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Seaweed-mediated synthesis of gold nanoparticles using *Turbinaria conoides* and its characterization

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Abstract

The synthesis of metal nanoparticles using algae has been unexplored, but it is a more biocompatible method than the other biological methods. Metal nanoparticle synthesis using algae extract shows rapid and non-toxic process which resulted to nano sizes having the greatest potential for biomedical applications. In this investigation, we studied the green synthesis of gold nanoparticles using the algae extract of *Turbinaria conoides*. Green synthesis of gold nanoparticles was preliminarily confirmed by color changing from yellow to dark pink in the reaction mixture, and the broad surface plasmon resonance band was centered at 520 to 525 nm which indicates polydispersed nanoparticles. Transmission electron microscopy and selected-area electron diffraction analysis show the morphology and crystalline structure of synthesized gold nanoparticles with the size range of 6 to 10 nm. The four strong diffraction peaks were observed by X-ray diffraction; it confirmed the crystalline nature of synthesized gold nanoparticles. The carboxylic, amine, and polyphenolic groups were associated with the algae-assisted synthesized gold nanoparticles which was confirmed using Fourier transform-infrared spectroscopy. This study eliminates the use of chemical substances as reducing and stabilizing agent. Because it has natural several constituents which are fucoidan and polyphenolic substances, it does a dual function as both reducing and stabilizing agent for nanoparticles. Thus, algae-mediated synthesis process of biomedically valuable gold nanoparticles is a one-spot, facile, convenient, large-scaled, and eco-friendly method.

Keywords: Green synthesis, Gold nanoparticles, *Turbinaria conoides*, TEM, Seaweed

Background

Nanotechnology is anticipated to be the beginning of many of the main technological innovations of this century. An essential area of research in nanoscience and nanotechnology deals with the synthesis of nanoparticles of different chemical compositions, dimensions, and controlled monodispersity [1,2]. Currently, there is a growing necessity to improve the environmentally benign nanoparticle synthetic processes that are gratis from toxic chemicals in the synthesis procedure [3]. As the nanorevolution persisted to unfold and it is very important

to manufacturing, the nanoparticles entrenched end products including environmentally beneficial and non-polluting technologies. Chemical and physical synthesis methods lead to the presence of some deadly chemical species adsorbed on the surface that may have unfavorable effects in medical applications [4]. In chemical-mediated synthesis of nanoparticles, some toxic substances could be used as reducing and stabilizing agent for stopping the agglomeration. As a result, researchers in the field of the synthesis of nanoparticles and assembly have turned to green system [5]. The use of eco-friendly materials like bacteria, fungi, plants, and parts of plant materials and algae for the synthesis of gold nanoparticles (AuNPs) offers several benefits of good environment and compatibility for biomedical applications as they do not use noxious chemicals in the synthesis procedure [6]. Green-mediated synthesis of metal nanoparticles provides

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progression over chemical and physical methods as it is economical and environment friendly, and in this method, there is no need to use high pressure, energy, temperature, and toxic chemicals [7]. Algae are otherwise called bionanofactories because they synthesized nanoparticles with high stability, are easy to handle, and eliminate cell maintenance [8]. Algae are the naturally available plant, which are important source of phytochemicals involved in the production of metallic nanoparticles. Recently, gold nanoparticle synthesized using the extract of algae such as *Sargassum wightii* [9], *Turbinaria conoides* [10], *Laminaria japonica* [11], and *Stoechospermum marginatum* [12] was reported.

Gold is one of the precious, inert, and less toxic metals, and it is utilized for curing various diseases. Gold nanoparticles play a vital role in nanobiotechnology as biomedicine because of convenient surface bioconjugation with biomolecular probes and remarkable plasmon-resonant optical properties [13-15]. Gold nanoparticles have an important function in the delivery of nucleic acids, proteins, gene therapy, *in vivo* delivery, targeting, etc. [16].

Among the marine sources, the macroalgae (seaweeds) occupy a significant place as a source of biomedical compounds. The compounds derived from macroalgae are reported to have a broad range of biological activities such as antibacterial [17], anticoagulant [18], and antifouling activity [19]. Seaweeds have been used since ancient times and have an exclusive place in traditional medicine of maritime nation as vermifuges, aesthetics, and antibiotics in the treatment of cough, wounds, gout, goiter, hypertension, venereal diseases, cancer, and a variety of other sickness [20]. *T. conoides* is the brown algae coming under the order of Fucales and in the family of Sargassaceae. The components found in such are a major amount sterols, some are fucosterol, and different molecules containing vinyl and ethyl cholesterol types, cyclohexane [21], and some sulfated polysaccharides such as fucoidan, neutral glucan, and guluronic and mannuronic acid residues containing alginic acid [22], rhamnose, fucose, arabinose, xylose, mannose, galactose, glucose, and uronic acids [23], providing a medicinal value for the brown algae.

