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Original Article

Dentistry Section

Biosynthesis and Characterisation of Ellagic Acid Coupled Silver Nanoparticles- An In-vitro Study

A SHAFIE AHAMED[1], M CHITTRARASU[2]

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ABSTRACT

Introduction: Nanomedicine dominated the world of therapeutics and diagnostics. Horizons are extending daily to identify a piece of the puzzle that helps us produce eco-friendly and cost-effective nanoparticles. In this, authors tried to synthesise a novel Silver Nanoparticle Coated with Ellagic Acid (EA-AgNPs) obtained from pomegranate using a simple method and explore its characterisation precisely.

Aim: To biosynthesise and assess the characterisation of Ellagic Acid coupled Silver Nanoparticles.

Materials and Methods: The study assessed the portrayal of EA-AgNPs were evaluated using various analytical techniques. Firstly, to analyse: the size of the EA-AgNPs using Ultraviolet (UV)-Visible Spectrometer; Secondly to determine the hydrodynamic size and its dispersity using Dynamic Light Scattering (DLS) and Zeta potential; thirdly to quantify the average size of EA-AgNPs by using Scanning Electron microscope (SEM); and lastly to identify the functional groups by using Fourier Transform Infrared Spectroscopy (FTIR) method. Descriptive statistics were used to analyse the results.

Results: Brownish colour change and shift of peak wavelength from 430 to 423 nm using UV-Visible Spectrometer analysis confirmed the formation and stability of EA-AgNPs. The DLS analysis revealed that EA-AgNPs were in nanosize (129.7 nm) with less aggregated polydispersity index (0.483). The Zeta potential confirmed that this newly synthesised nanoparticle was negatively charged -0.268mv. The SEM determination confirmed the formation of spherical-shaped nanoparticles with sizes ranging from 84.34-98.80 nm. The FTIR revealed EA-AgNPs exhibited different functional groups, which help to prevent particle aggregation.

Conclusion: In this work, the novel EA-AgNPs exhibited the apt characterisation needed for an effective and cost-efficient nanoparticle that could be effectively tapped in various fields of nanodentistry.

Keywords: Analytical technique, Capping agents, Ellagic acid, Green chemistry, Nanomedicine, Reducing agents

INTRODUCTION

Nanotechnology is the fast emerging field having a more significant impact on modern science that helps produce bioactive novel substances ranging in few nanometers [1]. These nanomaterials have a greater interest in the medical and dental industry because of their applications as an antimicrobial agents, local drug delivery system and biosensing [2]. An array of nanoparticles are synthesised commercially from their time of discovery, AgNPs have an immense role in nanomedicine due to their efficient activity against microbes [3,4]. Various techniques are used for AgNPs commercially produced on large scales. However, each method has its disadvantage. To begin with, traditional physical methods like pyrolysis and spark discharge method was used to synthesise AgNPs. Chemical industries were thriving in producing AgNPs by reducing silver ion and stabilising it with various chemicals like borohydride, thio-glycerol whose toxicity is well documented [5]. As both physical and chemical methods of extracting AgNPs reported various hazards (i.e., toxicity levels, difficulty in handling their, by product waste management demanding high labour and environmental pollution) at in-vitro and in vivo studies, biological methods with no or reduced toxicity levels were called for [6]. This recently led to green synthesis of nanoparticles utilizing various microorganism and plant based extracts. The biogenic impact of the nanoparticle is indirectly proportional to its particle size (i.e., smaller particle size greater its efficacy) [7].

For any metallic nanoparticles, physical properties like size, shape, and size distributions depend on three main factors: the synthesised method, reducing agent, and stabiliser present in it. Although the area of interest (AgNPs) can be produced on a larger scale with eco-friendly techniques from microorganisms, it is not as cost-effective as plant extracted AgNPs [8]. When compared to microbes and enzymes, the majority of plants have features that distinguish them as sustainable and renewable suppliers, as they can absorb nearly 75 percent of light energy and convert it into chemical energy, contain chemicals such as antioxidants and sugars, and play critical roles in the production of nanoparticles [9].

