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Green synthesis and characterization of La_2O_3 nanoparticles using *Eclipta prostrata* and its antibacterial activity

E. Amutha[1], M. Margret Leema[3], SP. Manobala[1], S. Amutha[1], S. Rajaduraipandian[2], M. Gandhimathi[2], G. Annadurai[*1]

[1]*Sri Paramakalyani Centre of Excellence in Environmental Sciences, Manonmaniam Sundaranar University, Alwarkurichi, India*

[2]*Department of Chemistry, Sri Paramakalyani College, Manonmaniam Sundaranar University, Alwarkurichi, India*

[3]*Department of Chemistry, Arignar Anna College, Manonmaniam Sundaranar University, Aralvoimozhi, India*

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Abstract

Advances in the field of nanoscience and nanotechnology to modulate materials at nanoscale level has continued to have great impact on different disciplines of science and engineering as well as agriculture and medical fields. Metallic and metal-oxide nanostructures have shown great potential due to their high surface to volume ratio and high reactivity. Among them, LaO has revealed wider applicability, including in nanomedicine, where LaO nanomaterials have shown great potential leading to effective interactions with biological membranes and exhibiting antibacterial and/or anticancer behaviors. In the current study, the biosynthesis of La_2O_3 NPs was attained by a biosynthesized method by using the aqueous leaf extract of *Eclipta prostrata*. La_2O_3 NPs were characterized by UV, FTIR, XRD, SEM, FL, PSA and TGA methods. The X-ray diffraction displayed the existence of La_2O_3 NPs which is confirmed by the incidence of peaks at 28.32 corresponds to 002 anatase form. The La_2O_3 NPs showed significant antimicrobial activity against all the tested microorganisms.

***Corresponding Author:** G. Annadurai ✉ gannadurai@msuniv.ac.in

Introduction

The use of biomolecules presents in plant extracts to reduce metal ions to nanoparticles in a single-step green synthesis process has been found to be cost effective and eco-benign over the physical and chemical methods. Development of bioinspired material for fabrication of nanoparticles is the cutting edge of research and is a suitable process for large-scale production (Kalishwaralal *et al.,* 2018, Anuradha *et al.,* 2014). Among the living organisms, the plant materials gain more attention in the nanoparticle production. In recent years, developments of nanotechnology field have triggered increased interest in using the unique properties of nanomaterials for environmental applications (Narayanan *et al.,* 2004). LaO NPs synthesis has attracted particular interest, compared with other NPs, as their useful properties are achievable at costs lower than silver and gold (Zhang *et al.,* 2013). Research into LaO NPs has made significant progress in the areas of nanotechnology and nanomedicine within the last decade due to their excellent catalytic, optical, electrical and antifungal/antibacterial applications (Han *et al.,* 2006, Ponce *et al.,* 2005). In addition, the plant-mediated synthesis was found to be rapid, flexible, and suitable process for large-scale nanoparticle production. Plant parts like fruit (Huang *et al.,* 2008), leaf (Ankamwar *et al.,* 2005), bark (Narayanan *et al.,* 2004), seed (Sathishkumar *et al.,* 2009), and stem (Bar *et al.,* 2009) extracts are being effectively used in green synthesis.

Eclipta prostrata (syn. *Eclipta alba*) commonly known as false daisy, yerba de tago, and bhringraj, is a species of plant in the family Asteraceae. Other common names include kehraj in Assamese and karisalaankanni in Tamil (Daisy *et al.,* 2012, Kritikar *et al.,* 1975). This plant has cylindrical, grayish roots. The solitary flower heads are 6–8 mm in diameter, with white florets. The achenes are compressed and narrowly winged. This species grows commonly in moist places as a weed in warm temperate to tropical areas worldwide. It is widely distributed throughout India, China, Thailand, and Brazil (Chopra *et al.,* 1955, Thorat *et al.,* 2010). Therefore, the present investigation was aimed to green synthesized La_2O_3 NPs using *Eclipta prostrata* leaf extract. The synthesized La_2O_3 NPs to assess its antimicrobial activity against *S. aureus, Enterobacter, Psedomonous, Bacillus* and *E. coli* (Prakash *et al.,* 2011). Henceforth, this method could be appropriate for evolving a biological method for mass scale generation of nanoparticles.