This present investigation deals with a simple, eco-friendly synthesis of gold nanoparticles by the reduction of aqueous AuCl_4 into nanoparticles using the extract of marine algae *T. conoides*. The optical property of synthesized gold nanoparticles was characterized using a ultraviolet-visible (UV-vis) spectrophotometer; morphological characters was studied by performing TEM; crystalline nature was analyzed using selected-area electron diffraction (SAED) and X-ray diffraction (XRD) patterns; and functional molecules involved in the reduction process were comprehensively studied using Fourier transform-infrared (FTIR) spectroscopy.

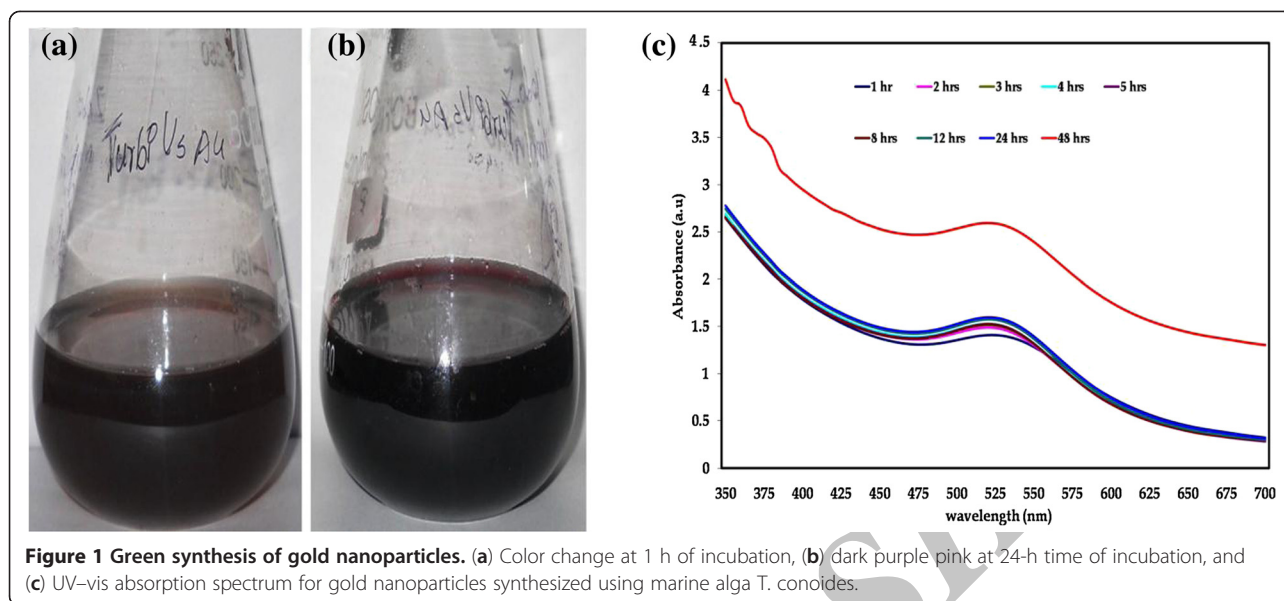
Results and discussion

Visual inspection

The reduction of gold ions into gold nanoparticles was visually identified by color change from yellow to dark pink during the exposure of algae extract into the gold ion solution. Generally, gold ion exhibits yellow color in distilled water. Since the algae extract was added into the gold ion complex solution, it started to change the color from yellow to pink which indicates the reduction of gold ions into gold nanoparticles. Variation in the color change was observed; it mainly depends on the reaction time and phytochemical components of the algae extract. In this observation, dark pink color was formed at 1-h time of incubation, and the completion of reduction process that occurred at 24 h was identified by the precipitation of nanoparticles at the bottom of the conical flask (Figure 1a,b). These color changes happen because of the excitation of surface plasmon vibrations with the gold nanoparticles [24]. Quite interestingly, Dharmotharan et al. reported gold nanoparticles synthesis started at 2 h using algae biomass of *Padina tetrastratica*. Some other biological resources such as fungi [9,25,26], bacteria [27], and plants [28] were used for synthesis of the gold nanoparticles by reduction process; they took time to complete the reduction which was about 24 to 120 h.