Literature showed evidence that Ellagic Acid (EA), a naturally occurring substances in various plants is a good antioxidant. Further, it has numerous additional medicinal values ranging from antiadipogenic to anticancerous. It is a diametric derivative of different readily available plant products [10]. There is a shortage of research using EA obtained from pomegranate as a reducing/stabiliser in green synthesis AgNPs. Pomegranate is a naturally available fruit that can be grown quickly [11]. Pomegranate reported various health benefits like antioxidants, anti-inflammatory, anticancerous, and so on. Most of the health benefits of this magic fruit are attributed to its physical-chemical constituents (EA) [12]. Hence, authors carried out the present study to evaluate the characterisation of EA stabilised AgNPs (EA-AgNPs). Hence objectives of the present study were: (i) to analyse the size of the EA-AgNPs using UV-Visible Spectrometer; (ii) to determine the hydrodynamic size and its dispersity using Dynamic Light Scattering (DLS) and Zeta potential; (iii) to quantify the average size of EA-AgNPs by using Scanning Electron Microscope (SEM); (iv) to identify the functional groups by using Fourier Transform Infrared Spectroscopy (FTIR) method.

MATERIALS AND METHODS

This in-vitro study was conducted in Department of Conservative dentistry and Endodontics, Vivekanandha Dental College for Women, Elayampalayam, Tamil Nadu, India, between July 2019

and December 2019. All chemicals, commercially available silver nitrate particles, and plant extract of EA of analytical grade were obtained from Himedia Laboratories Pvt., Ltd., Mumbai, India and Sigma-Aldrich (St. Louis, MA, USA). Throughout the study, double distilled water was used for all solution preparation.

Preparation of the Sample

EA-AgNPs sample was prepared as per method followed by Kasthuri J et al., with modifications [13]. Ellagic acid and a 1 mM silver nitrate solution in double distilled water were used to synthesise silver nanoparticles. Silver nitrate and Ellagic acid solution were combined in the following ratios: 5:5, 6:4, 7:3, 8:2, and 9:1. The reaction mixture was heated below its boiling point and stirred continuously at 800 rpm. with a magnetic stirrer. Within 1 hour, the mixture had turned reddish brown in colour. The entire reaction took place in complete darkness. The obtained suspension was centrifuged at 15,000 rpm for 15 minutes. To remove impurities, the pellet containing silver nanoparticles was washed three to four times with deionized water. The precipitated nanoparticles (EA-AgNPs) were lyophilized. Before further characterization, lyophilized nanoparticles were stored in a cool, dry, and dark place.

Analyses, qualitative and quantitative measurements involved the use of the following tools and systems. The formation of the EA-AgNPs during the reduction process was indicated by a change in the colour of the reaction solution from colourless to dark brown in [Table/Fig-1], that was visually observed using UV-Visible (UV-Vis) Spectrometer (Lambda 365, PERKIN ELMER, USA). The hydrodynamic size of the novel particle and its property in solution was assed by DLS (Particle Size Analysers Nano Plus – Micro metrics, USA). Precise size of the EA coupled AgNPs was confirmed by Scanning Electron Microscopy (EVO-18, CarelZeiss, USA). Fourier Transform Infrared Spectroscopy (Spectrum Two, Perkin Elmer, USA) technique was used for identifying the functional groups involved in the stabilisation of synthesised nanoparticles.



[Table/Fig-1]: Synthesised EA- AgNPS.

STATISTICAL ANALYSIS

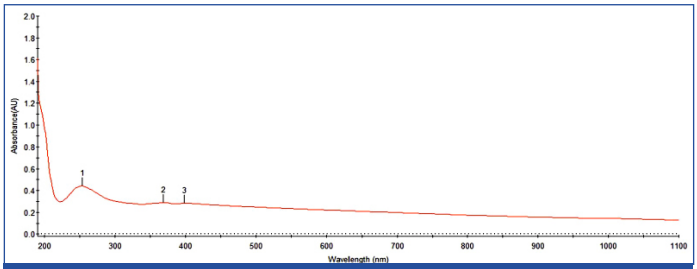
All data were obtained from mentioned above usage of instruments directly. Descriptive statistics were used to analyse the results.

