Anti-oxidant activity of Green synthesized AgNps prepared from Acacia *catechu* plant Extract by β-Carotene methods

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Abstract

Current study deals with the synthesis, and antioxidant activity of silver nanoparticles using aqueous extract of *acacia catechu* leave extract . The reaction mixture turned to brownish colour after 2 h of incubation and was confirmed by surface Plasmon spectra using UV-visible spectrophotometer around 490 nm characteristic of silver nanoparticles Nanoparticles have shown exceptional electronic, catalytic, optical, magnetic and other physical and chemical properties that are quite different from the bulk one. Silver nanoparticles have proven useful in antibacterial clothing, burn ointments and as coating for medical devices because of their mutation resistant antimicrobial activity. To fulfill the growing need of environmental friendly nanoparticles, researchers are using microorganisms for the synthesis of various silver nanoparticles ofhas been reported *acacia catechu* to possess antioxidant activity ..

Keywords: Green synthesis, anti oxidant activity of Silver oxide nanoparticles, UV- VIS, SEM. acacia catechu anti oxidant activity.

Introduction:

Nanoparticles exhibit new and improved properties based on size, distribution and morphology than larger particles of the bulk materials from which the nanoparticles are made1. The surface to volume ratio of nanoparticles is inversely proportional to their size. The biological effectiveness of nanoparticles can increase proportionately with an increase in the specific surface area due to the increase in their surface energy and catalytic reactivity. Studies have shown that the size morphology stability and chemical-physical properties of the metal nanoparticles are strongly influenced by the experimental conditions, the kinetics of the interaction of metal ions with reducing agents and the adsorption processes of stabilizing agents with metal nanoparticles. Hence, the design of the synthesis method in which the size morphology, stability and properties are controlled has become a major field of interest3-5. There are many established wet-chemical methods for the synthesis of metal nanoparticles. Most of these methods are quite easy to execute and offer facile means to control the shape, sizes and surface characteristics of the produced nanoparticles, enabling scientists to precisely tune the physico-chemical properties of nanoparticles However, inspite of these advances, these techniques still have major drawbacks, *e.g.*, use of starting materials, stabilizers and solvents used which are toxic and potentially hazardous. Moreover, adsorption of the toxic by-products on the surface of the synthesized nanoparticles is another problem associated with wet chemical methods Metal nanoparticles are currently being used in biomedical applications, such as novel

biosensing devices and cancer therapy. For these applications, synthesized nanoparticles need to be biocompatible, water soluble and easily functionalized for fine-tuning their surface chemistry. However, biocompatibility can be severely compromised if toxic by-products formed during nanoparticle synthesis are adsorbed onto the particle surface. Biological molecules have qualities by which they can undergo highly controlled and hierarchical assembly, which makes them suitable for the development of a reliable and eco-friendly process for metal nanoparticles synthesis. Therefore, the biological approach for the synthesis of nanoparticles becomes essential. One of the promising alternative synthetic routes for metal nanoparticles is biogenic synthesis, which employs non-toxic reactants derived from the biological sources ranging from unicellular organisms to higher plants. The key advantage of the use of plant extracts as the biogenic agents for metal nanoparticles synthesis is their easy availability.Moreover, simple laboratory set-up is required for the synthesis process and use of these biogenic materials potentially eliminates the elaborate process of cell culture and cell maintenance necessary for the biogenic synthesis of metal nanoparticles using unicellular organisms. Moreover, this synthetic protocol is applicable at room temperature and pressure, thus saving huge amount of energy.

Materials & Methods

Extract preparation and nanoparticle generation

In the present study the leaves of *Acacia Catechu leaves* were brought from the forest range at Lucknow and identified by NBRI, Lucknow.

Fig 1: *(a)* **Acacia Catechu leaves' powder**

For nanoparticle generation, we used plant leaves extract, prepared by mixing of 5g of plant powdered in 100ml of 95% ethanol, with 1-3 M aqueous solution of silver nitrate ($AgNO_3$) in 250 mL flask to give a pale yellow solution mixture at room temperature. The color of the solution mixture of silver nitrate and plant extract changes from pale yellow to deep color at 40℃ and 5 min of reaction time. Increasing the temperature of water bath to 60℃ and 10 min of reaction time, the color of mixture changed to deep brown color in 15 min of reaction time at 60℃ the deep

brown color changed to grey-black due to excitation of surface Plasmon. This color indicates the reduction of Ag^+ ions to Ag nanoparticles. Unmodified Ag+ separated as supernatant by process of centrifugation.