UV-vis spectrophotometer analysis

UV-vis spectroscopy is an important technique to determine the fabrication and stability of metal nanoparticles in an aqueous solution. Figure 1c shows the UV-vis spectrograph of the colloidal gold solution that has been recorded as the function of time. Initially, the broad surface plasmon resonance (SPR) band for gold nanoparticles occurred at 520 nm. The broad peak indicates the presence of nanoparticles with a large size distribution. After 1 h, increases in intensity occurred as a role of the time of reaction, the SPR band was observed at 525 nm (Figure 1c). This band blue shift occurred due to longitudinal excitation of surface plasmon resonance of the nanoparticles [29]. The plasmon resonance bands of gold nanoparticles are broad with an absorption tail in the longer wavelength that extends well into the near-infrared region attributing the excitation of the in-plane SPR and indicates considerable anisotropy in the shape of Au nanoparticles. According to the Mie theory, the small size and spherical shape of nanoparticles which were formed in the reaction mixture were identified by forming a single SPR band for gold nanoparticles [30] and were confirmed by TEM. The optical absorbance was increased while increasing the incubation time indicates increasing nanoparticle synthesis. Absorbance intensity and increased nanoparticles are directly proportional to the incubation time. The conversion of gold ions into gold nanoparticles was found 90% within



1 h, identified by the absorbance intensity of reaction mixture. Similarly, Ghodake and Lee [11] reported that the SPR band was located at 530 nm for gold nanoparticles synthesized using brown algae *L. japonica*.

Transmission electron microscope

The structure and size of the nanoparticles were shown in Figure 2a,b,c. The TEM images of *T. conoides*-synthesized gold nanoparticles show mostly large and small spherical-, triangle-, and pseudo-spherical-shaped particles. The spherical and undefined shapes are also found in the TEM images which indicated that these particles are synthesized at the beginning of the reaction, i.e., at 6-to18-h incubation. After 24-h incubation, the particles are aggregated and form a bulk structure. The low absorbance was observed after 24 h in the UV-vis spectrum which also proved the aggregation of the particles. Due to the absence of stabilizing agent in the brown algal extracts, the particles are aggregated with each other and form a bulk structure. The average size of resulting particles is about 6 to 10 nm. When compared to the previous reports of Noruzi et al. [31] and Raghunandan et al. [32], herein, the algal-mediated gold nanoparticle shape are varied as spherical, triangle, and pseudo-spherical. Similarly, Ghodake and Lee [11] synthesized gold nanoparticles in the size range between 15 and 20 nm. The SAED pattern also suggested that the synthesized gold nanoparticles are crystalline in nature. The result of SAED pattern coincided with the result of XRD. The appearance of rings is attributed to set of diffraction planes (1 1 1), (2 0 0), (2 2 0), and (3 1 1) of fcc gold (Figure 2d). Similar SAED pattern was obtained using the algae [32].

X-ray diffraction assay

The green-synthesized nanoparticle in crystalline nature was clearly analyzed using XRD patterns [6]. Figure 3 shows the XRD patterns of the AuNPs prepared using the algal extract and its simulated solution. The XRD patterns revealed that AuNPs corresponded to the crystalline gold fcc phase. The diffraction peaks obtained at $2\theta = 38.36^\circ$ (1 1 1), 44.13° (2 0 0), 64.78° (2 2 0), and 77.98° (3 1 1) are identical with those reported for the standard gold metal (Au^0) (Joint Committee on Powder Diffraction Standards-JCPDS, USA). The presence of these four intense peaks corresponding to the nanoparticles was in agreement with the Bragg's reflections of gold identified with the diffraction pattern [33]. Thus, the XRD pattern suggests that the gold nanoparticles were essentially crystalline nature. Hence, the simulated solution exhibited tremendous performance on the synthesis of AuNPs as that of the algae *T. conoides* extract. The XRD pattern of *T. conoides*-derived AuNPs shows some unidentified peaks that if raised with standard Au peaks reveals the association of algal biomolecules with the synthesized AuNPs. Similar result of XRD for gold nanoparticles synthesized using brown algae *S. marginatum* was found by Rajathi et al. [12].