RESULTS

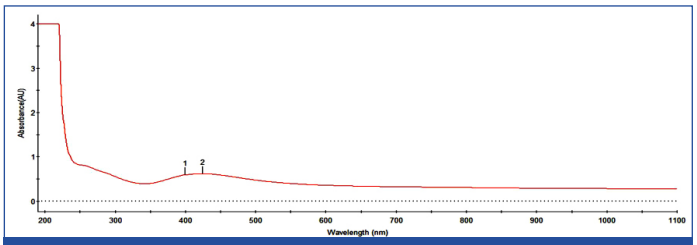
In this study, the EA-AgNPs particle obtained was placed in an Eppendorf tube and analysed using the following techniques.

UV-Visible Spectrometer

In this work, UV-visible absorption spectra of the Ag nanoparticles were synthesised from EA-AgNPs and pure EA. The absorption spectrum of EA showed three corresponding peaks at 252.60 nm (0.443 AU), 367.85 nm (0.291 AU), and 397.55 nm (0.285AU) in [Table/Fig-2]. Metal nanoparticles have free electrons; peaks have been observed due to the mutual vibration of electrons of metal nanoparticles. The absorption spectrum of EA-AgNps exhibit a gradual decrease in absorbance, accompanied by a shift in the wavelength from 430 to 423 nm (0.623 AU). The peak at 367.85 nm (0.291 AU) observed in EA has a change in the peak observed for EA-AgNPs at 398.80 nm (0.597 AU) in [Table/Fig-3], which indicates the participation of EA in the synthesis of Ag nanoparticles. The UV-Vis spectrum shows the role of $AgNO_3$ and the presence of active atoms in EA for the formation of silver nanoparticles. The concentration of EA at a 5:5 ratio increases the amount of energetic particles present in EA, which are necessary to convert Ag^+ to Ag^0.



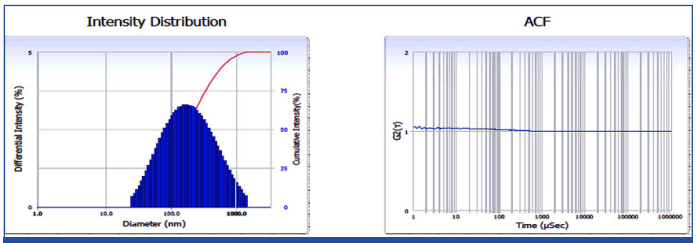
[Table/Fig-2]: UV-Vis spectra Ellagic acid (EA).



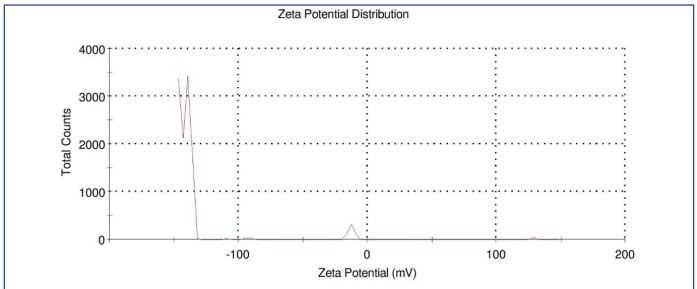
[Table/Fig-3]: UV-Vis spectra of Ag nanoparticles synthesised from Ellagic acid (EA-AgNPs).

Dynamic Light Scattering (DLS) and ZETA potential

DLS-Particle Size Analyser measurements were used to determine the size of the synthesised nanoparticles. The distribution curve of the synthesised nanoparticles is shown in [Table/Fig-4]. The average length of the silver nanoparticles and the statistical distribution of the size are determined using the particle size analyser. DLS shows the particle size of EA-AgNPs is 129.7 nm with a polydispersity index of 0.483, indicating the narrow distribution of the synthesised nanoparticles. Zeta potential showed a value of -0.268 [Table/Fig-5].