Materials and Methods

Chemicals, Reagents, and Media

Analytical grade Lanthanum nitrate hexahydrate was purchased from Sigma Aldrich Pvt Ltd, India. Bacterial isolates, Gram-positive *Staphylococcus aureus* and *Bacillus subtilus*, and Gram-negative *Escherichia coli, Enterobacter* and *Pseudomonas fluorescens* were procured from standard vendors.

Collection of Plant Samples and Preparation of Eclipta prostrata Extract

Fresh leaves of *Eclipta prostrata* was collected from the local area land, seval, India. The fresh leaves were then washed multiple times with tap water followed by distilled water. The leaves were then dried in an oven for an hour and then ground to form a fine powder. 5 grams of fine powders are boiled with 100ml of deionized water at 80^0C for 30 min and the extract is then filtered using Whatman No. 1 filter paper. The filtrate was stored at 40^0C for further use.

Green synthesis of La_2O_3 nanoparticles

Lanthanum nitrate was used as the precursor for the synthesis of the La_2O_3 NPs. 20 mL of the *Eclipta prostrata* leaves extract was added dropwise with 80 mL of 1 mM Lanthanum nitrate solution at room temperature. The resultant mixture was stirred using a magnetic stirrer for 30 min and the formation of intense pale yellow colored solution confirmed the synthesis of Lanthanum oxide nanoparticles. The nanoparticles were separated by centrifugation at 6000 rpm for 20 min and cleansed by subsequent washing with ethanol and water about 2-3 times. The NPs were finally dried in a hot air oven at 80^0 C for 3 hr and calcinated for 400^0 c as detailed as shown in Fig 1.

Fig. 1. Synthesis of Lanthanum oxide NPs.

Antibacterial Property

The antibacterial property of the Lanthanum oxide NPs was determined by using the bacterial species including the pathogenic bacteria such as Staphylococcus *aureus, Bacillus subtilus*, *Enterobacter,* and *Pseudomonas fluorescens*, by the well diffusion method. The different concentrations used were at 25µl, 50µl, 75µl and 100µl for the identification of antimicrobial activity of the above bacterial species. All the plates were incubated at 37°C for 24 hours, and the zone of inhibition of bacteria was measured.

Result and discussion

Optical analysis

The UV absorbance spectrum of La_2O_3 nanoparticles for the wavelength length range (200-800nm) was recorded using UV-visible spectrophotometer. The UV absorption spectrum of as-prepared and calcinated La_2O_3 nanoparticles prepared by green synthesis method were investigated. By this regard, the SPR band was noticed at 260 and 320nm and confirmed the formation of La_2O_3 nanoparticles by using Eclipta prostrata extract also shown in Fig 2.

It can be seen that there is a slight increase in the absorption and slight decrease in the transmittance when the sample is subjected to calcinations. The UV study illustrates the absorbance spectrum of *Eclipta Prostrata* and La_2O_3 with their cut-off wavelengths. (Saleem, 2017; Karthikeyan and Selvapandiyan 2018, Guguloth Ravi *et al.,* 2019; Manoj Kumar *et al.,* 2020; Maheshwaran *et al.,* 2021). This confirms that La_2O_3 can be used for optical applications.

Structural analysis

The crystalline nature of biosynthesized La_2O_3 nanoparticle was determined by X-Ray diffractometer. The XRD spectrum of nanoparticle was represented in Fig 3. The XRD patterns generally show the broad diffraction peaks, indicating nanocrystalline nature of the sample. The diffraction peaks corresponding to (001), (002), (101), (102), (211), (201), and (202) planes are shown for La_2O_3. The XRD patterns obtained in the present work confirmed the formation of hexagonal phase for La_2O_3 (JCPDS No. 05-0602). The crystalline peak at (211) is due to the presence of smaller quantities of La $(OH)_3$ (Wang *et al.,* 2014). The significant broadening of the peaks indicates that the particles have nanometer dimensions. Estimated from the Scherrer formula, calculation of particle sizes from the broadening of the XRD peaks (i.e., $D = 0.891 \lambda/\beta \cos \theta$, where D is the average grain size, λ is the X-ray wavelength (1.5418 A°), and h and b are the diffraction angle and full-width at half maximum of an observed peak, respectively (R. Jenkins *et al.,* 1996). From Scherrer's formula, the size of crystallite was calculated at 32nm.

