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Green Synthesis of Silver Nanoparticles from *Dracaena mahatma* Leaf Extract and its Antimicrobial Activity

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Abstract

Objective-In recent years, nanotechnology has evolved from a multidisciplinary research concept to primary scientific field. Nanotechnology is mainly concerned with the synthesis of nanomaterials using different systems and their applications. Green synthesis is the safe and easiest method of producing silver nanoparticles. Because of the production of the silver ions, silver nanoparticles are found to have the antibacterial activity.

Method-This paper describes a rapid and eco-friendly method for green synthesis of silver nanoparticles from aqueous solution of silver nitrate using Dracena Mahatma Leaf extract.

Result-Nanoparticles show an absorption maximum at 421nm. The particle size of silver nanoparticles and polydispersity index was calculated. The silver nanoparticles were found to be facing cubic centred and moderately stable. The silver nanoparticles were tested for antimicrobial activity using *Vibrio ponticus*.

Conclusion-This study suggests that successful synthesis of silver nanoparticles is possible using the ornamental plant *Dracaena mahatma*

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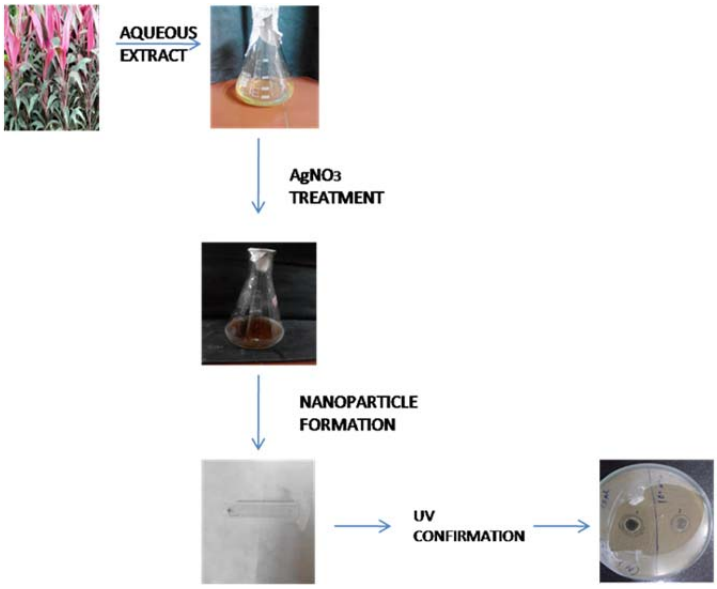

Keywords: *Dracaena mahatama,* silver nanoparticles, antimicrobial action.

1. INTRODUCTION

Nanotechnology is a rapidly growing science of producing and utilizing nano-sized particles. It can be defined as the science and engineering involved in the design, synthesis, characterization and application of materials and devices whose smallest functional organization in at least one dimension is on the nanometer scale [1]. Silver nanoparticles i.e. silver particles are of different sizes varying between 1 nm and 100 nm in size. Several methods have been devised in order to prepare metallic nanoparticles such as physical, chemical, photochemical and biological synthesis. Many of the nanoparticle synthesis have various disadvantages which involves the use of hazardous and harmful toxic chemicals, low rate of material conversions and high energy consumption. Thus, in order to overcome these limitations there is a growing demand for development of safe and environmentally friendly process for nanoparticle synthesis. Biological methods or green synthesis methods which uses either microorganisms like bacteria, fungi, etc or plant extracts have emerged as a simple and viable alternative to chemical synthetic and physical methods. The application of plant extract to the biosynthesis reaction is an important branch of biosynthesis of nanoparticles .The use of plants for the synthesis of nanoparticles has gained attention, because of its rapid, economical, eco-friendly procedure, and the biosynthesis process can be carried out by a single step technique. Many research papers reported the synthesis

of silver nanoparticles using plant extracts such pine, persimmon, ginkgo, magnolia, and platanus leaves [2]; *Acalypha indica* leaf [3]; *Chenopodium album* leaf [4]; *Murraya koenigii* (curry) leaf [5];*Ocimum sanctum* (Tulsi) leaf [6]; *Garcinia mangostana* (mangosteen) leaf [7]; *Stevia rebaudiana* leaves [8]; *Nicotiana tobaccum* leaf [9]; *Arbutus unedo* leaf [10] and *Olea europaea* leaves [11].

