



Usage of Moringa Oleifera Seed Extract in the Synthesis of Silver Nanoparticles and its Antibacterial Activity

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Abstract Researchers interested in exploring the green synthesis of AgNPs involving plant extract as it is eco-friendly. In the present work, we tried to get extract from seeds of a moringa oleifera seeds. Formation of AgNPs was confirmed by UV-Visible spectroscopic absorption and Infra-red absorption spectrometry. The morphology of AgNPs was confirmed by SEM. XRD analytic studies revealed the crystalline nature. The formation of AgNPs using moringa oleifera seed extract was unique work reported so far. Atomic silver was reduced with aqueous solution of moringa oleifera seed extract. The size of silver nanoparticles formed was analysed by Particle size analyser. Phytochemical test analysis of the moringa oleifera seed extract was done for the presence of natural products which imparts unique properties to the extract synthesised. 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.

Key words: Green synthesis, Silver nanoparticles, moringa oleifera seed extract extract, characterization techniques, Antibacterial activity

Introduction

Nanoscience deals with synthesis, characterization and exploration of nanostructured materials. Green Chemistry deals with the approach of synthesis of AgNPs using less hazardous solvents and chemicals.

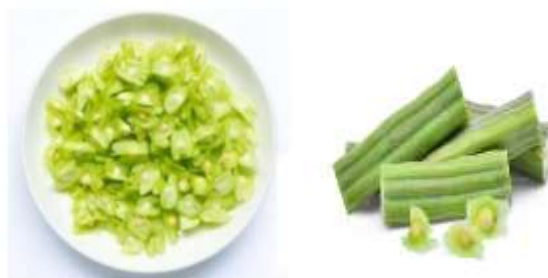


Figure 1: Picture of moringa oleifera seeds

Biologically –mediated synthesis of nanoparticle was environmentally safe. Much attention given to the high yield production of AgNPs of defined size using including various biological system including bacteria, fungi, plant extracts. The application in AgNPs in various biological and biomedical applications, such as antibacterial,



antifungal, antiviral anti-inflammatory anti- Cancer and antibacterial activity were of more significant nowadays. AgNPs seem to be the agents to overcome the ability to resistance against antibiotics [1,2,3].

Scope of the Work

Nanotechnology has the potential of improving the environmental condition through direct application of nanomaterials for detecting , preventing and removing pollutants. By this way, nanotechnology useful to conserve the safety of the environment. Nanoparticles exhibit higher reactivity due to their small size and high surface. The possibility of accumulation nanoparticles in the environment makes easy absorption and causes damage to various organs of the body. Positive Effect of Green Synthesis has various dimensions.including cost effective and alternation to chemical method. It also reduces cost of micro- organism isolation and their culture media. The disadvantage of green synthesis time consuming and difficult to control over size, shape and crystal. nanoparticles are not mono dispersed.

Materials and Methods

Silver nanoparticles were synthesized according to the chemical reduction method by using moringa oleifera 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 [4,5].

Experimental Section



Figure 2: Picture of silver nitrate, moringa oleifera seed extract and AgNPs

Synthesis of Silver Nanoparticles

Silver nano particles were synthesized according to the chemical reduction by using ground drumstick seed. This method can easily be performed in any chemical laboratory and economical, thus a cheaper methods of synthesizing silver nanoparticles.

Preparation of Moringa oleifera Seeds Extract

The extract was prepared by taking 10 grams of drumstick seed. The seeds were washed several times with distilled water. The drumstick seed were boiled in 100ml distilled water taken in a 250ml borosil beaker for 15 minutes. The boiled in a clean container. The extract is used as the reducing and stabilizing agent for the preparation of silver nanoparticles.

Preparation of Silver nitrate solution

Accurately weighing 1.6grams of silver nitrate (AgNO_3) was obtained for our chemical laboratory. It is made up to 100ml standard measuring flask using distilled water to get 0.01N AgNO_3 (1.7000g in 100ml)



Silver nanoparticles synthesis

0.01N aqueous solution of silver nitrate was prepared and used for the synthesis of silver Nano particles. The equal proportions 10ml of 0.01N silver nitrate solution is mixed with 10ml of drumstick seed extract after the 10min observations the mixture turned into reddish brown colour which confirms the presence of silver Nano particles.

Result and Discussion

UV Spectrum of AgNPs synthesised from moringa oleifera seed extract.

