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Green Synthesis of Silver Nanoparticles from Abelmoschus Esculentus Seeds Extract and their Antibacterial Activity

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Abstract The green synthesis of AgNPs involving plant extract was explored by a lot of researchers. In the present research work, we tried to get extract from seeds of a Abelmoschus esculentus seeds. Formation of AgNPs confirmed by UV-Visible spectroscopic absorption and Infra-red absorption spectrometry. The morphology of AgNPs was confirmed by SEM and XRD analytic studies revealed the crystalline nature. we are reporting a novel biological approach for the formation of AgNPs using Abelmoschus esculentus seeds extract. Silver nitrate was reduced with aqueous solution of Abelmoschus esculentus seeds extract. Particle size analyser done to analyse the size of silver nanoparticles formed. Phytochemical analysis of the Abelmoschus esculentus seed extract was done using standard test procedures. Antibacterial studies confirmed its potentiality against the gram-positive bacteria like *Bacillus subtilis*, *Staphylococcus aureus* and gram-negative bacteria like *Escherichia coli* and *Proteus vulgaris*.

Keywords Green synthesis, Silver nanoparticles, abelmoschus esculentus seeds extract, characterization techniques, Antibacterial activity

1. Introduction

Green synthesis are more advantages over chemical and physical methods. it is cost effective and environment friendly. In this method, there is no need to use temperature, sonicator and toxic chemicals [1]. Green synthesis is unique and their stabilization of the NPs. This creates motivation and interest in synthetic routes that allows better of the NPs formation. Optimising the reaction conditions control the shape and size for various nanotechnological applications. AgNPs nanoparticles are nanometer in size. Since they have large surface area, highly reactive and widely used in industries, packaging material. The present study illustrates the characterization of silver nanoparticles was done by using UV- Visible spectroscopy, IR spectroscopy, XRD analysis and Particle size analysis, which gives a preliminary confirmation of silver nanoparticles. An attempt has been made to study the antibacterial activity of silver nanoparticles prepared by green synthesis.

Figure 1: Abelmoschus esculentus seeds

2. Materials and Methods

Silver nanoparticles were synthesized according to the chemical reduction method by using Abelmoschus esculentus seed extract. This method can easily be performed in any chemical laboratory and economical, thus a cheaper method when compared with other methods of synthesizing silver nanoparticles.

3. Experimental Section

3.1 Preparation of silver nitrate solution:

Accurately weighing 1.6g of silver nitrate ($AgNO_3$) as obtained from our chemistry laboratory. It is made up to 100 ml standard flask using distilled water to get 0.01N silver Nitrate.

3.2 Preparation of Abelmoschus esculentus seeds extract:

The extract was prepared by taking 30g of Abelmoschus esculentus seeds. The seeds were washed twice with distilled water and dried. The Abelmoschus esculentus seed were boiled with 100 ml distilled water taken in a 400ml beaker for 15 minutes. Then the plant material is filtered by whatmann no: 40 filter paper and extract were collected in a clean container. The extract is used as reducing and stabilizing agent for the synthesis of silver nanoparticles. [12, 13, 14]

3.3 Silver nanoparticles synthesis:

0.01N Aqueous solution of silver nitrate ($AgNO_3$) as prepared and used for the synthesis of silver nanoparticles. The equal proportions 10ml of 0.01N silver nitrate solution is mixed with 5ml of Abelmoschus esculentus seeds extract. After the 15 minutes observation, the mixture turned into reddish brown colour which confirms the presence of silver nanoparticles [15,16].