Characterization of nanoparticles

Silver oxide nanoparticles were characterized by UV-Vis spectrophotometer spectrum. Spectrophotometric analysis of AgO nanoparticles was performed using SYSTRONICS, DOUBLE BEAM UV-VIS Spectrophotometer 2202 at Cytogene Research & Development, Lucknow.

Scanning Electron Microscope Analysis

The morphological features of synthesized silver nanoparticles from *Acacia catechu* plant extract were studied by Scanning Electron Microscope (Electron Probe micro Analyzer JEOL MODEL No JXA8100) at Allahabad University. The samples were characterized in the SEM at an accelerating voltage of 25.0KV

Antioxidant activity

Free radical scavenging activity of silver nanoparticles

Determination of total antioxidantcapacity

β- Carotene bleaching assay

The determination of β – carotene bleaching assay was carried out according to the method developed earlier **(Olugbami, *et al.*, 2015)** with some modification. In brief, β – carotene solution (0.2 mg/ml in chloroform) linoleic acid (0.02 ml) and Tween 20 (200mg) were added into a beaker.The mixture was then evaporated at 40 °C for 10 min to remove the solvent, immediately followed by the addition of distilled water (100 ml). After agitating vigorously the mixture, 5 ml aliquots of the resulting emulsion were transferred into test tubes containing different concentrations (0.25mg/ml) of extracts and standard. The mixture was stirred and placed in a water bath at 50 °C for 2 h while the absorbance of the tested sample was repeatedly measured every 20 min at 470 nm using a UV–VIS spectrophotometer. The blank solution contained the same concentration of sample without β-carotene. All determinations were performed in triplicates. Butylated hydroxytoluenewas used as a standard solution for this method.

Result & Discussion Antioxidant activity

Conformation of formation of nanoparticles

A 1mM aqueous solution of $AgNo_3$ when added to the extract of *leaves* le *Acacia Catechu* ads to color change of extract from colourless to brown. This color change is due to excitation of surface Plasmon vibration **(Irawwa *et al.*, 2012)**.

Fig 2: *Aqueous solution of 1mM AgNO3 with leaves extract Acacia Catechu*

The intensity of color increases with increase in time duration indicating the continuous reduction of silver ions. The formation of silver nanoparticle in the reaction mixture was confirmed by plasmon resonance of silver nanoparticles at 420nm by UV-Vis spectrophotometer. 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 AgNPs were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of Ago nanoparticles. The UV-Vis spectrum of the synthesized Ag nanoparticles carried out using SYSTRONIC, Double Beam UV-VIS Spectrophotometer-2202. The absorption peak is shown in **Fig 3**. The formation of silver nanoparticle in the reaction mixture was confirmed by plasmon resonance of silver nanoparticles at 420nm by UV-Vis spectrophotometer. **Gavhane *et al.* (2012)** reported approximately similar result in their study absorption spectra (at 420nm) of silver nanoparticle formed by reaction media. **Sastry *et al.* (2003)** reported the synthesis of silver nanoparticles and characterization of UV-VIS spectrophotometer was given the absorbance peak at 430nm which was showing approximately similar result.