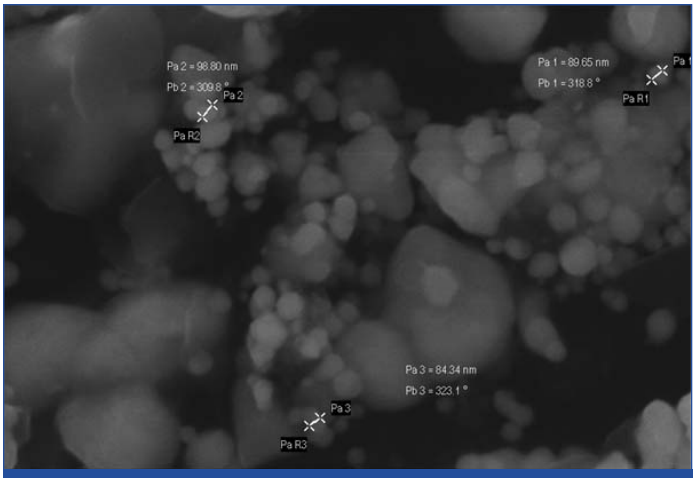
[Table/Fig-4]: Dynamic Light Scattering (DLS) of Ag nanoparticles synthesised from Ellagic acid (EA-AgNPs).



[Table/Fig-5]: Zeta potential of Ag nanoparticles synthesised from Ellagic acid (EA-AgNPs).

Scanning Electron Microscope (SEM)

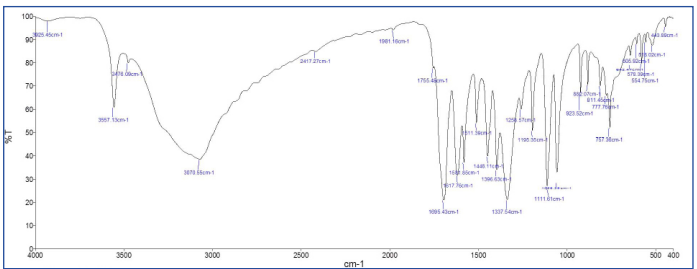
The spherical EA-AgNPS with various sizes ranging from 84.34 to 98.80 nm was observed using SEM as shown in [Table/Fig-6].



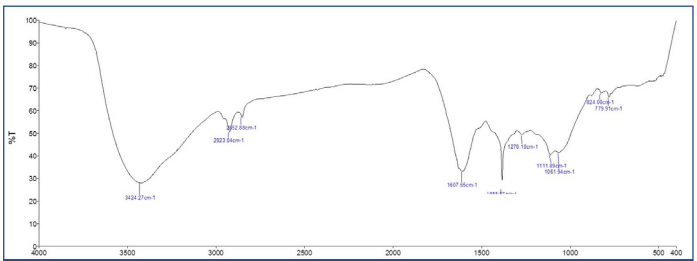
[Table/Fig-6]: Scanning Electron Microscope (SEM) of Ag nanoparticles synthesised from Ellagic acid (EA-AgNPs).

