



Biosynthesis And Characterization of Silver Nanoparticles Using Mint Leaf Extract And Its Antimicrobial And Antioxidant Activities

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Abstract: Nanotechnology is widely used in the field of research in modern material science. Green synthesis of nanomaterials is mostly emerging method of synthesis eventhough there are many chemical methods for synthesizing the products. In the present work, biosynthesis of silver nanoparticles using mint leaf extract is presented. This study also evaluates the antimicrobial and antioxidant properties of silver nanoparticles from mint leaf extract. The prepared nanoparticle has been characterized by Fourier transform infrared spectrophotometer, Scanning electron microscopy, Powder X-ray diffraction analysis and Ultraviolet visible spectrophotometer. The synthesized silver nanoparticles showed highest antimicrobial activity against Salmonella typhi and Klebsiella pneumonia, Aspergillus niger and highest antioxidant activity using 1,1-diphenyl-2-picryl hydrazyl (DPPH) assay.

Key words: Nanoparticles, Antimicrobial, Antioxidant

Introduction

Nanotechnology is the manipulation of matter on an atomic, molecular and supra molecular scale. Nanotechnology may be able to create many new materials and devices with a vast range of applications such as in medicine, electronics, biomaterials, energy production and consumer products [1]. Suspension of nanoparticles are possible since the interaction of the particle surface with the solvent is strong enough to overcome density differences. In nanotechnology, a particle is defined as a small object that behaves as a whole unit with respect to its transport and properties.

Nanotechnology refers to the projected ability to construct items using techniques and tools to make complete, high performance products. Nowadays there is a wide application of nanoparticles in diverse fields including catalysis, energy, chemistry and medicine [2]. Nanotechnology is taken as the scale range in nanometer. The term nanoparticle is used to describe a particle with size in the range of one to hundred nanometer. Synthesis and characterization of nanoparticles are important because of their role in basic research and technological applications [3-10]. In the present work, silver nanoparticles were synthesized by mixing the plant leaf extract with silver nitrate solution and incubated at room temperature under dark condition.

Results and Discussion

Initially the experiment was carried out under the following conditions.

5 grams of washed mint leaves were grounded with 50 ml of distilled water and filtered with Whatman filter paper no:1 and the filtrate was collected. Added 10 ml of mint leaf extract (2g/10ml) to 90 ml of aqueous

solution of 1mM silver nitrate for reduction into Ag⁺ ions. The solution was kept for incubation period of 16 hours at room temperature in dark conditions. The reaction was observed by monitoring the colour change in the solution. After the incubation period the silver nanoparticle solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 minutes followed by re-dispersion of the pellet in de-ionized water. The synthesized silver nanoparticles were characterized by UV-Spectral Analysis, FTIR Analysis, SEM Analysis, X-Ray diffraction Analysis. Pure culture of *Salmonella typhi* and *Klebsiella pneumonia* species of bacteria and *Aspergillus niger* of fungi were used for the anti microbial analysis. The anti-oxidant analysis was carried out by DPPH method.

UV-Vis spectroscopic analysis

UV-Vis spectroscopy measurements were carried out at room temperature, operated at a resolution of 1nm AND SHOWN IN Fig1 .The colour of the solutions changed from pale yellow to yellowish brown indicating AgNPs formation. It is well known that the AgNPs exhibit yellowish brown colour [11] in aqueous solution due to excitation of surface Plasmon vibrations in AgNPs. [12]The broadening of peak indicated that the particles are polydispersed. The spectra of AgNPs showed maximum absorption at 443nm to the surface Plasmon resonance (SPR) of the formed AgNPs. The results obtained from bio reduction of AgNPs using *spiruline platensis*, showed that a SPR silver band occurred at 400-480nm.

