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Synthesis and Characterization of Green Silver Nanoparticles Mediated by *Aegle marmelos* (L.) Leaf Extract

Sukumar Dandapat[1], Manoj Kumar and M.P. Sinha

Department of Zoology, Ranchi University, Ranchi, Jharkhand-834008
[1]Corresponding author
e-mail: [1]scholar.sukumar27@gmail.com

Abstract:

Synthesis of green silver nanoparticle mediated by medicinal plant extract is easier, cheaper and eco-friendly and the bioactive phytochemicals like phenols, saponins, flavonoids, tannin terpenoids, alkaloids etc. of *A. marmelos* acts as a reducing and capping agent during synthesis of green silver nanoparticles (AgNPs) for target specific action and delivery of drugs. Colour change from pale yellow to dark brown and highest absorption of spectrum at 200 nm and a broad spectrum at 474 nm of UV-visible spectroscopy provides the first confirmation about the synthesis of green nanoparticle. FT-IR spectroscopy showed transmission peak at 3275 cm^{-1} corresponding to O-H and H- stretch, 1604 cm^{-1} corresponding to C = C stretch, 1384 cm^{-1} corresponding to N = O bend, 1072 cm^{-1} corresponding to C = N stretch, 825 cm^{-1} corresponding to symmetric P-O-C stretching and 750 cm^{-1} corresponding C-Cl and = C-H bending provided the confirmation about the presence of alcohols and phenols, represents alkenes, as aliphatic nitro compound, represents aliphatic amines, aliphatic phosphate, chloro alkane respectively. Final confirmation obtained from Scanning electron microscopy showed the spherical and cubical shaped green AgNPs with diameter of 60 nm – 120 nm and the average diameter of the particles were of 70 nm.

Keywords: Drug, Nano, Plants, Disease.

INTRODUCTION

Antibiotics, other synthetic drugs and antibiotic chemotherapy has been one of the most important medical achievements used against pathogenic microbial diseases and other diseases since their introduction. However, over the past few decades commonly used antibiotics such as streptomycin, amoxicillin, tetracycline, etc. have become less effective due to the emergence of multi-drug resistant bacteria and also they associated with side effects [1, 2].

Many infectious diseases and disorders, especially intracellular infections, neurological disorder, cancer etc. remain difficult to treat with the antibiotics and other chemotherapeutic agents because they are difficult to transport through cell membranes and have low activity inside the cells, thereby imposing negligible inhibitory or bactericidal effects on the intracellular matrix of bacteria [3]. It is very challenging to target the drug in the central nervous system and another nervous tissue due to blood-brain barrier (BBB), which strictly restrict the delivery of most drugs to the brain because they do not cross the BBB in sufficient amount [4].

Over the last few decades, the applications of nanotechnology in medicine have been extensively explored as a broad area in the field of pharmacology. Nanotechnology in the field of medicine, concerns

the size of matters in the range between 1- 100 nm as a drug or natural or synthetic polymer loaded material acting as a carrier and within this scale materials have unique physicochemical properties including ultra-small size, large surface to volume ratio, high reactivity and unique interactions with structural components such as core, emulsion to work as carrier and functional groups includes the therapeutic molecules and ligands for targeting location of biological systems, which significantly improves the pharmacokinetics and therapeutic index of the drugs in contrast to the free drug counterparts [5-7].

Within few decades many advantages of nanoparticle-based drug delivery have been recognized, including improving serum solubility of the drugs, prolonging the systemic circulation lifetime, releasing drugs at a sustained and controlled manner, preferentially delivering drugs to the tissues and cells of interest, and concurrently delivering multiple therapeutic agents to the same cells for combination therapy [8-9].

Medicinal plants have been used as the chief source of treatment and disease management almost in all countries of the world because the herbal medicines are inexpensive, easily available and do not possess side effects [10-11]. Medicinal plants play an important role to improve the immunological response against many pathologies due to the presence of a wide variety of secondary metabolites, which are associated with therapeutic efficacy against various diseases and disorders [12-13].