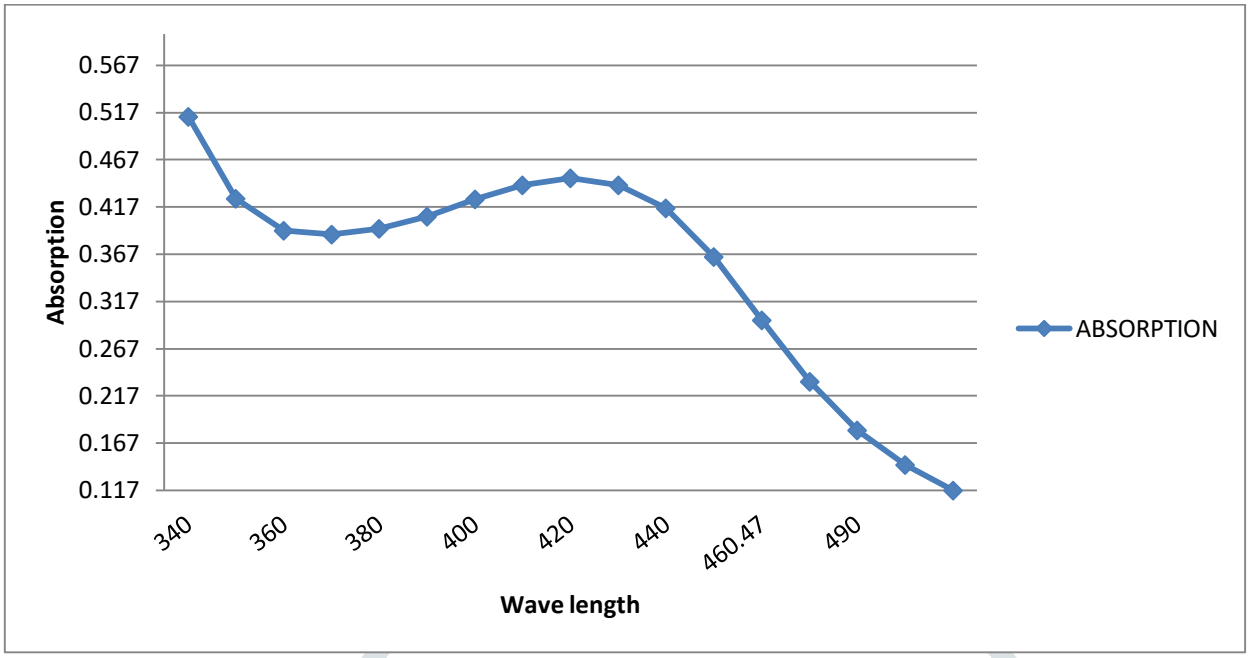

Fig 3: UV-Vis spectrum of synthesized AgNp

Scanning Electron microscopy (SEM) analysis of AgNPs

The SEM images of the AgNPs are shown in Figure 4. It is seen that AgNPs of different shapes were obtained in case of leaf extracts being used as reducing and capping agents. This reducing and capping agents of the extracts formed approximately spherical, triangular NPs, respectively which may be due to availability of different quantity and nature of capping agents present in the leaf extracts. An insight into the morphology and size details of the silver nanoparticles was done using Scanning Electron microscopy. Comparison of experimental results showed that the diameters of prepared nanoparticles in the solution have sizes of several µm in case of 10000rpm at 25mints and 30000rpm at 25 minutes the size is of several nm. The size of the prepared nanoparticles was more than the size of nanoparticle which should be; i.e.; between 1-100 nm. The bound proteins in the surface of the nanoparticles lead to increased size which was more than the desired size. The result showed that the particles were of spherical shape, the shape varies due to the concentration increased.

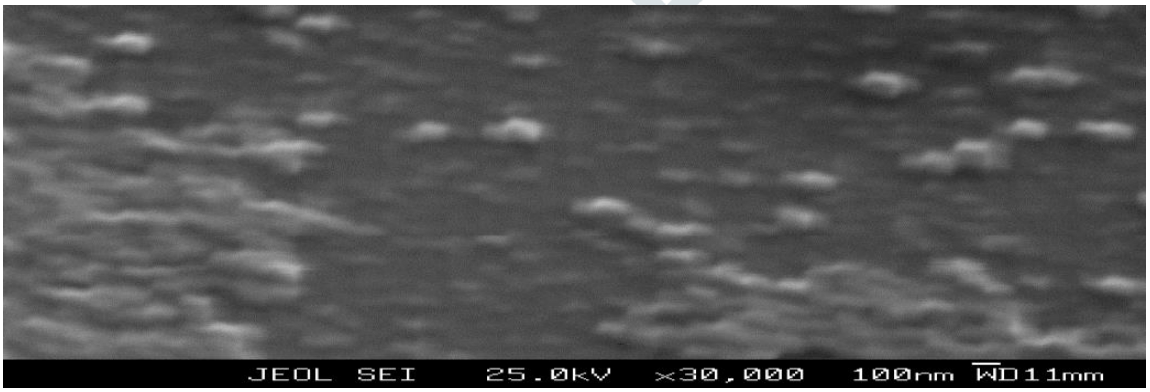

Fig 4: *SEM analysis shows that size and shape of the silver nanoparticles synthesized by at 1µm and Acacia Catechu 100nm.*