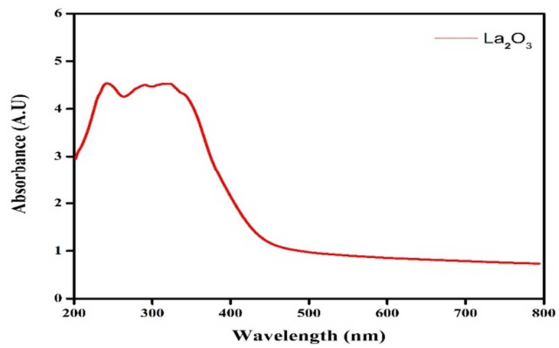

Fig. 2. UV spectrum of La_2O_3 nanoparticles.

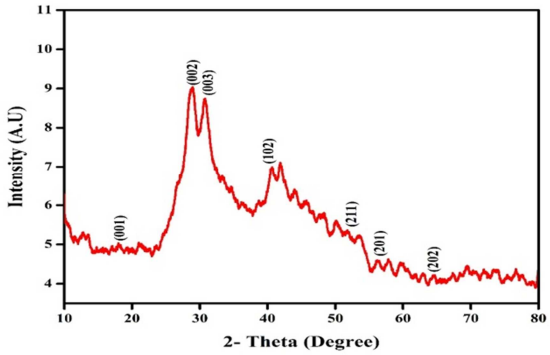

Fig. 3. XRD pattern of La_2O_3 nanoparticles.

Vibrational analysis

The FTIR spectrum of biosynthesized La_2O_3 nanoparticle was represented in the Fig 4. The functional groups responsible for the synthesis of nanoparticle and the functional groups corresponding to the peak value are presented in the Table 1. The peak at 3415cm^{-1} is due to the stretching vibrations of water molecules. The CN stretching of nitriles can be seen at 2212cm^{-1}.

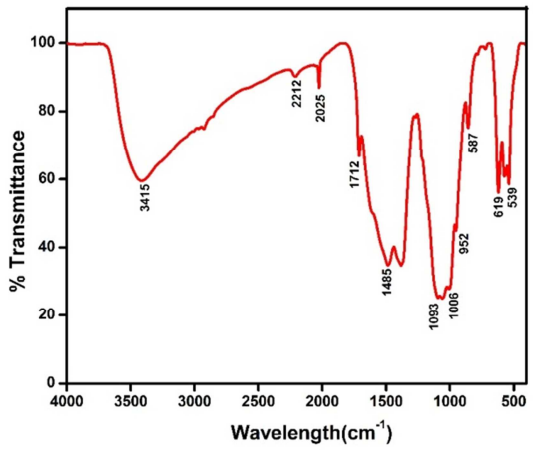

Fig. 4. FTIR spectra of La_2O_3 Nanoparticle.

The organic stretching of C=O can be found at 2025 and 1712cm^{-1}. The peak at 1485cm^{-1} is attributed to the vibrations produced due to the ions present in the solution. The vibrations due to alcohols, phenols and acids present in the plant extract can be found around 1093 and 1006cm^{-1}. The stretching due to la-O can be found around 619cm^{-1}. The halide impurities present in the water and plant extract vibrates at 539cm^{-1}.

Table 1. FTIR Peak value and corresponding function groups.

SN	Peak (cm^{-1})	Functional groups
1	3415	OH stretching of water molecules
2	2212	C≡N stretching of nitriles
3	2025	C=O stretching of organics
4	1712	C=O stretching of organics
5	1485	Stretching vibrations due to ions
6	1093	C-O stretching of acids, phenols, alcohols
7	1006	C-O stretching of acids, phenols, alcohols
8	952	=C-H bending of alkenes
9	887	=C-H bending of alkenes
10	619	La-O stretching
11	539	C-X stretching of alkyl halides

Morphological analysis

The shape and size of the morphology of synthesized La_2O_3 nanoparticles by using *Eclipta prostrata* extract were inspected via SEM micrographs which exhibited in Fig 5. The SEM micrographs labeled the synthesized La_2O_3 nanoparticles have mostly hexagonal and the remaining particles were rectangular rod, spherical and undefined shape (Jing *et al.,* 2017).