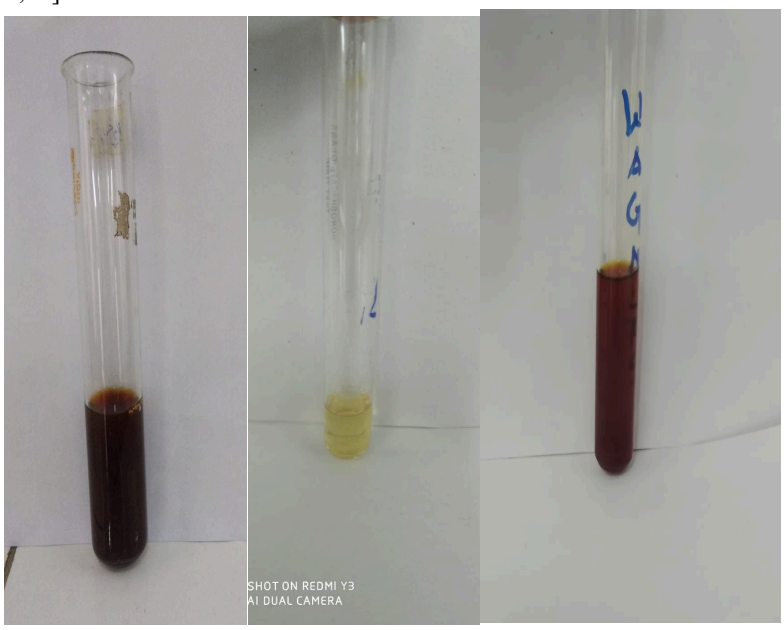

Figure 2: Picture of AgNPs (a) and Alkaloids present by Meyer's test (b) and Wagner's test (c)

4. Results and Discussion

Silver Nanoparticles were synthesized according to chemical reduction method using Abelmoschus esculents seeds extract. Silver Nanoparticles are characterized by phytochemical constituent extract test UV-Visible spectrometer, Scanning electron microscope (SEM) and XRD [1].

4.1 Phytochemical constituents of abelmoschus esculentus seed extract

Phytochemical Analysis preliminary phytochemical analysis for abelmoschus esculents seed extract was done using standard test procedures, it confirms the availability of active phytochemicals in the aqueous abelmoschus esculentus seed extract. The healthful properties of edible plants are perhaps due to the presence of a variety of phytoconstituents such as alkaloids, polysaccharides, flavonoids, glycosides, phenols, saponins, tannins etc. The preliminary screening tests are useful in the detection of these bioactive constituents. The result of phytochemical screening depicted [4].

Table 1: phytochemical test of the *Abelmoschus esculentus* seeds extract

S. No.	Husk Metabolite	Name of the Test	Colour/ Observance	Abelmoschus Esculentus Seeds Extract
1	Glycosides	Molish's	No change	Absence
2	Alkaloid	Wagner's	Brown	Presence
3	Tannins	Tannin's	No change	Absence
4	Steroid	Salkowski	No change	Absence
5	Saponin	Saponin's	No change	Absence
6	Carbohydrate	Fehling	No change	Absence
7	Alkaloid	Mayer's	Yellow	Presence

4.2 UV-Visible spectra

The nanoparticles were first analysed using UV-visible spectrometric techniques. The wavelength was scanned from 350 to 700nm giving the expected spectra. A broad spectrum around 430nm obtained for the silver nanoparticles formation, which is the surface Plasmon peak.

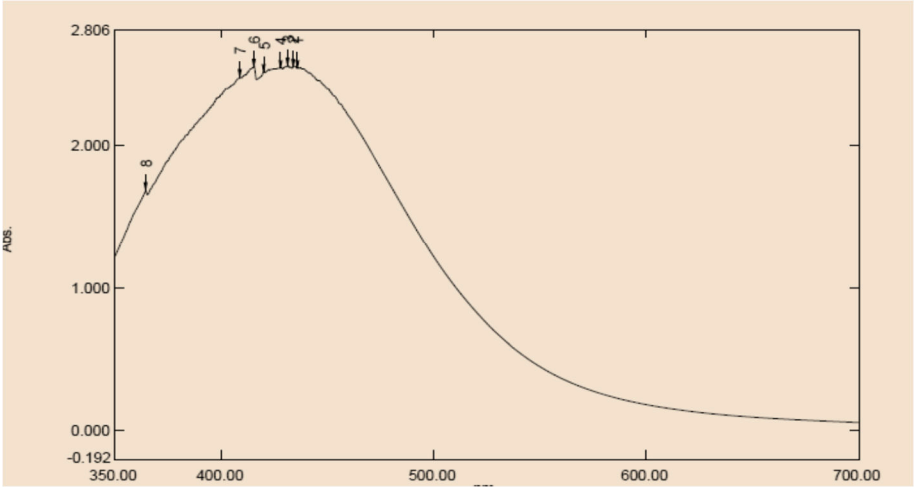

Figure 3: UV-VISIBLE Spectrum of Silver Nanoparticle

The colour changes 10 minutes of incubation after the addition of 5 ml of aqueous extract of abelmoschus esculentus and 10 ml of 0.01N silver nitrate solution. The colour of abelmoschus esculentus husk extract and silver nitrate mixture solution showed a colour changes from light yellow to brown colour. These changes indicated the formation of silver nanoparticles [5,6].

