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GREEN SYNTHESIS AND CHARACTERIZATION OF GOLD NANO PARTICLES USING *JUSTICIAADHATODA* AND EVALUATION OF ITS ANTIMICROBIAL ACTIVITY

Microbiology

B.Josephin Sheeba Assistant Professor,Udaya college of Arts&Science,Vellamodi.

T.Selva Mohan* AssistantProfessor,Rani Anna College,Tirunelveli. *Corresponding Author

ABSTRACT

Nanotechnology is one of the most active research areas in modern material science. Nanotechnology can be termed as the synthesis, characterization, exploration and application of nanosized (1-100nm) particles for the development of science.Green synthesis of nanoparticles is an eco-friendly approach which might pave the way for researchers across the globe to explore the potential of different herbs in order to synthesize nanoparticles.Present investigation conducts a study on the synthesis and characterization of gold nanoparticles using *Justiciaadhatoda.*Antibacterial efficacy of the gold nanoparticles against 4 different bacteria, *E.coli, Stapylococcus sp., Klebsiella sp., Pseudomonas sp.*has been performed.The UV visible spectroscopy of the synthesized nano particles were in the range of 400-800 nm. The plant extract showed to synthesize the gold nanoparticles by the indication of suitable surface Plasmon resonance (SPR)with high band intensities and peaks under visible spectrum. A strong resonance at 540 nm is clearly seen in The UV visible spectroscopy of the synthesized nano particles were in the range of 400-800 nm. The plant extract showed to synthesize the gold nanoparticles by the indication of suitable surface Plasmon resonance (SPR)with high band intensities and peaks under visible spectrum. A strong resonance at 540 nm is clearly seen in *Justiciaadhatoda*and arises due to the excitation of surface plasmon vibrations in the gold nano particles.The average size of the nanoparticles is ~37 nm for AuNPs using *Justiciaadhatoda*with spherical and cubic shape. Among the four pathogens *Klebsiella sp. andStapylococcus sp.* Showed the maximum activity.

KEYWORDS

Nanoparticles,*Justiciaadhatoda,*Antibacterial efficacy,*Klebsiella sp.,Staphylococcus sp.*

INTRODUCTION

Nanotechnology is one of the most active research areas in modern material science. Nanotechnology can be termed as the synthesis, characterization, exploration and application of nanosized (1-100nm) particles for the development of science. It deals with the materials whose structures exhibit significantly novel and improved physical, chemical, and biological properties and functionality due to their nano scaled size (Feynman,1991). Because of their size, nanoparticles have a larger surface area than macro-sized materials. Metallic nanoparticles are presently applied in different fields such as electronics, biotechnology, chemical and biological sensors, DNA labeling, drug delivery, cosmetics, coatings and packaging (Kohler *et al.,* 2001). Gold in nanoscale display novel properties and have diverse activities that make it appropriate for therapeutic use and broad applications in nanobiotechnology (Kim, *et al,* 2004, Sperling*et al.,* 2008). AuNPs have been found to be of immense use in the field of biological sciences owing to its physical, chemical and optical properties (Levy, 2010). Due to its non-toxic nature, it is used as catalyst (Grieser&Ashokkumar, 2003), cell markers, drug delivery etc. Gold nanoparticles are also used in the diagnosis of cancer cells (Chandra *et al.,* 2011).

Due to their extensive use the synthesis and characterization of nanomaterials has become a needful task. Although the physical and chemical methods produces a pure, well defined particles, these methods are not cost effective, not ecofriendly (Sastry*et al.,* 2004). Green synthesis of nanoparticles is an eco-friendly approach which might pave the way for researchers across the globe to explore the potential of different herbs in order to synthesize nanoparticles (Savithramma, 2011). Bio-inspired methods are advantageous compared to other synthetic methods as; they are economical and restrict the use of toxic chemicals as well as high pressure, energy and temperatures (Parashar, 2009). Nanoparticles may be synthesized either intracellularly or extracellularly employing yeast, fungi bacteria or plant materials which have been found to have diverse applications (Bhattacharya and Rajinder, 2005; Mohanpuria, 2008).

The use of phytochemicals in the synthesis of nanoparticles is an important symbiosis between nanotechnology and green Chemistry (Huang, 2007**).** In recent years, green approaches for the generation of AuNPs are on the rise and their antimicrobial properties have also been evaluated. Gold NPs may become useful in the development of antibacterial strategies because of their nontoxicity, versatility in surface modification, polyvalent effects and photothermal effects.

The present study focuses on a plant namely *Justiciaadhatoda*for the synthesis of gold nanoparticles by the reduction of aqueous $AuCl_4^-$ ions.The plant *Justiciaadhatoda*plant is used in indigenous medicine for jaundice, inflammation, rheumatism, anaemia and helminthic infestations.Some findings of support the applicability of *Justiciaadhatoda*in traditional system for its claimed uses and can be recommended by the scientific community as an accessible alternative to synthetic antibiotics (Jeeshna, 2009). The juice of fresh leaves is given to children to expel intestinal worms (Prusti, and Behera, 2007). *Justiciaadhatoda*leaves are said to have some action against tubercular and other bacteria, and in typhoid and haemorrhages. With nanotechnology emerging as a forefront area in integrated science, the use of Nanoparticle-based therapeutics for controlling pathogenic bacteria has emerged as an important alternative. Present investigation conducts a study on the synthesis and characterization of gold nanoparticles using *Justiciaadhatoda.* Antibacterial efficacy of the gold nanoparticles against 4 different bacteria, *E.coli, Stapylococcus sp., Klebsiella sp., Pseudomonas sp.*has been performed.