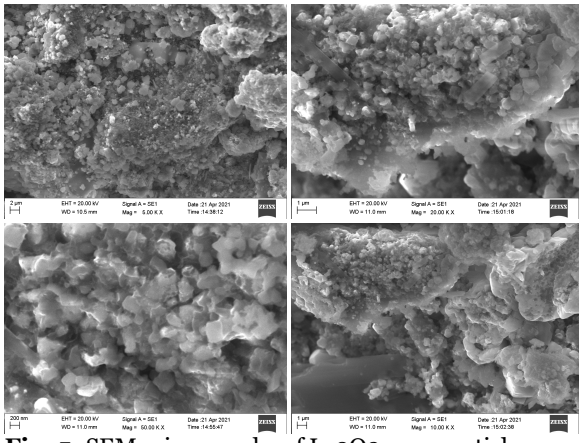

Fig. 5. SEM micrographs of La2O3 nanoparticles.

The slight aggregation of particles was detected overusing the biological capping agents in the *Eclipta prostrata* extract such as amino acids, flavonoids, proteins, carbohydrates, etc., moreover, the aggregation of small La_2O_3 nanoparticles was the reason for viewing of larger La_2O_3 nanoparticles. The particles were formed with small size including well-shaped and stabilized by the control of bioactive reducing, capping and stabilizing agents in the *Eclipta prostrata* extract (Maheshwaran *et al.,* 2020).

Thermogravimetic Analysis

The heat stability of the biosynthesize La_2O_3 nanoparticle was analyzed using a thermal analyzer and the results were given in the Fig 6. The fig. o indicates the differential thermal analysis of La_2O_3 nanoparticle. The first weigh loss was observed at 56.6°C and the loss was attained due to the loss of volatile organic moieties present in the sample. Second loss was observed at 134.2°C and the loss was due to the removal of moisture present in the sample. The third loss at 252.1°C was due to the oxidation of organic compounds present in the biomaterial chosen for the nanoparticle synthesis (Mahnaz *et al.,* 2015).

There was weight loss at regular intervals and the La_2O_3 nanoparticle was stable up to 607.5 °C and on further rise in the temperature no notable loss was obtained. Hence, the biosynthesized nanoparticle was stable to higher temperature.

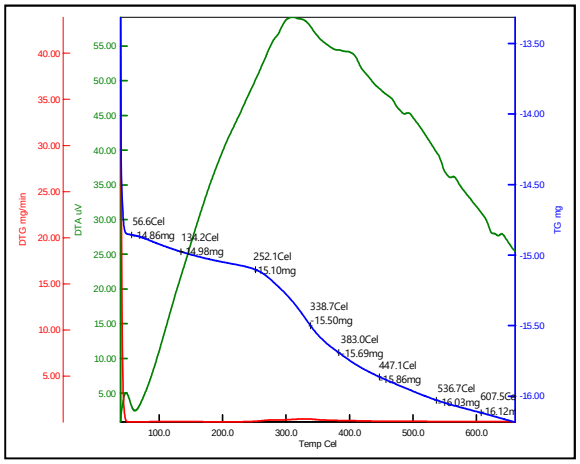

Fig. 6. TGA graph of La_2O_3 Nanoparticle.

Fluorescence spectroscopy

The fluorescence spectra of the La_2O_3 nanoparticle were recorded at room temperature by Fluorescence spectrophotometer. Fig 7, represents the fluorescence spectrum of La_2O_3 nanoparticles. Absorption bands is seen at 361, 480 and 645nm can be assigned to the intraligand π→π transition of the functional groups present in the reduction medium and the nitrates of lanthanum particles. There was any transition in visible region due to Lanthanum (III) has d^0 electron configurations.