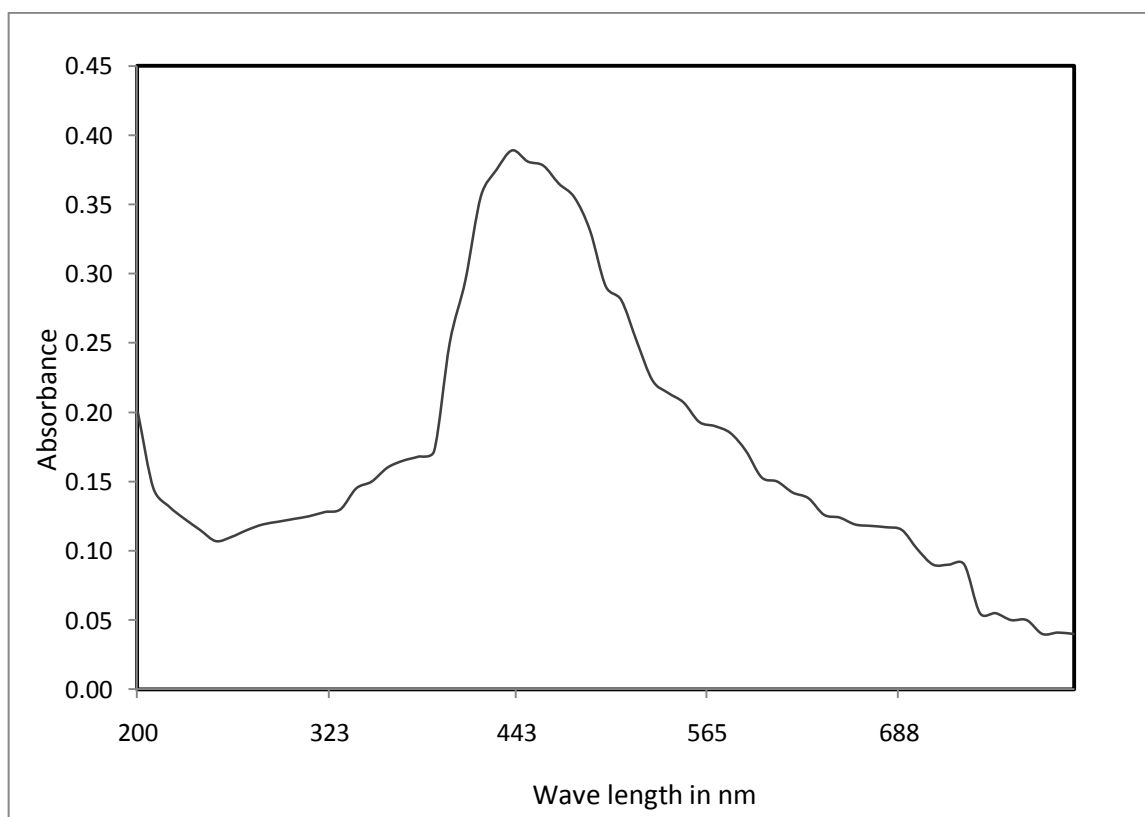


Fig 1: UV-Vis spectrum of AgNPs.

FTIR spectroscopic Analysis

FTIR spectroscopic analysis was used for the characterization of the extract and the resulting nanoparticles[13].

Absorbance bands were observed at 3383.5, 2357.1, 2331.0, 1640.1 cm^{-1} . The peak at 1640.1 cm^{-1} indicates amide group arising due to carbonyl stretching in proteins. The peak at 3383.5 cm^{-1} corresponds to -OH, due to hydrogen bonded phenols and alcohols in AgNPs. The peaks at 2357.1 and 2331.0 cm^{-1} corresponds to $-\text{C}\equiv\text{N}$ group. The -CH bending of alkenes can be seen at the peak at 1082.6 cm^{-1} . The peak at 1495.8 cm^{-1} of the FTIR spectrum shows $-\text{C}=\text{C}$ in an aromatic ring.

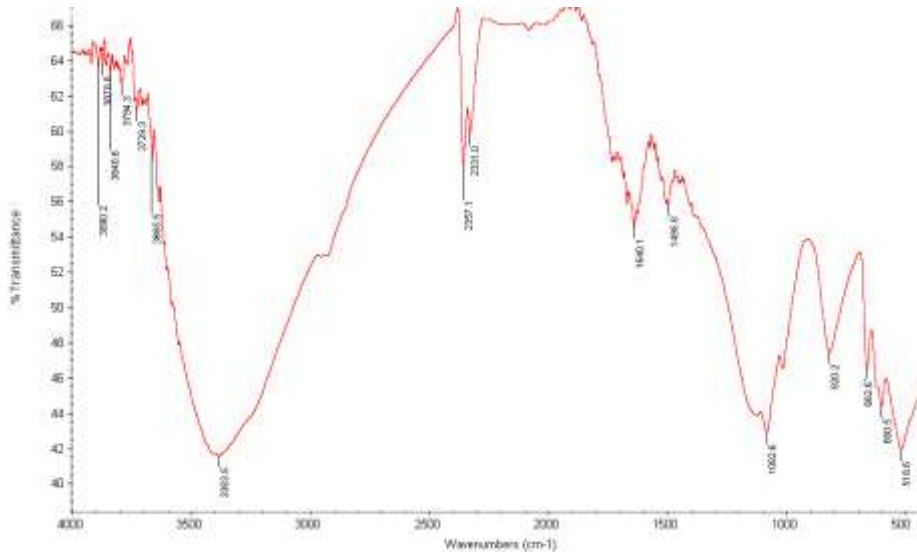


Fig 2: FTIR spectrum of Ag NPs

SEM Analysis

The formation of AgNPs as well as their morphological dimensions in the SEM study demonstrated that the size of the nanoparticles ranges from 22.87nm to 42.11nm. The average particles size of the individual silver nanoparticle using SEM analysis is estimated to be 29.15nm. It is observed that most of the silver nanoparticles were spherical and irregular in shape [14].