Total antioxidant capacity of *acacia catechu* is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the silver nanopartilces . The observed scavenging effect AgNPs and standard on the total antioxidant activity decreases in the following order: AgNPs >L ascorbic acid > plant extract. Among this AgNPs possess potential antioxidant activity as compared with ascorbic acid. PositiveHydrogen peroxide tests demonstrated that AgNPs and ascorbic acid are free radical scavengers. The Hydrogen peroxide scavenging assay exhibited effective inhibition activity of AgNPs and ascorbicacid. The Hydrogen peroxide activity of the nanoparticles was found to increase in a dose-dependent manner. The identification of antioxidant is beneficial to biological system against ROS ravage. Recently importance has been given for *in vitro* antioxidant study to understand the pharmacological role of medicinal plant and its isolate. *In vitro* techniques have been used for detection of antioxidants, which are based on the ability of compounds to scavenge peroxy radicals (Duran,et al., 2005).

Total antioxidant activity

Total antioxidant capacity of *Acacia Catechu* ver nanopartilces is expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to *Acacia Catechu* silver nanopartilces (Vigneshwaran, et al., 2001). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the *Acacia Catechu* plant extract, ascorbic acid and AgNPs. Among this AgNPs possess potential antioxidant activity as compared with silver *oxide nanoparticle*

β-Carotene Bleaching Assay

(Lu, *et al*., 2014)reported that the*β*-carotene bleaching method is based on the loss of the yellow colour of b-carotene due to its reaction withradicals which are formed by linoleic acid oxidation in an emulsion. The rate of *β*-carotene bleaching can be declinedin the presence of antioxidants.According to the result shown in Fig.4.6 shows that AgNPs **of Acacia catechu plant extract** at 0.25 mg/ml concentration had the higher inhibition percentage of beta carotene. Figure 4.6 shows 60% inhibition during 50min. which were as strong as BHT. Antioxidant activity of ZnO nanoparticle increased with their increasing Time interval. Their antioxidant activities were shown in graph **(Salah, *et al.*, 2012).**

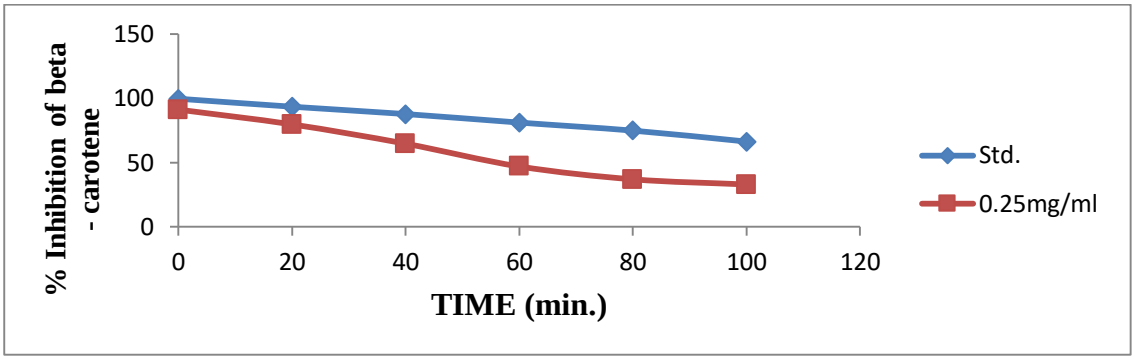

Figure :scavenging activity (%) versus Time of AgNps of Acacia catechu plant extract .

CONCLUSION.

Green synthesis of Zinc Oxide nanoparticles (ZnO Nps) was carried out using the aqueous extract of *Chrysanthemum dendranthema* leaves. In this study, the characterization of the nanoparticles was examined by XRD and scanning electron microscope (SEM).The morphology structure and stability of the synthesized ZnO nanoparticles were studied using scanning electron microscope (SEM). The synthesized nanoparticles were of hexagonal shaped and the estimated crystallite sizes were in4.5nm range.This study indicates that zinc oxide nanoparticles could potentially. Antioxidant activity was done by DPPH and β- carotene bleaching method result show that particle has good antioxidant activity.

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