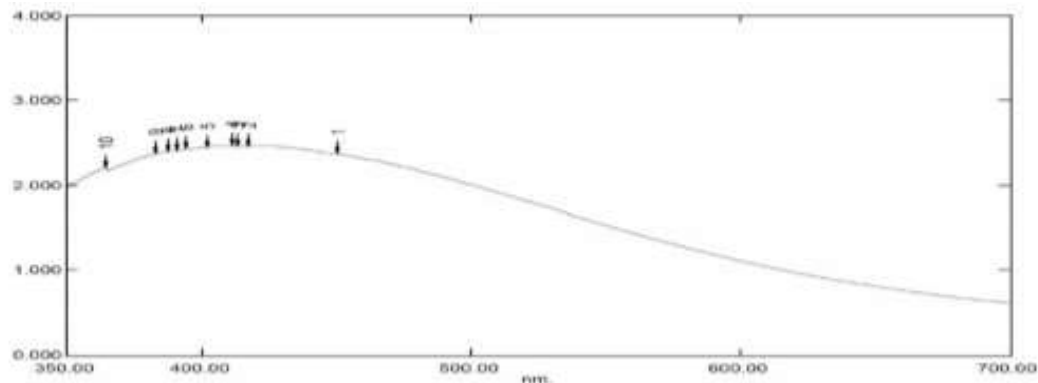


Figure 3: UV Spectrum of AgNPs synthesised from moringa oleifera seed extract

Reduction of silver ions into silver nanoparticle was observed as a result of the reaction accompanied by colour change. The colour change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The broad bands of silver nanoparticles were observed around 417 nm using moringa oleifera seed extract. From our studies, we found the SPR peak at 417 nm [6]. It was confirmed that moringa oleifera seeds extract has more potential to reduce silver into silver nanoparticles. The intensity of absorption peak increases with increasing time period. This characteristic colour variation is due to the excitation of the SPR in the metal nanoparticles. The reduction of the metal ions occurs rapidly and more than 90% of reduction of Ag^+ ions is completed at almost 24hrs.

Phytochemical Constituents of Drumstick Seed Extract

Phytochemical analysis preliminary phytochemical analysis for drumstick seed extract was done using standard test procedures to confirm the availability of active phytochemicals in the aqueous seed extract. The healthful properties of edible plants are perhaps due to the presence of a variety of photo constituents such as polysaccharides, flavonoids, glycosides, saponins, tannins etc. the preliminary screening tests are useful in the detection of these bioactive constituents. The results phytochemical tests were reported as below.

Table 1: Phytochemical constituents of moringa oleifera seed extract

S. No.	Phytochemical constituents	Name of the test	Colour/ observance	Drumstick seed extract
1.	Flavonoids	Lead acetate	No change	Absent
2.	Carbohydrates	Molisch's	No Change	Absent
3.	Alkaloids	Mayer's	Yellow precipitate	Present
4.	Tannins	Tannins	Brown	Present
5.	Triterpenoids	Liberman-Burchard's	No change	Absent
6.	Saponins	Saponins	No change	Absent

Scanning Electron Microscopy

SEM was used the view morphology and size of the silver nanoparticles.



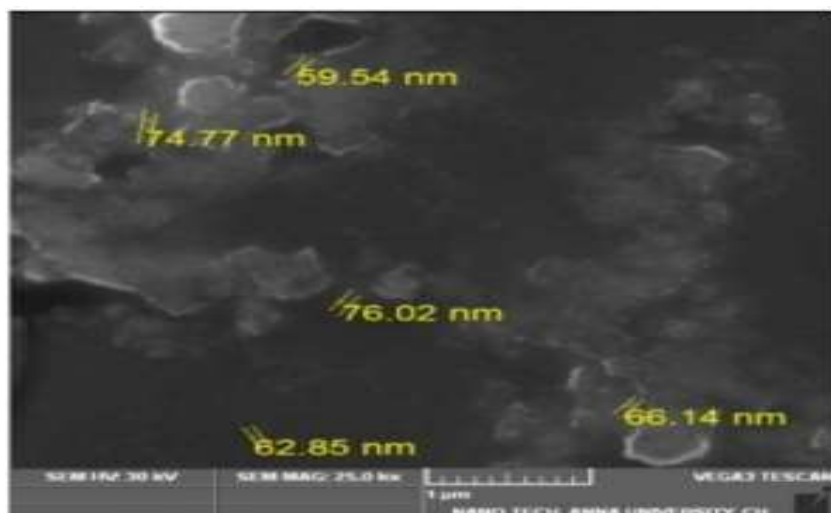


Figure 4: Scanning Electron Microscopy of AgNPs formed from moringa oleifera seed extract

