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Original Article

GREEN SYNTHESIS OF SILVER NANOPARTICLE FROM LEAF EXTRACT OF *AEGLE MARMELOS* AND EVALUATION OF ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

Objective: The synthesis of metal nanoparticle is a growing area of research in modern material science and technology. Utilization of the silver nanoparticles in the field of biomedical nanotechnology and nanomedicines is rapidly growing because of their antimicrobial, anticancer, antioxidant property and less toxicity. Nanoparticles are synthesized by chemical methods, but are not eco-friendly. The objective of the study is to develop a fast, eco-friendly and convenient method for silver nanoparticle synthesis.

Methods: In this method utilization of the reducingproperty of *Aegle marmelos* leaf extract was done for synthesis of stable silver nanoparticles. Characterization of the metal nanoparticles was carried out by UV- Vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), X-ray Diffraction microscopy (XRD), Energy Dispersive X-ray spectroscopy (EDX) and zeta potential analysis.

Results: This result showed the average particle size of 15 -30 nm and spherical structure of stable silver nanoparticles. Green synthesized nanoparticles tested for its antibacterial activity by the well diffusion method. Silver nanoparticles had shown a more inhibitory effect against *Streptococcus pyogenes, Escherichia coli,* and *Pseudomonas aeruginosa* than *Staphylococcus aureus* and *Aeromonas hydrophila at* 25, 50 and 100 µg/ml concentrations.

Conclusion: This study is recommends the use of *Aegle marmelos* leaves for the synthesis of silver nanoparticles and can be applied as an antimicrobial agent.

Keywords: Nanoparticles, *Aegle marmelos,* Characterization, Antibacterial activity.

INTRODUCTION

Nanotechnology is one of the fastest developing science during the last few years. It is an interdisciplinary science that connects knowledge of biology, chemistry, physics, engineering and material science [1]. Remarkable advances are made in the field of biotechnology and nanotechnology to harness the benefit of life sciences, health care and industrial biotechnology [2]. It also have extensively been achieved for the treatments of cancer [3], diabetes [4], allergy [5], infection [6], inflammation [7] and cancer [8]. Novel properties of nanoparticles have been exploited in a wide range of potential applications in medicine, cosmetics, renewable energies, environmental remediation and biomedical devices [9]. It is a well-known fact that silver ions and silver-based compounds are highly toxic to microorganisms which include 16 major species of bacteria [10].

There are many ways to synthesize nanoparticles such as solid reaction, chemical reaction, co-precipitation and sol gel method, etc. The problem with most of the chemical and physical methods of nanosilver production is that they are very expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks [11]. In recent years, green synthesis of nanoparticles has several advantages over chemical synthesis, as green synthesis technique eliminates the use of energy, high pressure, temperature, and toxic chemicals. Moreover, green synthesized nanoparticles are compatible for biomedical and food applications [12]. The use of plant extracts to synthesize nanoparticles is receiving attention in recent times because of its simplicity. Also, the processes are readily scalable and may be less expensive. Plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles [13].

Aegle marmelos (Linn) Correa commonly known as Bael, belongs to family Rutaceae [14]. It is cosmopolitan, distributed the deciduous forests of India. It found almost in all the states of India [15]. The therapeutic value of the plant has been referred by almost all the ancient Ayurvedic treatises like Siddha, Unani, Sushruta Samhita and Charaka Samhita etc. Bael tree is a medium sized deciduous tree with unusual branches surrounded by aromatic trifoliate leaves [16]. The products obtained from the bale being highly nutritive and therapeutic getting popularized in Indian and international market. In the last five decades, these plants have been extensively studied by advanced scientific techniques and reported the various medicinal properties, which are anticancer, antibacterial, antifungal, antidiabetic, antioxidant, hepatoprotective, hemolytic, larvicidal and anti-inflammatory activity [17]. It has chemicals like caumarins, steroids, alkaloids, tannins etc. [11].

The present work describes the easy and simple method of green synthesis of silver nanoparticles by reducing silver nitrate solution using an aqueous leaf extract of *Aegle marmelos.* Also assessment of the antibacterial activity of synthesized silver nanoparticles against *Streptococcus pyogenes, Staphylococcus aureus, Aeromonas hydrophila, Escherichia coli,* and *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

Materials

For the synthesis of silver nanoparticles, *Aegle marmelos* leave was collected from the area of Madukkarai, Coimbatore, South India. The sample was authenticated by a botanical survey of India, the southern regional center, Coimbatore. Chemicals, media and solvents were purchased from Merck Limited, India.