FTIR analysis

FTIR measurement was carried out for functional molecules or constituents in the *T. conoides* responsible for the reduction of gold ions to nanoparticles and stabilizing the nanoparticles. The gold nanoparticles synthesized from *T. conoides* exhibit a lot of biomolecules which were involved in the synthesis of AuNP process (Figure 4).

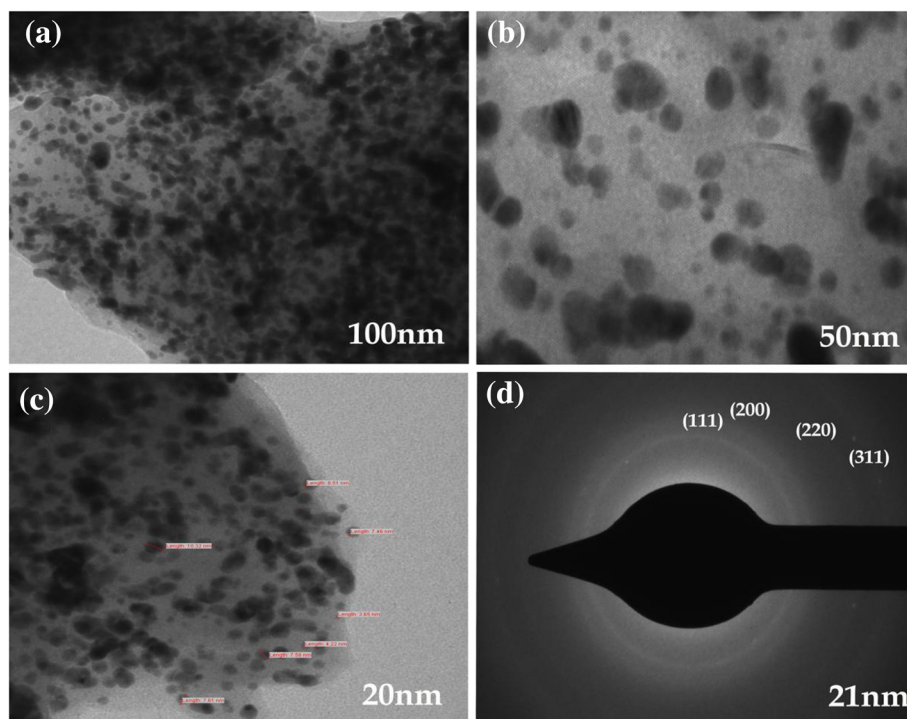


Figure 2 TEM images of AuNPs synthesized using *T. conoides* at different scale bars show spherical-shaped nanoparticles. The (a) 100 nm, (b) 50 nm, (c) 20 nm, and (d) SAED pattern of AuNPs.

FTIR shows the presence of different functional groups, which give rise to the well-known signatures in the IR region of electromagnetic spectrum. The strong and broad band observed at $3,414\text{ cm}^{-1}$ indicates the presence of polyphenolic O-H group and primary amine O-H band [34], C-H stretching vibrations of alkanes group was formed at the absorption band at $2,924\text{ cm}^{-1}$, a narrow band at $1,638\text{ cm}^{-1}$ indicates the presence of amide I, $1,418\text{ cm}^{-1}$ corresponding to C-C stretching aromatic

ring, C-O stretching carboxylic acid group assigned at $1,243\text{ cm}^{-1}$, the narrow peak at $1,030\text{ cm}^{-1}$ is because of the presence of C-N stretching vibrations of aliphatic amines of proteins [35], and 813 cm^{-1} is assigned to S-O stretching of sulfonates. The weak bands at 639 and 586 cm^{-1} correspond to alkyl halides. This study also confirms that the carbonyl group from amino acids or proteins has stronger ability to bind metal so that the proteins or enzymes could most possibly cap the metal nanoparticles

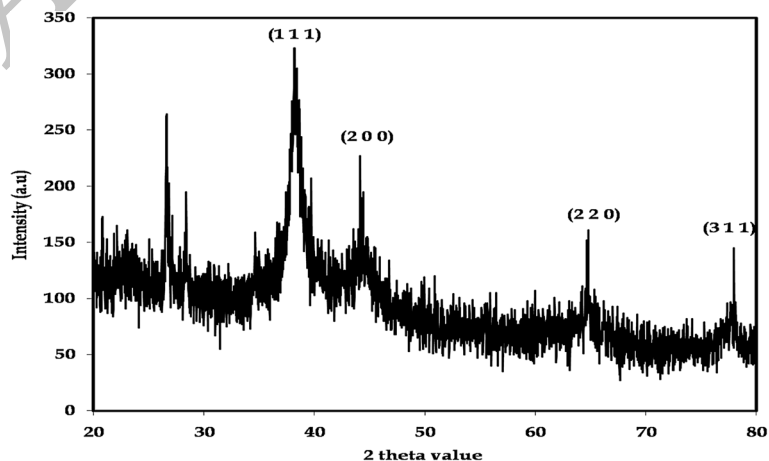


Figure 3 XRD spectra of gold nanoparticles synthesized using *T. conoides* algal extract.