UV-Visible spectroscopy was used to confirm the formation and stability of abelmoschus esculentus husk extract and 10ml of 0.01N silver nitrate solution. The highest absorbance peaks at approximately 436nm, 434nm, 431nm, 428nm, 420nm, 415nm, 409nm and 364nm formation of silver nanoparticles. Peak at 436nm due to silver nanoparticle and peak at 364 nm due to the alkaloid present.

4.3. FT-IR spectra

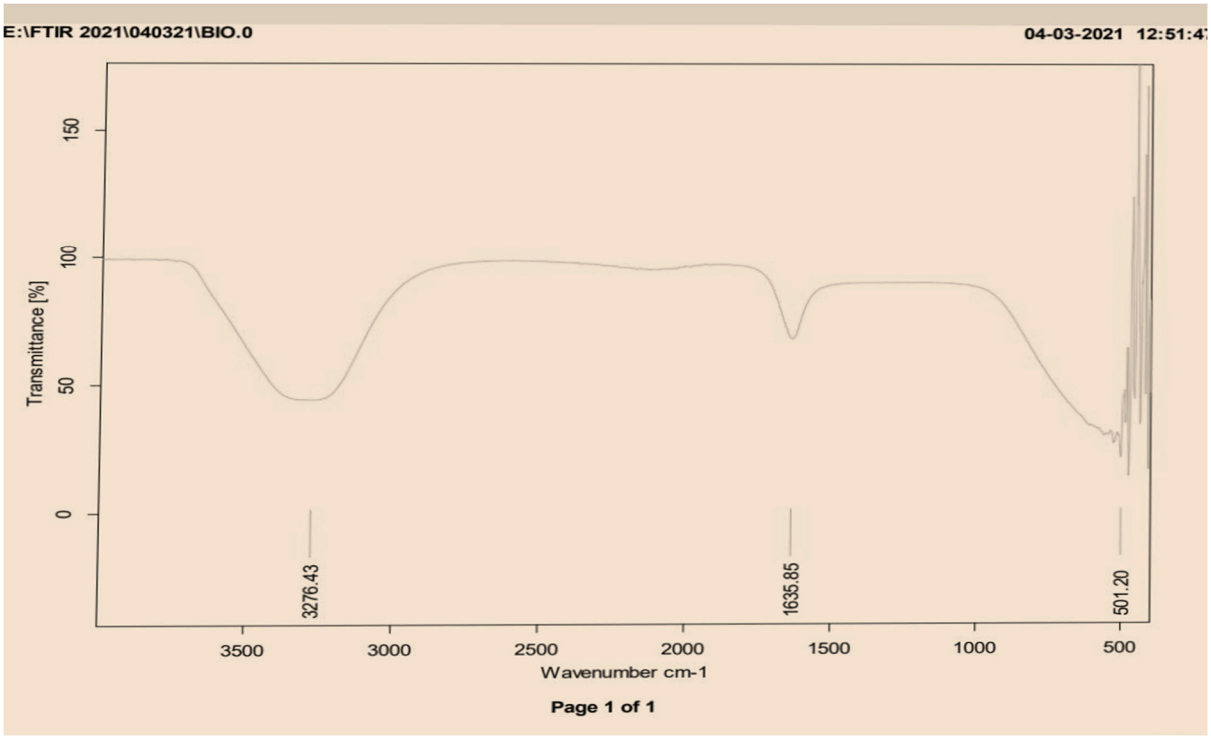

Figure 4: FTIR spectra of the aqueous Abelmoschus esculentus seeds extract and AgNPs samples were analysed by FTIR spectroscopy (Hitachi Ltd., Tokyo, Japan).