Methods

Preparation of the extract

The extract was prepared by using 10 g fresh leaves of *Aegle marmelos*. Washed thoroughly thrice with distilled water, cut into fine pieces and transferred into a 500 ml Erlenmeyer flask, then 100 ml of distilled water was added in it and boiled for 10 minutes. Then the extract was cooled to room temperature and filtered with Whatman no. 1 filter paper.

Synthesis of nanoparticles

Silver nitrate ($AgNO_3$) was used as a precursor for the synthesis of silver nanoparticles. 0.1 mM aqueous solution of silver nitrate was prepared. Silver nanoparticles were prepared by adding 25 ml of plant extract to 75 ml of silver nitrate to give the final concentration 0.1 mM at room temperature. A distinct colour change was observed after 2 min the solution turned to dark yellow from normal colorless solution, suggesting the synthesis of silver nanoparticles. The colour became darker and turned into dark brown after 5 minutes. The reduction of silver ions was confirmed by the UV–Vis spectrum of the solution. The synthesized nanoparticles were separated out from the mixture by centrifugation at 10,000 rpm for 20 min [18]. Centrifugation process was repeated for 3 to 4 times by dispersing pellet in distilled water for removing organic matters of leaf extract. The pellet was collected carefully from the bottom of the centrifuge tube in a watch glass and dried in hot air oven at 60^{0} C.

Characterization of silver nanoparticles

The reduction of pure silver ions was observed by measuring the UV-Vis spectrum of the reaction mixture. In this study, a Shimadzu spectrophotometer was used for observing the spectrum at 200 to 800 nm wavelength range. For detection of functional groups responsible for reducing and stabilizing silver nanoparticles FTIR analysis was carried out. FTIR spectrum in the range 4000–600 cm^{-1} at a resolution of 4 cm^{-1} was used for analysis of the nanoparticles. The particle size of silver nanoparticles was determined by X'Pert Pro X-ray diffractometer. Elemental analysis of green synthesized silver nanoparticles was done by EDX. Morphology of synthesized silver nanoparticle was observed by Scanning Electron Microscopy (SEM) at different magnifications. Zeta potential of the silver nanoparticle aqueous suspension was analyzed by the Hobira zeta potential analyzer for checking its stability.

Antibacterial activity

The antibacterial study of silver nanoparticles was estimated by the well diffusion method[19] against S*treptococcus pyogenes, Staphylococcus aureus, Aeromonas hydrophila, Escherichia coli,* and *Pseudomonas aeruginosa*. The synthesized silver nanoparticles was suspended in water as 25 µg/ml, 50µg/ml and 100 µg/ml. Tetracycline 10 µg/ml was used as a control Plates were incubated for 24 h at 37 °C. After an incubation period zone of inhibition was measured around the well.

RESULTS AND DISCUSSION

UV-Vis Spectroscopy

Silver nanoparticles appear brown in colour in aqueous medium as a result of surface plasmon vibrations [20]. In previous studies, similar colour change was observed [21-23]. Synthesis of silver nanoparticles in sterile distilled water was confirmed by using UV-vis spectrophotometer in a range of wavelength from 200 to 800 nm. As an *Aegle marmelos* leaf extract was mixed in aqueous solution of silver ion the reduction of pure silver ions to silver nanoparticles was confirmed by measuring UV–vis spectrum of the reaction media. The UV-Vis absorption spectrum of silver nanoparticles in the leaf extract is shown in fig. 1. The spectroscopic band of silver nanoparticles solution was found to be close to 450 nm which confirms the synthesis of silver nanoparticles. This absorption strongly depends on the particle size, chemical surrounding and dielectric medium [24].