However the delivery and efficacy of many herbal drugs is often limited to reach the site of therapeutic action and they require few modifications such as changing the molecular structure of the drug or their proper distribution by incorporation in carrier system [14]. Mathur and Govind [15] reported that, when the materials are incorporated into nano carriers, they require in lesser quantity to exert the action in target area, and this is useful, when dealing with effective phytomolecules.

Aegle marmelos commonly known as bael, belonging to the family rutaceae have been tested against the pathogenic bacteria. This plant is frequently used as folk medicine for treatment of various ailments like, antidiabetic, antihyperlipidemic, cardioprotective, radioprotective, antiulceranticancerous, antimicrobial and male contraceptives [16, 17].

In the last two decades, a number of nanoparticle-based therapeutic and diagnostic agents have been developed for the treatment of cancer, diabetes, pain, asthma, allergy, infections, and so on [18, 19].

Therefore, the present study was carried out the process of synthesis of green silver nanoparticles using aqueous extract of *Aegle marmelos* leaf extract.

MATERIALS AND METHODS

Collection of Plant Material

The fresh tender leaves were collected from Ranchi district, washed and disinfected by treating with $HgCl_2$ and washed again. The leaves were dried in the shade under room temperature for six to seven days, powered and sieved [20].

Extract Preparation

50 g of a fine powder was subjected to extraction by soxhlet using methanol and distilled water for the aqueous extract. The extract obtained was filtered, concentrated and dried in rotary flash evaporator maintained at 45 ºC for proper dehydration methanol free because methanol induce toxicity to living organisms. Percentage yield of each extract was calculated, and the dried extract was stored in air tight containers at room temperature for further studies [21].

Phytochemical Screening

Estimation of phyto phenols, tannins, flavonoids, alkaloids, steroids, terpenoids, saponins, carbohydrates, protein, cumarins content was done following Sofowara [22]. The details have been described elsewhere Kumar *et al.* [23].

Synthesis of Green AgNPs

The reaction mixture was prepared by adding 1 mL of the plant extract to 99 mL of 1 mM $AgNO_3$ (169.87 mg) solution in a 250 mL round-bottom flask, which was mounted with a cooling condenser and magnetic stir bar. The mixture was allowed to stir for 2 hours at 90 °C (immediate color change was observed from light yellow to dark brown, and thereafter no further color change was observed even after 2 hours). After 2 hours, the mixture was allowed to cool down before being centrifuged. The centrifugation was performed at room temperature and a speed of 9000 rpm. After washing three times with distilled water, a black powder was obtained that was dried overnight in an oven at 80 °C [24-26].

Characterisation of Silver Nanoparticles

UV-visible spectra analysis: The reduction of pure Ag^+ ions was monitored by measuring the UV-visible spectrum of the reaction medium at 5 h after diluting a small aliquot of the sample into Milli-Q water. UV-visible spectral analysis was done by using Parkin Elmer lambda 25 UV-Vis spectrophotometer.

Fourier Transforms Infrared Spectroscopy (FT-IR analysis) Measurements

FT-IR analysis was carried out on IPR resting-21 (Shimadzu) in the diffuse reflectance mode operated at a resolution of 4 cm^{-1} in the range of 400 to 4 000 cm^{-1} to evaluate the functional groups that might be involved in nanoparticle formation.

SEM Analysis of Silver Nanoparticles

SEM (Scanning electron microscope) analysis was done using JEOL JSM-6390 LV (Japan) 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 was allowed to dry by putting it under a mercury lamp for 5 min and was coated with gold using ion sputter.

RESULTS AND DISCUSSION

Synthesis of green nanoparticles mediated by aqueous *A. marmelos* leaf extract and $AgNO_3$ solution is presented in Fig-1, showed the change in light yellow colour of plant extract into dark brown which indicate the formation of green nanoparticle [26, 27]. Mohan *et al.* [28] also reported the changes in light colour of solution containing $AgNO_3$ and plant extract into dark brown which increases with an increase in temperature and incubation period.