Fourier Transform Infrared Spectrometry

The FTIR spectrum of EA and EA- AgNPS was shown in the [Table/Fig-7,8], repectively. For EA, the band was observed at 3557.13 cm^{-1}. There is a decrease in the O-H stretching frequency from 3600.00 cm^{-1} to 3557.13 cm^{-1}, and the broadening of the same band indicates the presence of intermolecular hydrogen bond between the oxygen and hydrogen present in EA and water molecules. The bands at 1695.43 cm^{-1} and 1111.61 cm^{-1} are due to C=O stretching and aromatic C=O stretching of >C=O carbonyl group. The band observed at 1617.76 cm^{-1} is due to aromatic C=C stretching. The narrow bands obtained at 1396.63 cm^{-1} and 1337.54 cm^{-1} are due to –C- H bending. A band at 1056.39 cm^{-1} is may be due to the C-O stretching of primary alcohol. The bands observed at 882.07 and 811.45 cm^{-1} are due to ring oxygen present in the aromatic ring. 923.52 cm^{-1} band is due to aromatic C-C stretching. The intense band appeared at 3070.55 cm^{-1} are due to aromatic C-H stretching. For EA-AgNPs, the band was observed at 3424.27 cm^{-1}. There is a decrease in the O-H stretching frequency from 3600.00 cm^{-1} to 3424.27 cm^{-1}, and the broadening of the same band indicates the presence of an intermolecular hydrogen bond. The band at 1111.89 cm^{-1} is due to the aromatic C= O stretching of >C=O carbonyl group. The band observed at 1607.55 cm^{-1} is due to aromatic C=C stretching. The narrowband obtained at 1383.87 cm^{-1} is due to –C- H bending. A band at 1061.91 cm^{-1} is may be due to the C-O stretching of primary alcohol. The band observed at 824.00 cm^{-1} is due to ring oxygen present in the aromatic ring. There is a disappearance of a band for aromatic C-C stretching. The intense bands that appeared at 2923.04 cm^{-1} and 2852.88 cm^{-1} are due to aromatic C-H stretching.



[Table/Fig-7]: Fourier Transform Infrared Spectroscopy (FTIR) Ellagic acid (EA).



[Table/Fig-8]: Fourier Transform Infrared Spectroscopy (FTIR) Ag nanoparticles synthesised from Ellagic acid (EA-AgNPs)

DISCUSSION

We live in the era of nanotechnology. Synthesising ecofriendly "One-pot synthesis" of biogenic nanoparticles from plant phytochemicals is found to be an efficient and cost-effective technique. Among the various metaloxides, silver nanoparticles occupy a unique postion due to its application in medicine, pharmacy, nursing, dentistry, biosensing and bioengineering. This wide horizon application neccsiaties its synthesis at a larger sale with efficient properties [2]. Literature shreds support when AgNPs are green synthesised not only increases their antibacterial efficacy but also significantly reduces their toxic potential [5,14]. Numerous plant based extracs were being tried in this aspect. This study was performed to evaluate the characteristics of green synthesised EA-AgNPs. [Table/Fig-9] shows different plant extracts that have been previously used for biosynthesis of silver nanoparticles [15-19].

Author name and year	Place of study	Plant extracts used for synthesis	Properties of synthesised nanoparticles
Das CGA et al., [15] 2020	Not mentioned	*Pelargonium graveolens* (Geranium) Leaves	Antibacterial
Haqq SM et al., [16] 2018	Not mentioned	*Prunus japonica*	Antibacterial
Hasnain MS et al., [17] 2019	Not mentioned	Aloe vera leaf	Antibacterial
Ahmad N et al., [18] 2010	Not mentioned	*Citrus sinensis*	Antibacterial
Nagar N and Devra V [19] 2018	Not mentioned	*Rosa indica* Leaf	Antibacterial
Present study	Tiruchengode, Namakkal (District), Tamilnadu	Ellagic acid	Antibacterial

[Table/Fig-9]: Plant extracts that were used for biosynthesis of silver nanoparticles [15-19].

In UV spectrometric analysis,the formation of EA-AgNPs was indicated by a colour change from colourless to dark brown. Our findings corroborate with the colour change of AgNPs synthesised from various plant extracts as reported by Sastry M et al., Shankar SS et al., and Krithiga N et al., maximum absorbance peak of EA-AgNPs 398.80 nm (0.597 AU) [20-22]. The present study though comparable was lesser in the range reported by Prasad T and Elumalai EK (430nm), Verasamy (438nm), Logeshwari (425 nm), Vilchis-Nestor (430nm) [7,23-25]. Further, it is notable that the maximum peak length of EA(367.85nm) shifted to 398.80 nm indirectly indicated the change in absorbance spectra that occurred due to the integration of EA into silver nitrate particles [26].