MATERIALS AND METHODS

Reagents and chemicals

Tetrachloroauric acid ($HAuCl_4 \cdot XH_2O$) was obtained from Sigma Aldrich. Aquaregia is used for the cleaning of glasswares. Freshly prepared triple distilled water was used throughout the experimental work. All experiments were conducted under laminar hood with strict aspectic conditions.

Collection of extracts

The leaves were washed and cleaned and shade dried. Then it was powdered and dispensed in 10 ml of sterile distilled water and boiled for 10 minutes at 70-80°C. Then the *Justiciaadhatoda leaf* extract were filtered and centrifuge for 10 minutes at 5000 rpm. Now extracts were collected in separate conical flasks by standard sterilized filtration method and were stored at 4°C.

Biosynthesis of gold nanoparticles

1mM Aqueouschloroauric acid (HAuCl4) solution was added to the extract with ratio of 2:1. Then it was placed in a magnetic stirrer .Within 30min colour changes from dark yellow to wine red indicating the presence of gold nanoparticles.

UV-VIS spectra analysis

The reduction of pure Au^{3+} ions to Au^0 was monitored by measuring the UV-Vis spectrum by sampling of aliquots (0.3 ml) of AuNPs solution diluting the sample in 3 ml distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer Systronics 118 at the range of 300-600 nm and observed the absorption peaks at 530-550 nm regions due to the excitation of surface plasmon vibrations in the AuNPs solution, which are identical to the characteristics UV-visible spectrum of metallic gold and it was recorded.

SEM analysis of gold nano particles

Scanning Electron Microscopic (SEM) analysis was done using Hitachi S-4500 SEM machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Antimicrobial activity

Antimicrobial activity was studied by the agar well diffusion method. Nutrient agar plates were prepared and plates were swabbed with different pathogens such as *E.coli, Staphylococcus sp., Klebsiellasp.and Pseudomonas sp.*With the help of well cutter, 5 holes were made equidistant from one another. The wells were then incubated for 37°C for 24-48 hrs.

RESULTS AND DISCUSSION

Biosynthesis of gold nano particles

Figure1. Gold nanoparticles synthesized using *Justiciaadhatoda*

The plant extract was used to produce gold nanoparticles.The reduction of gold ions into gold particles during exposure to the plant extract is followed by colour change from yellow to different color depends on the plant extract.As the plant extract was mixed in the aqueous solution of the gold ion complex,it started to change the color from yellowish to different colors due to reduction of gold ion,indicating the formation of gold nanoparticle.

UV-VIS spectra analysis of gold nano particles.

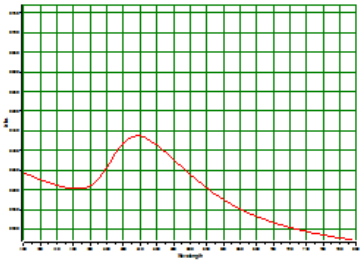

figure 2. UV-vis absorption spectra of AuNPs synthesized using *Justiciaadhatoda*

The UV visible spectroscopy of the synthesized nano particles were in the range of 400-800 nm. The plant extract showed to synthesize the gold nanoparticles by the indication of suitable surface Plasmon resonance (SPR)with high band intensities and peaks under visible spectrum. A strong resonance at 540 nm is clearly seen in *Justiciaadhatoda*and arises due to the excitation of surface plasmon vibrations in the gold nano particles.

Scanning electron microscopy of gold nano particles

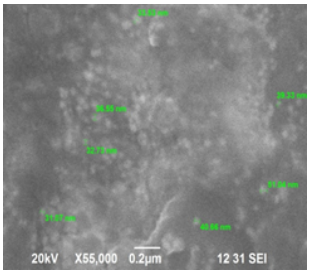

Figure 3.SEM photograph of AuNPs synthesized using *Justiciaadhatoda*

The SEM photograph of gold nanoparticles is shown in figure 5. SEM photograph of gold nanoparticles clearly indicates that in the room temperature synthesized samples, the average size of the nanoparticles is ~37 nm for AuNPs using *Justiciaadhatoda*with spherical and cubic shape.

1.Antibacterial activity of Gold Nanoparticles from *J. adhatoda* leaf

Bacterial strains	Zone of inhibition (diameter in mm)			
	25µl	50µl	75 µl	100 µl
Escherichia coli	-	11.00.08	12.50.09	13.40.14
Klebsiellasp	-	11.40.12	13.50.08	15.50.14
Staphylococcus sp	-	12.4	14.70.08	16.90.04
Pseudomonas sp	-	10.20.14	11.20.08	11.20.16

CONCLUSION

Gold nano particles with mean sizes of 20-45 nm were successfully synthesized using *Justiciaadhatoda*extract. The present study represents a clean, non-toxic as well as eco-friendly procedure for synthesizing AuNPs. This technique gives us a simple and efficient way for the synthesis of nanoparticles with tunable optical properties governed by particle size. AuNP functionalized with ampicillin (AuNP-AMP) were bactericidal. With the ever increasing resistant strains of microorganisms to the already available and synthesized antibiotics, this could be a potential alternative. Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially stimulating for the large-scale synthesis.

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