SEM image show the silver nanoparticles synthesized by drumstick extract are spherical in shape varying from 59.54nm to 76. 02nm. Agglomeration increases the particle size of the AgNPs formed. The AgNPs were not uniformly distributed in the picture. This shows that the AgNPs formed were 10nm in size as confirmed form particle size analyser. Later, Agglomeration leads to the formation of AgNPs of varying size [7].

X-ray Diffraction

Analysis through x-ray diffraction was carried out to confirm the crystalline nature of silver nanoparticles shown fig 5. The XRD pattern showed numbers of bragg reflection that may be indexed on the basis of the face- centred cubic structure of silver.

A comparison of our XRD spectrum with standard confirmed that the silver nanoparticles formed in our experiments were in the form of nanocrystals, as evidence by the peaks at 2θ values 31° , 38° , 40° , 45° , 65° , 80° , 83° , and 89° corresponding to Bragg reflections, respectively, which may be indexed based on the face- centred cubic structure of silver [8,9].

x-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag^+ ions by the drumstick seed extract are crystalline in nature. The unassigned peaks thought to be related to amorphous organic phases. Peaks obtained show disturbances at various points and scattered. It may be due to agglomeration of silver nanoparticle since we don't used any capping agent .

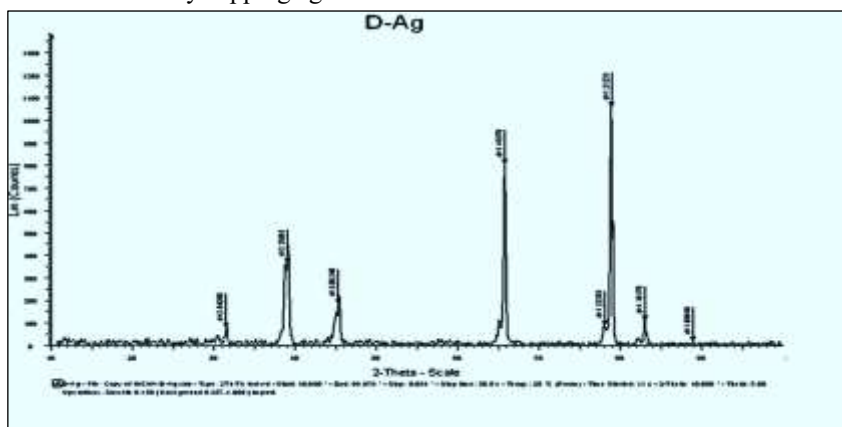


Figure 5: XRD Images of silver nanoparticle from moringa oleifera seed extract



Partical Size Analysis

Partical size analysis confirmed the formation of AgNPs of 10nm size. it also confirmed the uniform size distribution of AgNPs of 10nm as 100%. This method of green synthesis produces a uniform size distribution