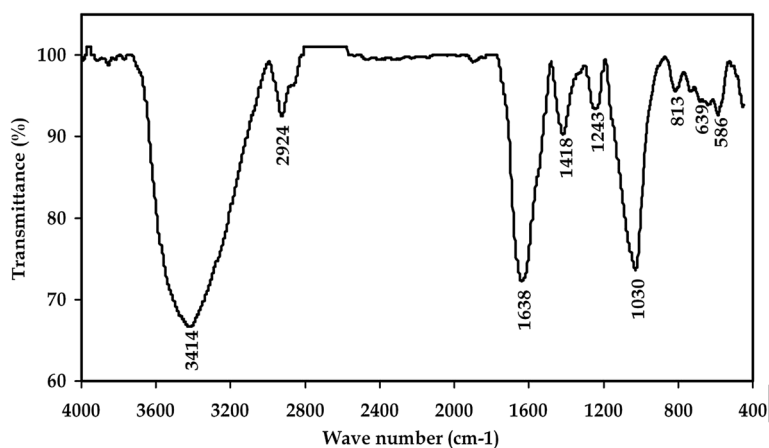


Figure 4 FTIR spectra of *T. conoides*-synthesized gold nanoparticles.

to prevent the agglomeration of the particles. This evidence reveals that sulfonated polysaccharide compounds and amide linkage proteins molecules could possibly be involved in the reduction of gold ions to nanoparticles and stabilization of gold nanoparticles in the aqueous medium. Previously, similar report has been presented [11,36]. Thangaraju et al. [37] reported that the presence of carboxylic, amine, phosphate, and hydroxyl functional groups is involved in the reduction of silver ions in the algae extract of *Sargassum polycystum*.

Polysaccharide and protein biomolecules in the algae extract do a dual function as reducing the gold ions and stabilizing the gold nanoparticles. Algal-mediated gold nanoparticle synthesis mechanism was proposed to involve electrostatic interactions between gold anions and algal functional groups. The AuCl_4^- anion binds to positively charged functional groups, such as amino groups (NH_2), on the algal surface. After 40 and 50 min, algal extracts reduced Au (III) to gold nanoparticles. Hydroxyl groups present in the brown algal polysaccharides were involved in the bioreduction of Au (III) ions into Au (0) [38]. The hydroxyl groups are present on the secondary metabolites, polysaccharides, and monosaccharides [22,23] of the algal material which might be involved in the synthesis of gold nanoparticles [9].

Conclusions

In conclusion, the gold nanoparticle was synthesized using the algae extract of *T. conoides* as this seaweed is an easily available marine source. The synthesized gold nanoparticles were preliminarily examined by the color change of reaction mixture to dark pink. Furthermore, UV-vis spectrophotometer shows that the strong band at 520 to 525 nm indicates small spherical-shaped polydispersed nanoparticles, which were also confirmed by TEM, and the size ranges from 6 to 10 nm. Four intense peaks shown in the diffraction analysis indicate

the crystalline structure of synthesized gold nanoparticles. The polyphenolic and amide linkages in the proteins or enzymes present in the algae extract may be responsible for the reduction of gold ions to gold nanoparticles that was identified using FTIR spectrum. This green synthesis is an efficient, environmentally reliable, less time-consuming, and single-step process, and it reveals that algae extract as a competent resources for manufacturing nontoxic biomimetic and biocompatible gold nanoparticles.

Methods

Chemicals

Gold chloride was obtained from Hi Media, Mumbai, India. All glasswares have been washed with distilled water and dried in oven before use. Dried algae of *T. conoides* have been collected from the Gulf of Mannar, South India.

Preparation of algae extract

The dried algae of *T. conoides* were washed several times with distilled water to remove the waste materials. Algae extract used for the synthesis was prepared from 5 g of thoroughly washed seaweed in a 500-mL Erlenmeyer flask and boiled in 100 mL double-distilled water for 15 min. Filtered algae extract was stored at -15°C for further use, being usable for several weeks.

