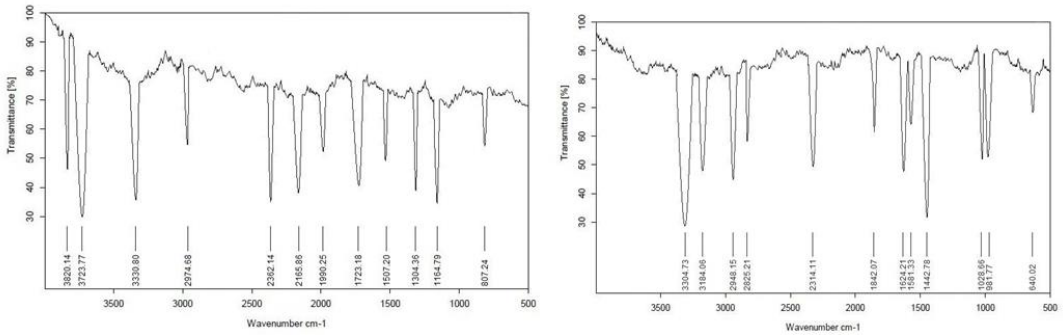


Figure 3: FTIR Analysis for Synthesis of Silver Nanoparticles ofSenna Alata & Senna Hirsuta

In Senna Alata the absorbance bands are 3820.14 cm^{-1} is Intermolecular hydrogen bondedOH (Strong), 3723.77 cm^{-1} 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), 1304.36cm^{-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), 1581.33cm^{-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

Review of the scanning electron microscope used to measure the size of silver nanoparticles.

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In this study the size of silver nanoparticles with different magnifications was between 1µm-0.5µm (Fig.4). The scanning electron microscopy (SEM) image showed nanoparticle of a fairly spherical shape shaped with a diameter of 1 µm. The SEM image recording from drop coated films of the Ag nanoparticles synthesized with Senna Alata and Senna Hirsuta leaf extract. Transmission electron microscopy (TEM) analysis was carried out forthe determination of morphology, size and crystalline nature of the synthesized Ag Nano-particles. The TEM image and size distributions of Ag-NPs showed that the mean diameter ofthe nanoparticles ranged from about 80 nm.

Figure 4: SEM Analysis for Synthesis of Silver Nanoparticles ofSenna Alata & Senna Hirsuta

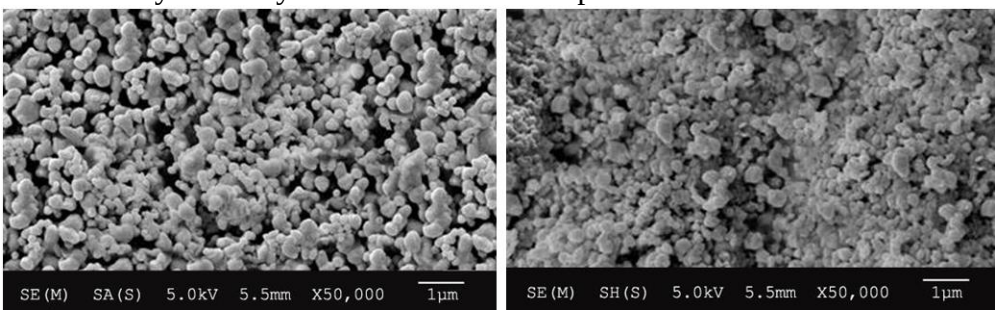
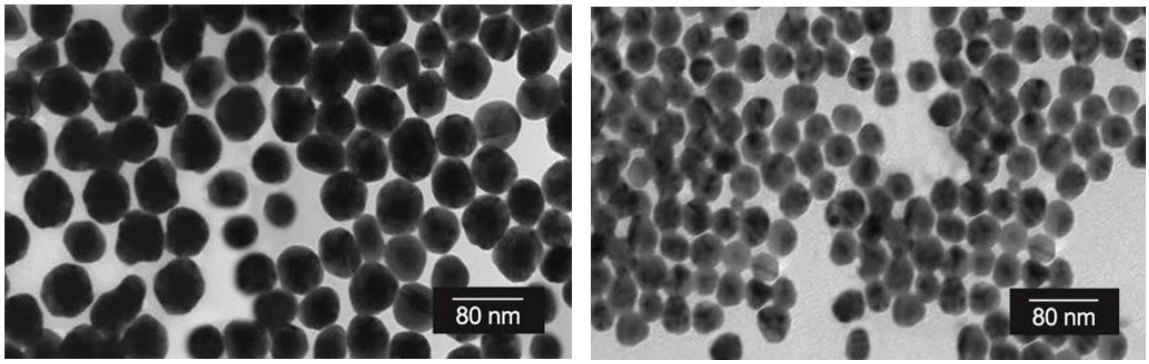