In this study, we have synthesized silver nanoparticles for the reduction of Ag^+ ions to Ag^0 nanoparticles from silver nitrate solution using *Dracaena mahatma* leaf extract at ambient temperature. *Dracaena mahatma* is an ornamental plant. *Dracaena* remains colourful in semi shade and in bright light. Dense shade results in dull colour and direct sun causes scorching of leaves, particularly in dry heat .In moderate temperatures, humidity, moist and porous compost, the leaves remain colourful and attractive for a longer period. Plants grow well in pots as well as ground. [12]. The mode of propagation of this plant is by cutting.

Human beings are susceptible to infection by microorganisms such as bacteria. Due to MDR strains and continuing emphasis on various cost effective efforts have been taken as a solution to these problems. Silver nanoparticles can be used for silver-based antiseptics that have usually a broad-spectrum activity and induce low microbial resistance as compared to other antibiotics. [13]. the important enzymes are associated with the bacterial plasma and cytoplasmic membrane and DNA and are the important target site of silver ions. Silver ions cause the release of K+ ions from bacteria cell and results in strong bacteriostatic and bactericidal effects [14]. Silver ions were deposited into the vacuole and cell walls as granules after the inhibition .They inhibited cell division and damaged the cell envelope and cellular contents of the bacteria. Free-radical is involved in the antibacterial activity of silver nanoparticles (Ag-NPs) [15], but the underlying mechanism and characteristics remain unclear. The interaction between reactive oxygen species (ROS) and bacterial cell death was revealed in previous study [16]. Reactive oxygen species affect the bacterial DNA and mitochondria. There are different modes of action of silver nanoparticles [21].Further, silver nanoparticles are found to be highly effective against *Vibrio ponticus* bacteria.

2. MATERIALS AND METHODOLOGY

2.1. Preparation of *dracaena mahatma* leaf extract

Dracaena Mahatma Leaves (Figure 1) were collected from D. Mahatma plant at nursery, VIT University, Vellore. The leaves were washed several times with distilled water to remove the dust particles. The leaves were cut into small pieces and 10g were boiled in a 500-ml glass beaker along with 50 ml of sterile distilled water for 20 min as shown in Fig.2 & 3. The extracted was concentrated by evaporation. After boiling, the colour of the aqueous solution changed from watery to yellow colour. The aqueous extract was separated by filtration with Whatman No. 1 filter paper. The leaf extract was stored at room temperature to be used for biosynthesis of silver nanoparticles from silver nitrate.

Fig1: *Dracaena mahatma* coloured leaves

Fig2: washed 10g of *Dracaena mahatma* leaves

fig3 : Leaves after hot water bath treatment

2.2. Synthesis of silver nanoparticles

In a typical reaction procedure, 20 ml of leaf extract was added to 100 ml of 1×10^{-3} M aqueous $AgNO_3$ solution at room temperature as shown in fig.4&5. The yellow coloured mixture of silver nitrate and leaf extract was then kept at 80^o Celsius for 3 hours in hot water bath. After 3 hours a marked colour change was observed when compared to the control silver nitrate solution with no leaf extract, brown suspended mixture was obtained. The colour change confirms the indication for the synthesis of the silver nanoparticles as shown in fig.6.

UV-visible (UV–vis) spectra showed strong surface plasmon resonance (SPR) band at 420 nm and thus indicating the formation of silver nanoparticles. The AgNPs obtained leaf extract were centrifuged at 10,000 rpm for 15 min.

Fig4: 20 ml of leaf extract

fig5: 20ml leaf extract with 100 ml of $AgNO_3$ solution

Fig 6: Conversion process of silver nanoparticle shows appearance of reddish brown colour

2.3. Separation of nanoparticles

The synthesized silver nanoparticles were separated by centrifugation. The solution was centrifuged at 10,000 rpm for 15mins. The process was repeated by dispersion of pellets in water, to obtain coloured supernatant solutions. The sample was then stored at -4° Celsius.

3. CHARACTERIZATION OF SILVER NANOPARTICLES

3.1. UV-visible spectroscopy

The formation and completion of silver nanoparticles was characterized by UV-visible spectroscopy using a Double beam spectrophotometer. The reduction of the Ag+ ions by the supernatant of the test plant extracts in the solutions and formation of silver nanoparticles were characterized by UV visible spectroscopy monitored by sampling the extract (1ml) and measuring the UV –VIS spectrum of solutions. The bioreduction of silver ions in aqueous solution was monitored by UV-VIS spectra of the solution between 360nm to 600nm.