Figure 1: (A) Photograph of Plant extract and (B) $AgNO_3$ and Plant extract mediated silver nano particle solution after 2 hours of heating at 80 ºC.

Phytochemical Screening

Results of phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, phenols, etc. presented in Table 1. Major bioactive components present in phytochemicals are acts as reductant to react with silver ions, and, therefore, leaf extract has been used as a reducing and stabilizing agent for the biosynthesis of silver nanoparticles [29].

Table 1: Qualitative phytochemical composition of *Aegle marmelos* leaf extract

Phytochemicals	Prresent (+) / Absent (-)
Alkaloids	+
Steroids	+
Terpenoids	+
Flavonoids	+
Saponins	+
Phenolic compounds	+
Tannin	+
Carbohydrates	+
Protein	+
Cumarins	+

UV-visible spectra analysis

UV- vis absorption spectroscopy is an important bio-physical technique to monitor the formation and stability of green nanoparticles with the help of the absorption spectrum. The absorption spectrum of nanoparticles obtained from UV-visible absorption spectroscopy was presented in Figure-2, which showed a broad peak at 474 nm and highest absorption of spectra represent highest peak at 200nm corresponds to the plasmon resonance. Kumar *et al.* [27] reported the highest absorption of spectrum at 200nm of alion mediated AgNPs solution by UV- vis absorption spectroscopy. Khan *et al.* [26] reported broad peak at higher wavelength indicates and increases the nanoparticle size and narrow line at shorter wavelength represents smaller particle size.

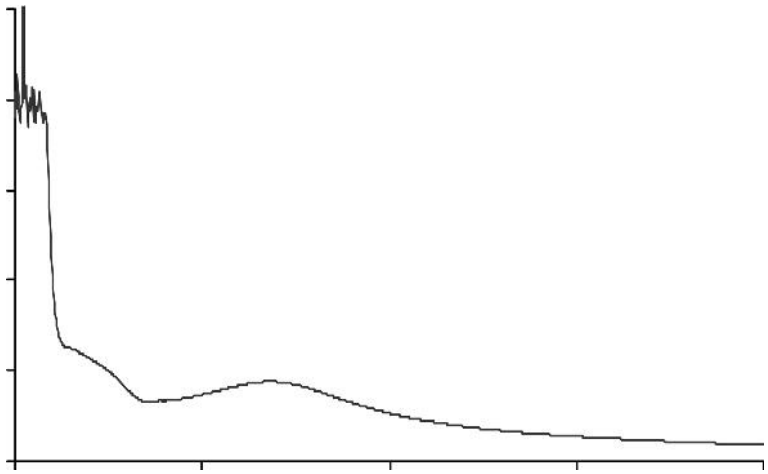

Figure 2: UV-Visible spectrum of A. marmelos and $AgNO_3$ mediated green nanoparticles.

FTIR- Analysis

FT-IR analysis was carried out to analyse the dual role of plant extract as capping agent and high bioreductant [26] and to analyse common types of molecular bonds and functional groups [30, 31].

FT-IR absorption spectra of green nanoparticle mediated *A. marmelos* leaf extract and $AgNO_3$ is presented in Figure 3. The spectra showed broad transmission peak at 3275 cm^{-1} corresponding to hydrogen bonded hydroxyl group (O-H and H- stretch) of alcohols and phenols, 1604 cm^{-1} corresponding to C = C stretch represents alkenes, 1384 cm^{-1} corresponding to N = O bend as aliphatic nitro compound, 1072 cm^{-1} corresponding to C = N stretch represents aliphatic amines, 825 cm-1 corresponding to aliphatic phosphate symmetric P-O-C stretching and 750 cm^{-1} corresponding chloro alkane (C-Cl) and = C-H bending [32, 33].