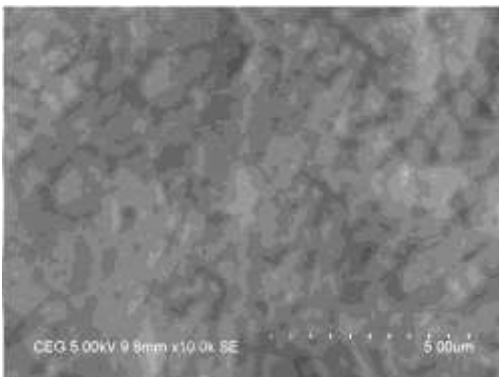


Fig 3: SEM image of AgNPs

XRD Analysis

The characterization of silver nanoparticles is done by powder x-ray diffraction analysis. From the XRD analysis, it is clear that the silver nanoparticles formed using mint leaf extract is purely crystalline. The average crystalline size calculated is found to be 28nm.

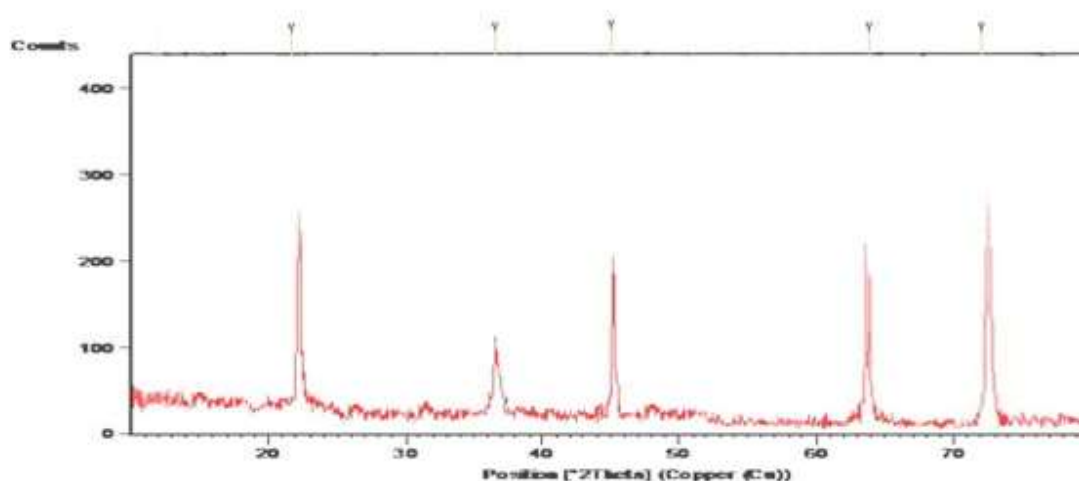


Fig 4: XRD spectrum of Ag NPs

Anti- Oxidant Analysis

DPPH method is faster and it can be helpful in the investigation of anti-oxidants for a rapid estimation and preliminary information of radical scavenging abilities [15]. The method is sensitive and requires small sample amounts [16]. Here, DPPH method is used to check the anti-oxidant property of the plant extract. DPPH has been widely used in the determination of the anti-oxidant activity of single compounds as well as different plant extracts [17]. The anti-oxidant activity was found to be better for the higher concentration of the leaf extract.

Table 1: Results of Anti-oxidant activity by DPPH method

S.No	SAMPLES	DPPH activity(%)	
1	Sample	5mg	83.15
		3mg	60.34
		1mg	48.51
2	Standard -BHT	99.9	

Anti – Microbial Analysis

Anti – microbial activity of silver nanoparticles against salmonella typhi and klebsiella pneumonia were investigated. The anti-bacterial activity is demonstrated by disc diffusion method against the two species of bacteria. The anti-bacterial effect of AgNPs synthesized by green route showed better results. Ag-NPs may attach to the surface of the bacterial cell membrane via interacting with sulfur containing proteins [18]. It is also possible that Ag NPs not only interact with the surface of membrane, but can also penetrate inside the bacteria [19]. The anti – fungal activity of Ag NPs depends on the types of fungal along with size of Ag NPs and are loosely associated with the formation of pits in the cell wall of micro organism. The biologically synthesized AgNPs prepared by reduction method showed anti-fungal activity against *Aspergillus niger*. Silver nanoparticles affect fungus cells by attacking their membranes [20], thus disrupting the membrane potential.



Fig 5: Antibacterial activity against *Salmonella typhi*



Fig 6: Antibacterial activity against *Klebsiella pneumonia*



Fig 7: Anti-fungal activity against *Aspergillus niger*

Table 2: Result of anti-microbial activity

S.No	Microorganism	Treatment – Zone of Inhibition (inmm)			
		Negative Control DMSO	Positive control – Streptomycin	Plant extract	Silver nanoparticles solution
1	<i>Salmonella typhi</i>	0	3	4	6
2	<i>Klepsiella pneumonia</i>	0	2	4	6
3	<i>Aspergillus niger</i>	0	3	6	9

Conclusion

The present work confirms the excellent capability of synthesizing the silver nanoparticles using mint leaf. This work proved the ability of using the bio material by the synthesis of silver nanoparticle with the principle of green chemistry. The synthesized nanoparticle shows effective antimicrobial and antioxidant activity. Here, the biological reducing agents used are the plant extract. The silver particles were spherical in shape.

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