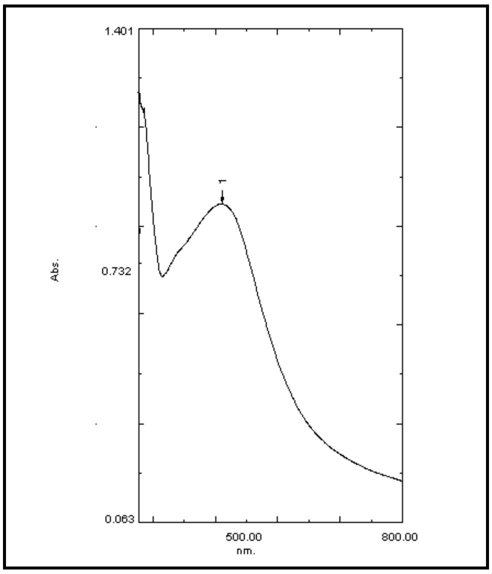

Fig. 1: UV-Vis spectrum of green synthesized silver nanoparticles

FT-IR Spectrum

To investigate the functional groups of *Aegle marmelos* leaf extract responsible for synthesis and stabilization of silver nanoparticle, a FT-IR study was carried out and the spectra are shown in fig. 2. It shows a number of absorption peaks, reflecting the complex nature of the extract. A peak at 3286 cm^{-1} is results from the stretching of the–OH bond of alcohol groups and it indicates bonded hydroxyl (-OH) group. The absorption peak at 3471 cm^{-1} could be due to–NH stretching of amine functional groups The peak at 2222 cm^{-1} assigned for a C=N group of nitrile. The peak at 1689 cm^{-1} indicates the C–O stretch. The absorption peaks at 1527 cm^{-1} could be assigned for the presence of N–O stretching in nitro. The band at 1195 cm^{-1} can be by the C=N stretching vibrations of aliphatic amines. The peak 736 $cm^{-1}$is due to–C-Cl stretch of alkyl halide FTIR study represents the hydroxyl (-OH), carboxyl (-C=O) and amine (-NH) groups of caumarins, tannins or alkaloids of the *Aegle marmelos* leaves extract are mainly involved in reduction of silver ions to silver nanoparticles. In FTIR spectra of synthesized silver nanoparticles bands of absorbance observed at around 740, 999, 1246 and 3153 cm^{-1} and these bands are matching to plant extract FTIR spectrum. This denotes coumarones and tannins from plant extract may responsible for reduction and stabilization of silver ions to silver nanoparticles.

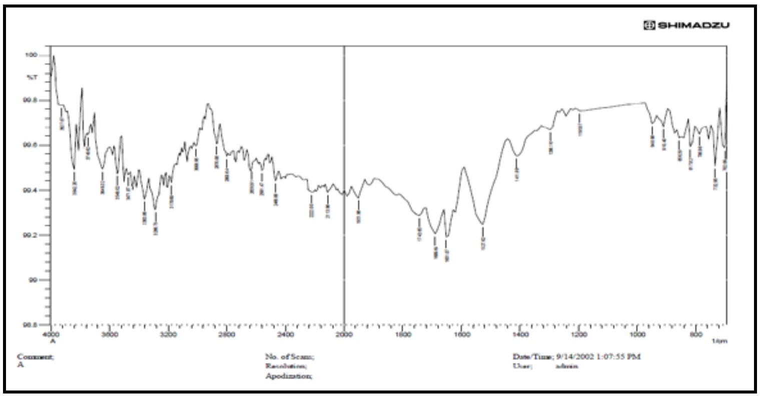

Fig. 2: FT-IR spectra of silver nanoparticles

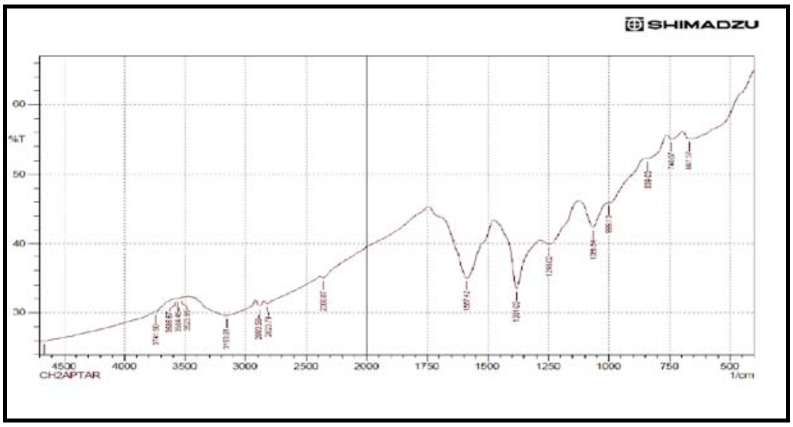

Fig. 2: FT-IR spectra of silver nanoparticle

SEM analysis

Shape and morphology of the synthesized nanoparticles were identified by scanning electron microscope analysis. The nanoparticles were examined under various magnifications of ×15,000, ×30,000, ×45,000 and ×55,000 SEM images of the synthesized silver nanoparticles are shown in fig. 3. It shows relatively spherical-shaped nanoparticles.