3.2. X-ray diffraction

The crystalline structure of the synthesized silver nanoparticles were investigated through powder x-ray diffraction using an X-ray powder diffractometer using Cu Kα radiation operating between 10º and 80º at the scanning rate of 2º per minute. The silver nanoparticles were distributed over a glass slide and the solvent was evaporated to form the thin film of silver nanoparticles for XRD analysis. The crystalline size was calculated using line broadening profile and Scherrer's formula:

$$\tau = \frac{K\lambda}{\beta \cos\theta}$$

Where:

T is the mean size of the ordered (crystalline) domains, which may be smaller or equal to the grain size, K is a dimensionless shape factor, with a value close to unity. The shape factor has a typical value of about 0.9, but varies with the actual shape of the crystallite, λ is the X-ray wavelength, β is the line broadening at half the maximum intensity (FWHM),after subtracting the instrumental line broadening, in radians. This quantity is also sometimes denoted as $\Delta(2\theta)$,θ is the Bragg angle.[17]

3.3 Zeta potential

The sample was dispersed in deionised water and tween 20 followed by ultrasonication. Then solution was filtered and centrifuged for 15mins at 25° Celsius with 5000rpm and the supernatant was collected. The supernatant was diluted for 4 to 5 times and then the particle distribution in liquid was studied in a computer controlled particle size analyser. [18], the Zeta potential of dispersion is measured by applying an electric field across the dispersion. Particles within the dispersion with a zeta potential will migrate toward the electrode of opposite charge with a velocity proportional to the magnitude of the zeta potential.

4. ANTIMICROBIAL ACTIVITY

4.1 Preparation of Inoculum

Nutrient agar was prepared in a conical flask and it was sterilized. The strain of *Vibrio ponticus* was added to the plates containing nutrient agar. The inoculated bacterial culture was kept for incubation at room temperature.

4.2 Antimicrobial activity assay by diffusion method

The culture plate was taken to study antimicrobial activity of silver Nanoparticles. 2 wells were dug and 10µl of antibiotic gentamicin (1mg/10ml) and silver nanoparticles were added to first and second well respectively. The plates were kept for incubation at 37° Celsius for 24 hours to observe zone of inhibition.

5. RESULT

5.1 UV-Vis spectrum Analysis

UV-Vis spectra recorded during synthesis of nanoparticles shows an absorption maximum at 420nm, which is typically attributed to plasmon resonance of silver Nanoparticles (fig.7). 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. Due to the tiny dimensions, silver nanoparticles have distinctive colour in colloidal solution. Silver nanoparticles exhibit reddish-brown in water. Fig. 6 shows the color changes before (a) and (b) after the process of reduction of Ag+ to Ag nanoparticle. After 3h of the conversion process silver nanoparticle showed reddish-brown colour, suggested the formation of silver nanoparticles in solution. The sharp bands of silver nanoparticles were observed around 421nm in case of *Dracena mahatma*

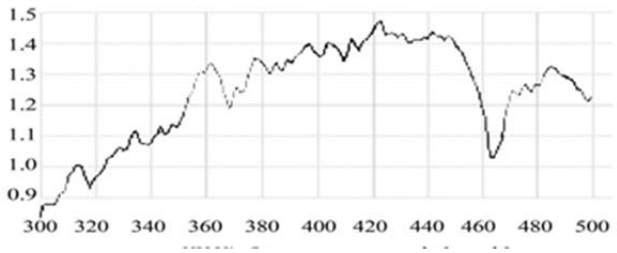

Fig7: graph showing plot of absorbance

5.2 Particle size distribution

The average size of the particles and polydispersity index (PDI) of the synthesized AgNPs were determined by particle size analyzer Horiba, sz100 and the results are shown in figure 8. The particle size of silver nanoparticles was found to be 87.1nm. It shows the average particle diameter, 3969.4nm and Polydispersity index, 3.586