Green synthesis of gold nanoparticles

To prepare gold nanoparticles, 10 mL of algae extract was added into 90 mL of 1 mM aqueous solution of gold chloride. The reduction was started at 10 min in room temperature, resulting in a color changing of solution indicating the formation of AuNPs. The bioreduction of gold ions in the solution was monitored periodically by measuring the UV-vis spectra (350 to 700 nm) of the solutions.

Characterization studies

UV-vis spectroscopy measurements (Lambda double-beam UV-Spectrophotometer, Perkin Elmer, Waltham, MA, USA) was carried out as a function of time of the reaction at room temperature operated at a resolution of 1nm. The reduction of gold ions was confirmed by qualitative testing of supernatant, obtained after centrifugation. Transmission electron microscopic analysis was done using a TEM (CM200, PHILIPS, Amsterdam, The Netherlands). Three microliters of the sample was placed on the carbon-coated copper grid, making a thin film of sample on the grid, and an extra sample was removed using the cone of a blotting paper and kept in a grid box sequentially. XRD measurements of film of the green-synthesized and washed gold nanoparticle solution cast onto glass slides were done on a Bruker AXS D8 Advance X-Ray Powder Diffractometer (Bruker AXS, Inc., Madison, WI, USA) operating at a voltage of 40 kV and current of 30 mA. The FTIR spectra of algae extract-synthesized AuNPs were analyzed which discussed for the possible function groups for the formation of AuNPs. The gold nanoparticle pellet obtained after centrifugation was redispersed in water and washed (centrifugation and redispersion) with distilled water for three times. Finally, the pellet was redispersed in water prior to FTIR (RX1 spectrophotometer, Perkin Elmer, Waltham, MA, USA) analysis.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SR carried out the nanoparticles synthesis and characterization. CM, MV, and GG carried out the sample collection. KP carried out the manuscript preparation. GA and CK supervised the research work and corrected the manuscript. All authors read and approved the final manuscript.

Authors' information

SR completed M.Sc. Biotechnology in Periyar University and obtained his Ph.D. degree in Nanotechnology under the guidance of GA. He published 11 research articles in nanoparticles synthesis. He is interested in the metallic nanoparticles synthesis using algae and algae-derived compounds and their potential applications in the biomedical field. CM completed B.Sc. in Zoology and M.Sc. Environmental Biotechnology in SPKCES, Manonmaniam Sundaranar University, Alwarkurichi, Tirunelveli. She achieved her Ph.D. degree under the guidance of GA in the field of nanotechnology. Her research interests include synthesis of semiconductor nanomaterials and metal nanoparticles and its biomedical applications. MV obtained her M.Sc. in Environmental Biotechnology in Sri Paramakalyani Centre for Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, Tirunelveli. She obtained her Ph.D. degree under the guidance of GA in the field of Nanotechnology. Her research interests include eco-friendly synthesis of nanoparticles and its application in the environment. KP received his B.Sc. (2005) and M.Sc. in Biotechnology (2007) from Madurai Kamaraj University and Periyar University, India, respectively. He is currently working to obtain his Ph.D. degree at Manonmaniam Sundaranar University in India. He is interested in the green and immobilized microbe-mediated synthesis process of nanoparticles and nanocomposites and their biomedical and textile industry applications. GG completed M.Sc. in Biotechnology in Periyar University and obtained her Ph.D. degree in Nanotechnology under the guidance of GA. Her research interests include green-mediated synthesis of nanoparticles and its agricultural applications for controlling plant diseases. CK had done his master's degree and doctoral degree in the discipline of Chemistry. His research interests are in the field of Green Chemistry, Environmental Chemistry, and synthesis of

nanoparticles. Currently, he works as an Associate Professor in MSU, Tirunelveli. GA received his M.Sc. in Applied Chemistry (1992) and Ph.D. in Environmental Biotechnology (1997) from Anna University, India. He had 10 years (1999 to 2008) post-doctoral experiences from the National Taiwan University in Taiwan, National Institute of Advanced Industrial Science and Technology in Japan, and National Central University in Taiwan. He has received many research awards from Indian and other country governments. He is an associate editor in five international journals. At present, he is an Associate Professor of the Environmental Biotechnology and the leader of Environmental Nanobiotechnology Division at SPKCES, MS University, India. His main research interest is on biosynthesis of nanoparticles and nanomaterials, nanobiocatalyst, and environmental chemistry.

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