Accumulation of two or more reducing moieties bound on the surface of the pre formed nuclei of particles could have contributed to the formation of elongated large spherical nanoparticles [25].

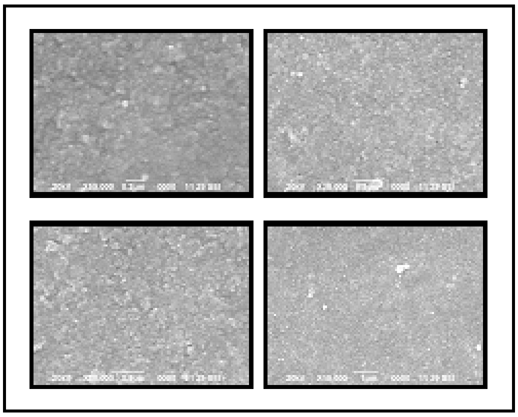

Fig. 3: SEM images of silver nanoparticles at different magnifications

EDX analysis

In this study, the elemental analysis of silver in the suspension of nanoparticles was done by EDX. The EDX result showed a peak of silver that confirmed its presence in the suspension. The EDX spectrum shown in fig. 4. Other peaks may be due to the attached groups from the leaf extract on the surface of nanoparticle.

XRD analysis

The X-ray diffraction (XRD) pattern of silver nanoparticles synthesized from aqueous *Aegle marmelos* leaf extract clearly shows the crystalline nature displaying the structural information. The XRD spectrum analysis indicated two different diffraction peaks at 32.12 °, 46.14 °, 38.01 ° and 43.9 ° (Fig.-5).

These diffraction lines are obtained at 2θ angles, which have been indexed as (98), (101), (111) and (200) plane of fcc silver by comparing with JCPDS data. Similar observations were reported by Ondari and Nalini for silver nanoparticles synthesized using *Trdax procumbens* [26] The Debye-Scherrer equation was used to determine the average particle size of the nanoparticles [27]. D = K λ/β cosθ. Were, D is the crystal size of nanoparticles, λ is the wavelength of the X-ray source (1.54 nm) used in XRD, β is the full width at half maximum of the diffraction peak (FWHM) K is the Scherrer constant with a value from 0.9 to 1, and θ is the Bragg angle. Assccording to Debye Scherrer equation the average particle size was found to be 15-30 nm.

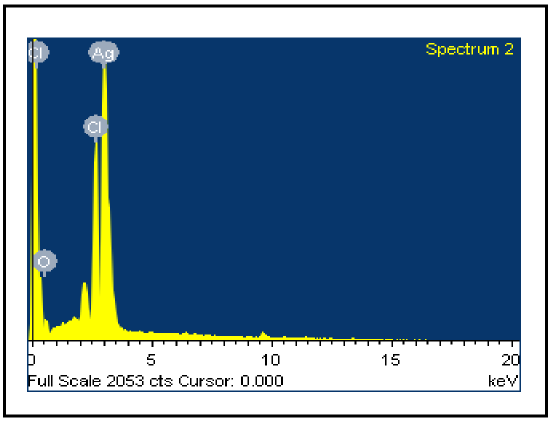

Fig. 4: EDX curve with three dominant peaks for Cl, O and Ag

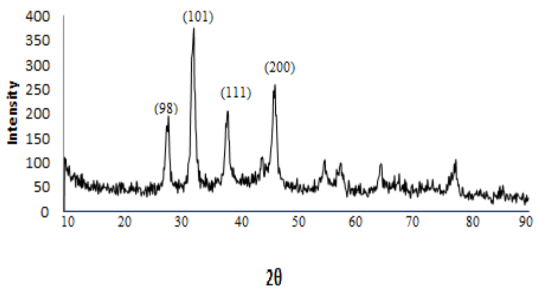

Fig. 5: XRD pattern of silver nanoparticles synthesized by *Aegle marmelos* leaf extract

Zeta potential analysis

Silver nanoparticles synthesized by green method are stable in nature as it has zeta potential-35.4 mV shown in fig.6. Measurement of zeta potential is depends on the movement of nanoparticles under influence of an applied electric field. This movemet depeds upon surface charge and the local environment of the particle[28].