The FTIR analysis was performed with KBr pellets and recorded in the range of 400–4,000 $cm^{-1.}$ The presence of some alcoholic group gives peak around 3200cm^{-1} and 1700cm^{-1} due to some carbonyl group present in the extract.

4.4. XRD Analysis

XRD analysis was done to determine the crystal structure of $AgNP_S$. Figure 5 shows the XRD diffraction pattern of silver nanoparticles. The silver nanoparticle solution coated with in an aluminium foil and dried well. This coated sample used for the XRD analysis.

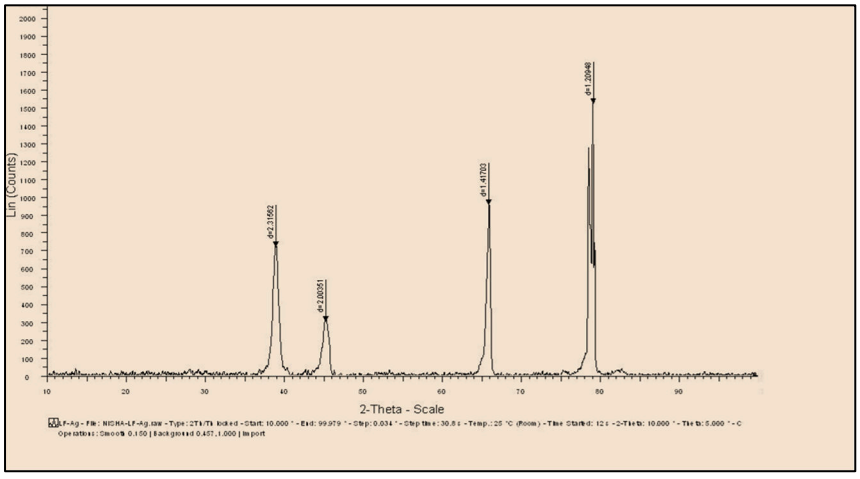

Figure 5: XRD Images of silver nanoparticle

The silver nanoparticle solution coated with in an aluminium foil and dried well. This coated sample used for the analysis and XRD analysis was conducted to determine the crystal structure of AgNPs. Figure 5 shows the XRD diffraction pattern of silver nanoparticles. Four distinct diffraction peaks at 2θ with values of, 39.1°, 45.0°, 65.5° and

78.5° were indexed with the planes respectively which interface that the particles were face-cantered cubic silver. Further analysis was performed to determine the size of the silver nanoparticles using particle size analyser [10,9].

4.5. Scanning electron microscopic analysis:

SEM was used to view the morphology and size of silver nanoparticles, Images shows the nanoparticles synthesized by abelmoschus esculentus seed extract were relatively spherical in shape. silver nanoparticles are associated with one another which leads it to clusters. As a result of agglomeration of silver nanoparticles, after 24 hours. Figure 6 shows the nanoparticles in the range of 5 to 450 nm. This range of size of nanoparticles confirmed the silver nanoparticles formation [11]. Polydispersed AgNPs obtained were confirmed by SEM studies.