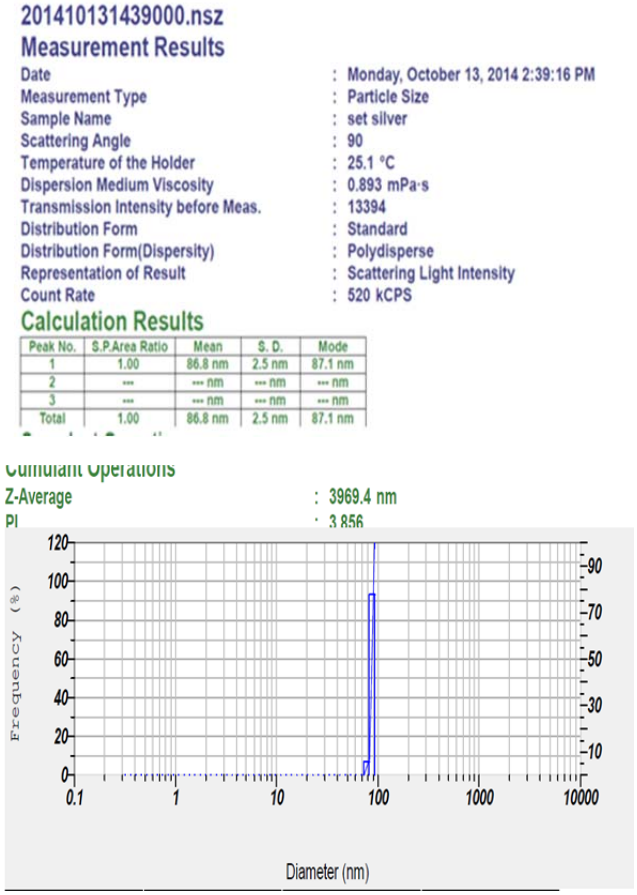

Fig.8 Graphs showing Particle Size distribution of silver nanoparticles.

5.3 XRD Analysis

The analysis of silver nano particle was also done by X-Ray Diffraction method. The silver nanoparticle solution thus obtained was purified by repeated centrifugation at 10000 rpm for 15min followed by redispersion of the pellet of silver nanoparticles into 10ml of deionized water. After centrifugation we kept in the hot air oven for drying of the purified silver particles, the structure and composition were analyzed by XRD. These sharp Bragg peaks might have resulted due to capping agent stabilizing the nanoparticle. Intense Bragg reflections suggest that strong X-ray scattering centres in the crystalline phase and could be due to capping agents. Independent crystallization of the capping agents was ruled out due to the process of centrifugation and redispersion of the pellet in millipore water after nanoparticles formation as a part of purification process. Therefore, XRD results also suggested that the crystallization of the bio-organic phase occurs on the surface of the silver nanoparticles or vice versa. Generally, the broadening of peaks in the XRD patterns of solids is attributed to particle size effects. Broader peaks signify smaller particle size and reflect the effects due to experimental conditions on the nucleation and growth of the crystal nuclei. The XRD spectrum analysis indicated two different diffraction peaks 885^{o}, 900^{o} as shown in Fig.9.

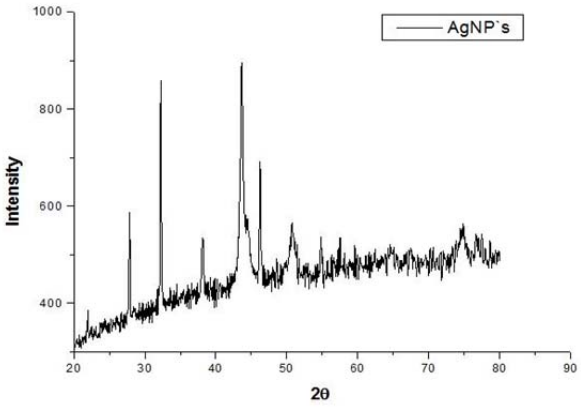

Fig. 9: XRD graph result

5.4 zeta potential

The stability of the silver nanoparticles was found out using Zeta potential analysis.The zeta potential value was found to be -31.1mv

Table I. Relationship between zeta potential and the stability of the colloid

Zeta potential [mV]	Stability behavior of the colloid
from 0 to ±5,	Rapid coagulation or flocculation
from ±10 to ±30	Incipient instability
from ±30 to ±40	Moderate stability
from ±40 to ±60	Good stability
more than ±61	Excellent stability

Measurement Results

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Measurement Results

Date	:	Monday, October 27, 2014 4:37:04 PM
Measurement Type	:	Zeta Potential
Sample Name	:	Silver NP SET
Temperature of the Holder	:	25.1 °C
Dispersion Medium Viscosity	:	0.893 mPa·s
Conductivity	:	0.158 mS/cm
Electrode Voltage	:	3.4 V

Calculation Results

Peak No.	Zeta Potential	Electrophoretic Mobility
1	-31.1 mV	-0.000241 cm2/Vs
2	--- mV	--- cm2/Vs
3	--- mV	--- cm2/Vs

Zeta Potential (Mean)	:	-31.1 mV
Electrophoretic Mobility Mean	:	-0.000241 cm²/Vs

Fig 10. Zeta potential result.