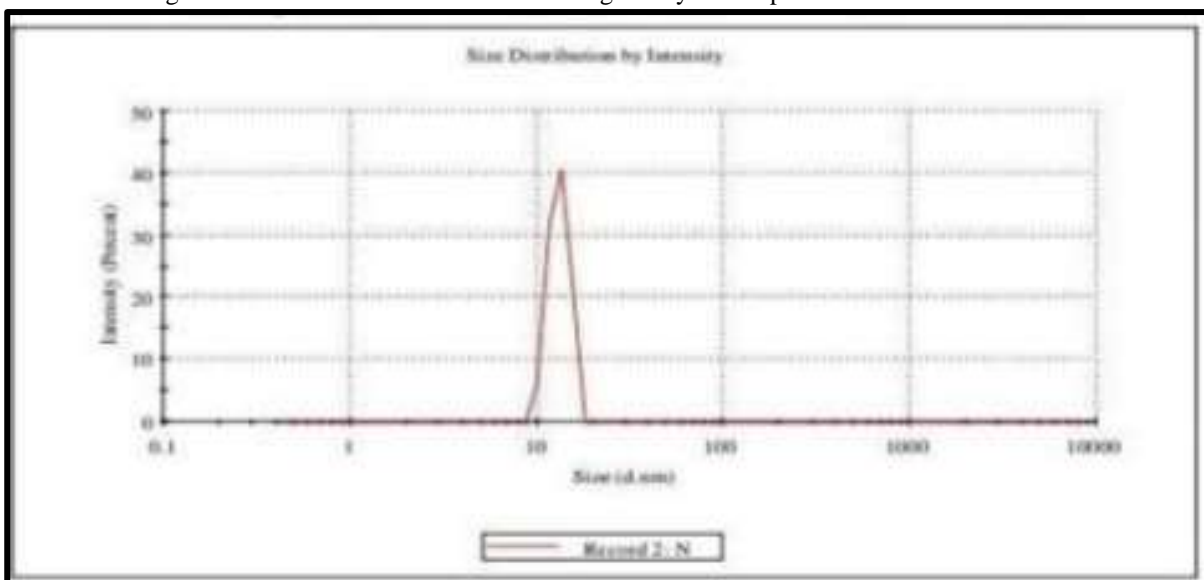


Figure 6: Particle size analyser of AgNps synthesised from moringa oleifera seed extract

Particle size analysis confirmed the formation of AgNPs of 10nm in size. It also confirmed the uniform distribution of AgNPs of 10nm as 100%. This method of green synthesis produces a uniform size distribution [10].

Antibacterial studies

Well diffusion assay was used to determine the antibacterial effect. The inhibition diameter was measured and units are mm [6,7,8]. The potentiality of the silver nanoparticle against two-gram positive bacteria and two-gram negative bacteria was studied.

Figure 7: Antibacterial studies of silver nanoparticle. Antibacterial studies picture of the following bacteria. gram positive - a. *Bacillus subtilis* and b. *Staphylococcus aureus* .gram negative- c. *Escherichia coli* and d. *Proteus vulgaris* [11].

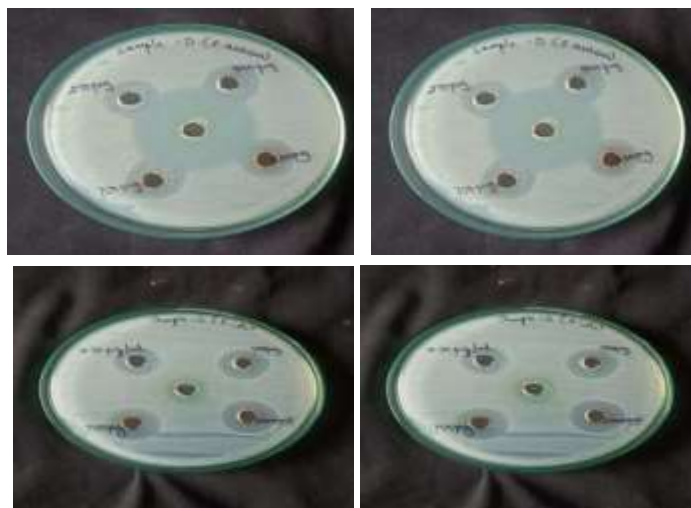


Figure 7: Antibacterial activity results of AgNPs formed from moringa oleifera seed extract



Table 2: Antibacterial activity results of AgNPs formed from moringa oleifera seed extract

Name of the Sample	Concentration of the sample ($\mu\text{g/ml}$)	Zone of inhibition (mm)			
		<i>B.s</i>	<i>S.a</i>	<i>E.coli</i>	<i>P.v</i>
D	250	14	13	14	13
	500	16	15	17	15
	750	18	18	18	18
	1000	20	20	20	20
	Control	16	16	16	16

The Green synthesized AgNPs obtained by their method is effective against the gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Pseudomonas aeruginosa*). As the concentration of AgNPs increases the zone of inhibition increases. This shows that it is effective against the bacterial activity [12,13].

Conclusion

In the present study, we have demonstrated that use of a natural, low-cost biological reducing agent Moringa oleifera seeds extract can produce metal nanostructures, through efficient green chemistry synthesis, avoiding the present of toxic solvents and wastes. The biosynthesized nanoparticles using Moringa oiliefera seeds extract proved to be excellent against pathogens. The silver nanoparticles synthesised from moringa oleifera seed extract showed higher activity. The present study shows as a simple, rapid, and economical route to synthesize silver nanoparticles.

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