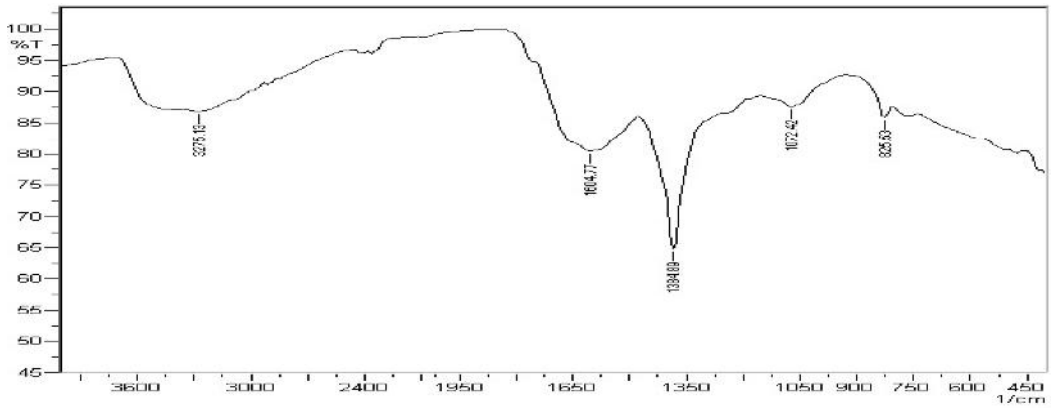

Figure 3: FTIR Spectrum of *A. marmelos* and $AgNO_3$ mediated green nano particles.

SEM analysis of green nano particles

Scanning electron microscopy was provided the final conformation about the morphology of synthesized green nano particles. SEM image of *A. marmelos* and $AgNO_3$mediated green nano particles was shown in figure -4. The green nano particles were of spherical and cubical in shapes and were formed with a diameter of 60 nm – 120 nm in diameter and the average diameter of the particles were of 70 nm. Kumar *et al.* [27] reported the size of green nano particles synthesized from alion of Aloe vera and $AgNO_3$ in the range of 287–293 nm and average size of nanoparticls were70 nm synthesized from. Firdhouse *et al.* [25] reported the size and shape of green nano particle were 20 nm -150 nm in diameter and spherical respectively.

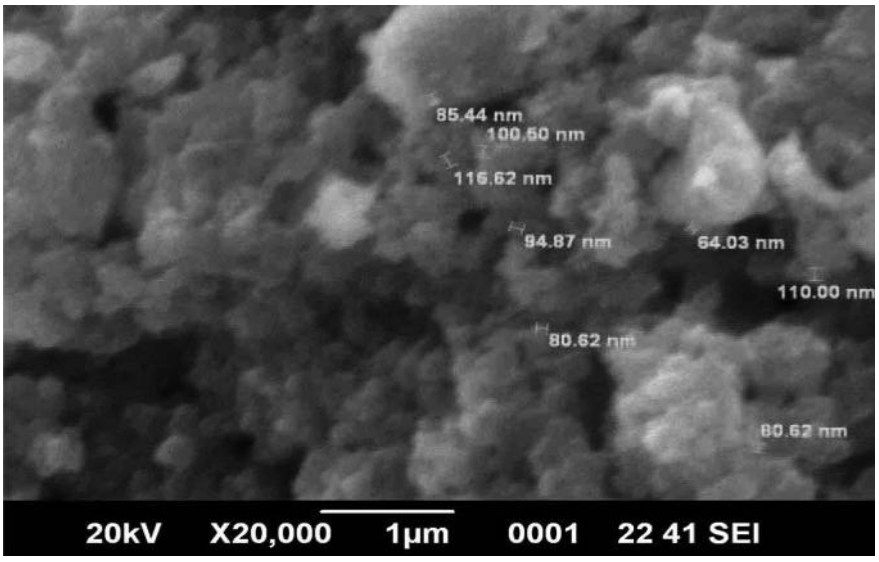

Figure 4: SEM image of *A. marmelos* and $AgNO_3$ mediated green nano particles.

CONCLUSION

This is the first ever reported green nano particles synthesized mediated by aqueous leaf extract of *A. marmelos* and $AgNO_3$. which is ecofriendly, cheap, easy to synthesize and can be used in the preparation of new pharmaceuticals due to its tiny size, capping ability of bioactive compounds, and possess therapeutic efficacy against various disorders and diseases.

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