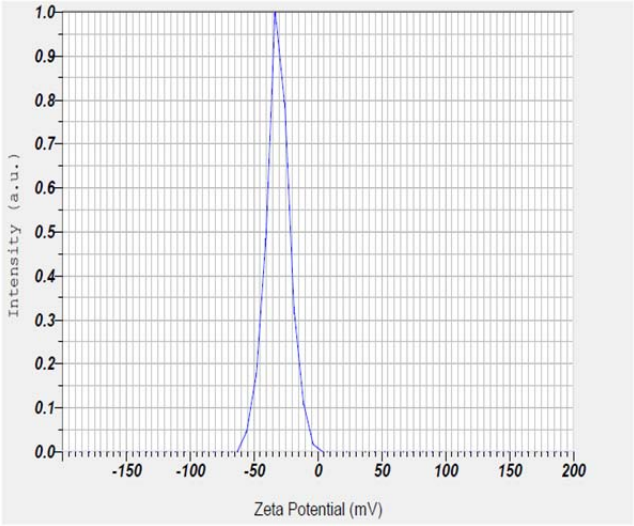

Fig 11 Graph result for zeta potential.

5.5 Antibacterial activity

The antibacterial activities of silver nanoparticles were carried out. In the nutrient agar plate the zone of inhibition was observed around both the wells (1 and 2) as shown in fig.11. The zone of inhibition of both silver nanoparticles and antibiotic gentamicin was compared. It was found that the zone of inhibition of nanoparticles was almost same as compared to gentamicin.

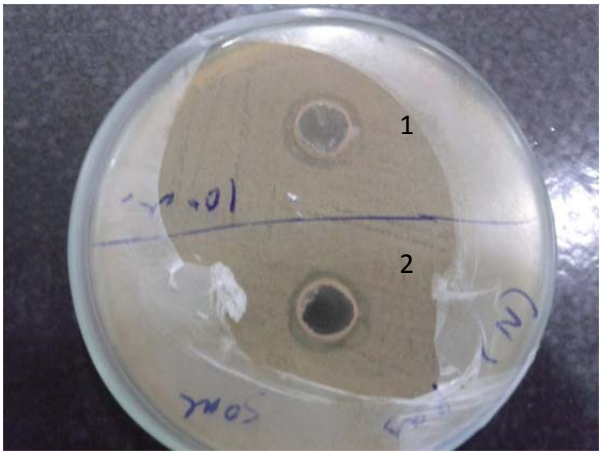

Fig.11: Showing zone of inhibition around the wells: 1(antibiotic- gentamicin), 2(silver nanoparicles)

6. DISCUSSION

The colours arise due to excitation of surface plasmon vibrations in the silver metal nanoparticles . The sharp bands of silver nanoparticles were observed around 421nm in case of *Dracena mahatma***.** From different literatures it was found that the silver nanoparticles show SPR peak at around 420nm. So we confirmed that *Dracena mahatma* leaf extract has potential to reduce Ag ions into Ag nanoparticles. The intensity of absorption peak increases with increasing time period. This characteristic color variation is due to the excitation of the SPR in the metal nanoparticles the insets to Figure of graph represent the plots of absorbance at λ_{max} (i.e., at 420 nm) versus time of reaction.The average particle size and PDI revealed that the produced AgNPs were polydispersed . All diffraction peaks correspond to the characteristics of cubic face centred AgNPs The zeta potential value was found to be -31.1mv which falls from ±30 to ±40. The silver nanoparticle was found to be of moderate stabilitySilver is known for its antimicrobial properties and has been used for years in the medical field for antimicrobial applications. Additionally, silver has been used in water and air filtration to eliminate microorganisms [19]. Being nanoparticles size it ensures that significantly large surface area of the silver nanoparticles is in contact with the bacterial cell. It causes membrane disruption affecting the permeability and alters the respiratory functions of the cell. [20]

CONCLUSION

The rapid biological synthesis of silver nanoparticles using *Dracaena mahatma* leaves extract provides environmental friendly, simple and efficient route for synthesis of benign nanoparticles. From the technological point of view these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

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