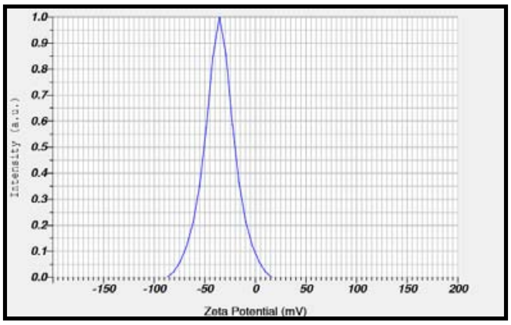

Fig. 6: Zeta potential of silver nanoparticles synthesized by *Aegle marmelos* leaf extract

Antibacterial activity

The antibacterial activity of synthesized silver nanoparticles against pathogenic microbes was shown in table 1. Zone of inhibition increases with concentrations 25, 50 and 100 µg/ml. A highest zone of inhibition was observed at 100 µg/ml with *Streptococcus pyogenes, Escherichia coli,* and *Pseudomonas aeruginosa*. While the lowest zone of inhibition was observed at 25 µg/ml with *Staphylococcus aureus* and *Aeromonas hydrophila*. The Fig.7 shows the zone of inhibition given by Silver nanoparticles and tetracycline as a control at different concentrations.

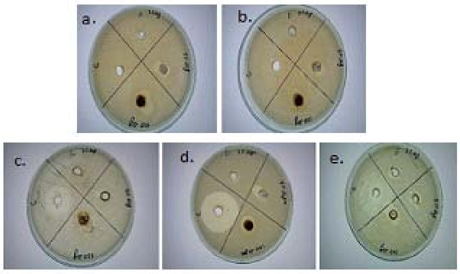

Fig. 7: Antibacterial activity of silver nanoparticles-a. *Streptococcus pyogenes,* b. *Staphylococcus aureus,* c. *Aeromonas hydrophila,* d. *Escherichia coli,* and e. *Pseudomonas aeruginosa*

Table 1: Zone of inhibition (mm) at different concentrations of silver nanoparticles

S. No.	Name of bacterial species	25 µg/ml	50 µg/ml	100 µg/ml	Control tetracycline (10 µg/ml)
1.	*Streptococcus pyogenes*	8±0.3	9±0.2	10±0.05	--
2.	*Staphylococcus aureus*	6±0.1	8±0.2	9±0.1	7±0.4
3.	*Aeromonas hydrophila*	6±0.05	7±0.1	8±0.5	12±0.5
4.	*Escherichia coli*	7±0.05	9±0.1	10±0.5	14±0.6
5.	*Pseudomonas aeruginosa*	7±0.4	8±0.1	10±0.6	15±0.6

CONCLUSION

In the field of nanotechnology, development of reliable and eco-friendly processes for the synthesis of metallic nanoparticles is prime need. The present study fulfills the objective of 'Green' synthesis of silver nanoparticles by a simple method. We have developed a fast, eco-friendly, simple and economical approach for preparation of stable silver nanoparticles by reduction of silver nitrate solution with a bio-reduction method using *Aegle marmelos* aqueous extract. The characteristics of the obtained silver nanoparticles were studied using UV-Vis, FTIR, XRD, EDX, SEM and zeta potential analysis techniques.

The experimental results showed that the synthesized silver nanoparticles are stable with an average size of about 15-30 nm. It was confirmed that the synthesized nanoparticles has antimicrobial activity and proved to be active against *Streptococcus pyogenes, Staphylococcus aureus, Aeromonas hydrophila, Escherichia coli,* and *Pseudomonas aeruginosa* microbes. Green synthesized silver nanoparticles could be very useful in the medical field for their antimicrobial properties.

CONFLICT OF INTERESTS

Declared None

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