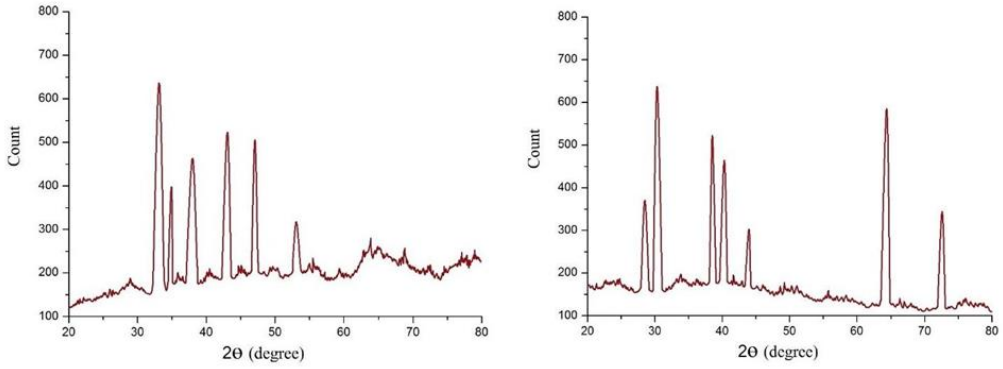
Figure 5: TEM Analysis for Synthesis of Silver Nanoparticles ofSenna Alata & Senna Hirsut



XRD Analysis

The phase identification and characterization of the nanoparticles crystal structure can bedone using XRD technique (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 Nanoparticlesof Senna Alata & Senna Hirsuta



Antibacterial Activity

Silver (Ag) exhibits potential applications in the fields of biological systems and medicine among the noble metals (e.g., Ag, Pt, Au and Pd) (Mulvaney et al., 1996). Biosynthesisof plant extracts with metal ions has produced metallic nano participants; surrounded by proteins and metabolites of various functional groups of amines, alcohols, aldehydes and carboxylic acids(Dhermendra et al.,

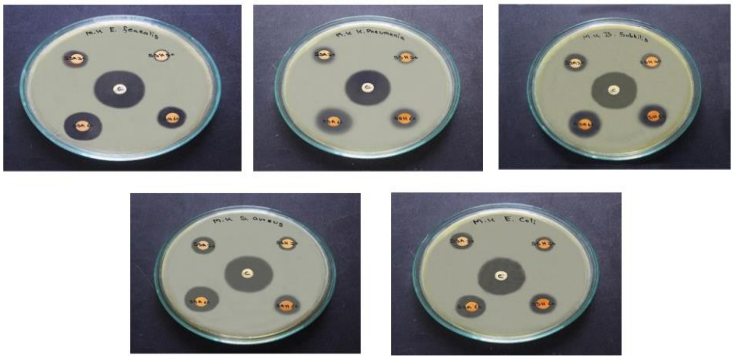
2008). The antibacterial activity was tested for Senna Alata and Senna Hirsuta synthesized sample against five different bacterial organisms (Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli). The sample was loaded in two different concentrations 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.

The antimicrobial activity of nanoparticles also depends on the nanoparticle's shapes.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

In this work, bio-reductive production of silver nanoparticles is demonstrated using leaf extract from Senna alata and Senna hirsuta. The ecologically benign nature of Senna Alata's methanolic extracts makes it possible to employ this method to produce silver nanoparticles quickly. The environmentally friendly method used in this work to create the methanolic Senna Alata extracts was created utilising silver nanoparticles. The Senna alata extract has the capacity to control the scale in addition to lowering the Ag ions. Consequently, the AgNPs that are compatible with XRD, FTIR, SEM, and UV-Visible. Future biomedical applications that realise the potential of natural remedies in nanoscience may find a new platform in the choice of such medicinal plants. AgNPs suspension antibacterial activity demonstrated an improved activity against Klebsiella pneumoniae and Staphylococcus aureus. Therefore, the method built has severaladvantages such as regulating the size of metal nanoparticles being environmentally friendly and cost-effective.

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