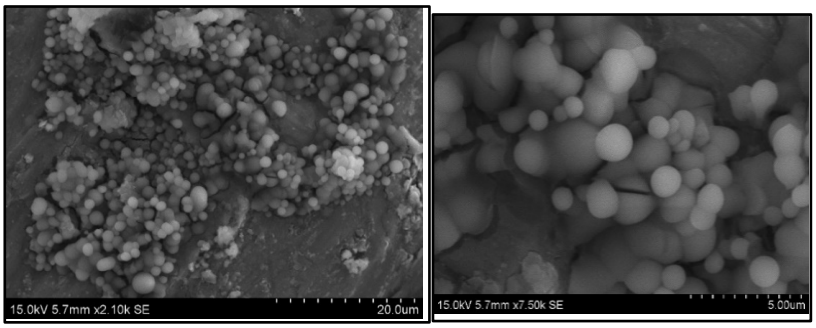

Figure 6: SEM Images of silver nanoparticle

4.6. Particle size analyser

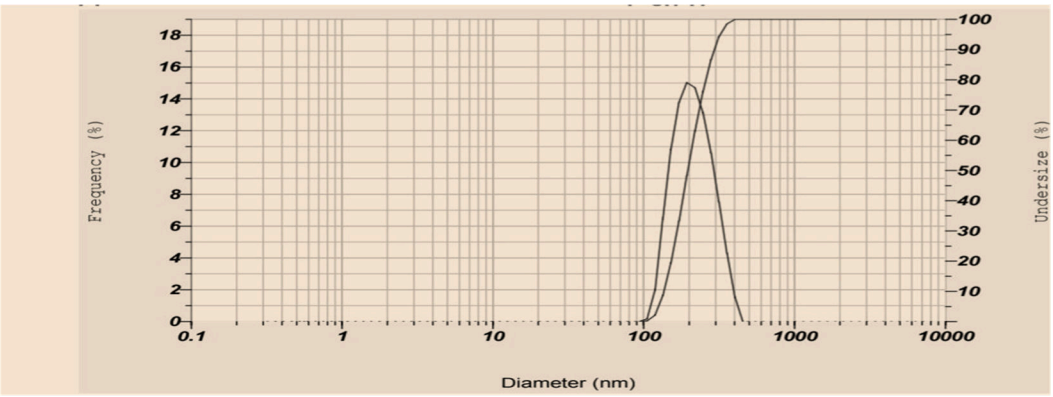

Figure 7: Particle size analyser of silver nanoparticles

The particle size of AgNPs using DLS analysis, as indicated as a size range of 200–300 nm, with an average size of 258 nm. DLS analysis showed that the average size of AgNPs synthesized from 0.2 and 0.3 mol/L was around 526 and 653 nm.

4.7. Antibacterial Activity

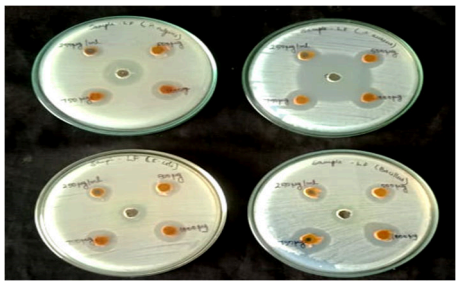

Figure 8: Antibcterial studies of silver nanoparticle

The method used was Well diffusion assay (Eloff, 1998) to determine the antibacterial effect. Nutrient agar was prepared and poured in the sterile Petri dishes and allowed to solidify. 24 hours growing bacterial culture of bacterial

pathogens was swabbed on it. Then, the test sample in different concentrations was loaded in the wells made using cork borer. Tetracycline (20µL) was used as positive control. The plates were then incubated at 37ºC for 24hours. After incubation the inhibition diameter was measured and units are mm [6,7,8].

Table 2: Antibacterial Activity Results

Name of the sample	Concentration of the Sample (µg/mL)	Zone of inhibition (mm)			
		B. s	***S. a***	***E. coli***	***P. v***
Aqueous solution of	250	12	13	12	10
silver nano-particles	500	14	16	14	14
	750	18	18	16	16
	1000	20	19	18	19
	Control	14	14	14	14

B. s – Bacillus subtilis S.a – Staphylococcus aureus P.v- Proteus vulgaris

The zone of inhibition confirmed the potentiality of the nanoparticles against gram positive and gram negative bacterias [17]. The results confirmed its potentiality against the gram-positive bacteria like *Bacillus subtilis*, *Staphylococeus aureaus* and gram-negative bacteria like *Escherichia coli* and *Proteus vulgaris*.

5. Conclusion

We have developed a fast, eco-friendly and convenient green synthesis method for the synthesis of nanoparticles from nitrate using abelmoschus esculentus seed extract at ambient temperature. The method of synthesis of silver nanoparticle from abelmoschus esculentus seed extract by a novel method and by green synthesis route. Phytochemical analysis confirms presence of alkaloid in the extract. SEM, UV, IR, XRD studies confirmed the formation and spherical shape of the silver nanoparticles. particle size analyser done for the average size distribution of NPs. polydispersed silver nanoparticle were obtained. The anti-bacterial activity biological synthesized silver nanoparticles as evaluated and active against the gram-positive bacteria like *Bacillus subtilis*, *Staphylococcus aureus* and gram-negative bacteria like *Escherichia coli* and *Proteus vulgaris*.

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