DLS was done to assess the EA-AgNPs size in the solution phase based on the free diffusion coefficient of suspended particles. Hence, DLS gives us an estimate of the hydrodynamic state. In this study, DLS revealed EA-AgNPs hydrodynamic particle size of 129.7nm with the polydispersity index of 0.483. The size of the nanoparticles obtained from DLS is slightly bigger than that measured by the SEM due to its diffusing property in solution [27]. Lower the polydispersity higher is the precision of the particle aggregation [28]. In this work, the zeta potential of EA-AgNps was -0.268 mv. Zeta potential denotes the stability of the nanoparticle by the measure of its electric discharge [29]. The negative charged potential value was imparted to EA-AgNPs because of reducing agents present in newly synthesised particles.

SEM analysis revealed [Table/Fig-6] EA-AgNPs were ranging from 84.34 to 98.80 nm with spherical morphology. In the present study, nanoparticle was a little larger than others reported from work done by Savithramma (30-40nm) in *Boswellia ovalifoliolata* and 40 nm in *Shorea tumbuggaia* [30]. The Larger diameter not only allows more functionality in a given volume of substance but possesses better physical properties than their bulk counterpart as reported in other plant extracts. Surface changes have significant implications

on their interaction with biological fluids and samples, indirectly indicating their antimicrobial efficacy [31].

This novel EA-AgNPs size, as confirmed in SEM, is similar to Gangatharan R and Natesan SK, having the range of 80-100nm [32]. Another study was done by Badar W and Khan MAU also revealed Silver nanoparticles synthesised ranging from 5-100nm with observed using SEM [33]. The larger particle size in the present study may be due to the increased number of EA, which in turn increases the number of nucleation in silver nanoparticles [11]. Studies revealed larger the size of coupling the nanoparticles completely, the faster is the rate of reaction for the formation of silver nanoparticles. Another added advantage with the fact larger sized nanoparticles are reported to have a high density of surfactants and capping agents, i.e., EA dispersed into the silver matrix [34-36].

The FTIR analysis confirmed the bioreduction of Ag^{+} ions to silver nanoparticles due to the reduction by capping material EA obtained from plant extract. Similarly, Gole A et al., reported that proteins in the quote could bind to silver nanoparticles through either the free amino or carboxyl groups in the proteins [37]. Further, Prasad KS et al., reported that the carboxyl (–C=O), hydroxyl (–OH), and amine (–NH) groups of leaf extracts are mainly involved in the fabrication of silver nanoparticles [38]. The stability of this new nanoparticle could have been due to the free functional groups that interacted with the silver particles [5]. The strength of the present work is analytical techniques are used systematically as recommended. Care was taken to objectively and preciously determine each parameter. i.e., the visual colour change observed was complimented with spectrometric analysis. Further, DLS findings were complemented with Zeta potential and SEM analysis to precisely report the characterisation of EA-AgNPs obtained from pomegranate plant extract. This novel biogenic nanoparticles having spherical shape with less polydispersity can be used as a antibacterial agents in biological samples requiring substance for drug delivery.

Limitation(s)

The present study has its own limitation of external validity until in vivo studies substanties it.

CONCLUSION(S)

In the present study, authors synthesised novel EA-AgNPs that were found to have all physiochemical characterisation qualifying it to be an apt nanomaterial with desirable spherical shape ad functional group for its interaction and stability in solution phases using an eco-friendly approach. Further studies targeting specific microorganisms of concern in nanomedicine and in vivo experimentation are further needed to transform this laboratory work into real world substantiation.

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PARTICULARS OF CONTRIBUTORS:
1. Professor and Head, Department of Conservative Dentistry and Endodontics, Rajah Muthiah Dental College and Hospital, Annamalai University, Chidambaram, Tamil Nadu, India.
2. Associate Professor, Department of Conservative Dentistry and Endodontics, Vivekanandha Dental College for Women, Elayampalayam, Tiruchengode, Namakkal, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
M Chittrarasu,
Associate Professor, Department of Conservative Dentistry and Endodontics,
Vivekanandha Dental College for Women, Elayampalayam, Tiruchengode,
Namakkal, Tamil Nadu, India.
Email Id: dr.chittrarasu@gmail.com

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