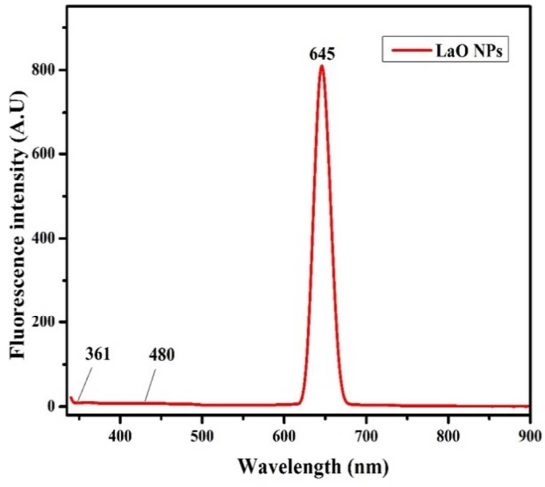

Fig. 7. Fluorescence spectrum of La_2O_3 nanoparticles.

DLS Particle size analyzer

The particle size of nano powders is done by particle size analyzer (Nano particle size analyzer SZ100). The size distribution of the Lanthanum nanoparticles was measured by Dynamic Light Scattering (DLS). Dynamic light scattering (DLS) uses a light source that emits through a solution containing particles and measures the amount of light reflected from the particles. The graph consists of size measurements with respect to intensity of scattered light, volume of the sample, or the measured number of particles in the sample. Fig 8 shows the distribution of particle size of La_2O_3 nano powders. The size is estimated as 60nm.

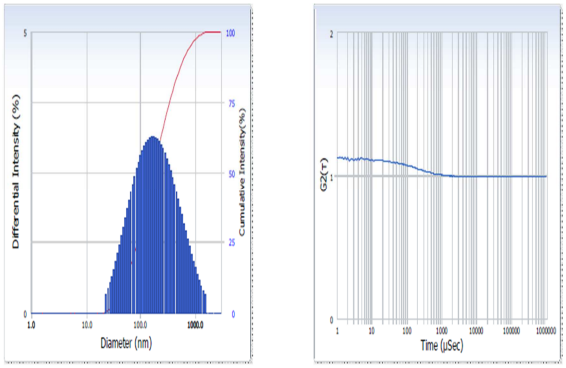

Fig. 8. DLS Particle size image of La_2O_3 nanoparticles.

Antibacterial activity

The medicinal efficiency was known from the antibacterial effect of formed La_2O_3 nanoparticles by using Eclipta prostrata extract upon *staphylococcus aureus, Pseudomonas* sp, *Bacillus subtilis*, *Enterobacter* and *Escherichia coli*. This zone of inhibition was displayed in Fig 9 and Fig.10.

Pathogenic bacteria are grown in nutrient broth and 24h culture of these strains were swabbed uniformly onto the individual's plates containing muller hinton agar using sterile cotton swabs. About 5 wells were made and the purified La_2O_3 NPs at different weight like 25µl, 50µl, 75µl and 100µl were added into each well on all plates as shown in Table 2. The plates were incubated for 24h at 37°C in an incubator. After incubation the different levels of zone formation around the well was measured. The antibacterial effect was further prominent against Gram-positive bacteria than Gram-negative bacteria.

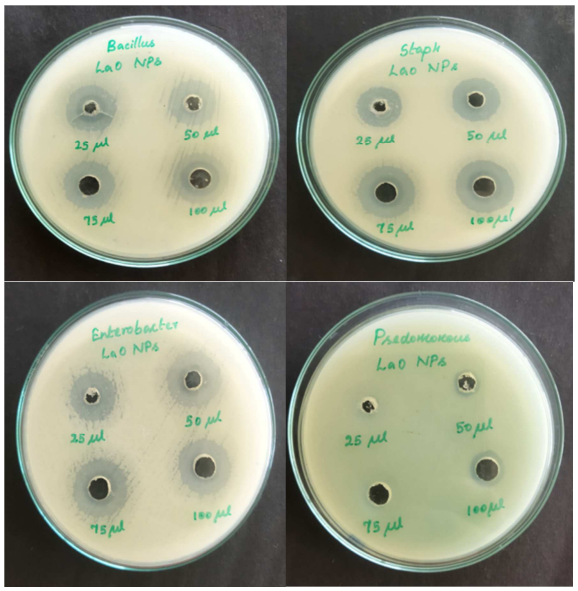

Fig. 9. Zone of inhibition of La_2O_3 NPs against various bacterial strains.

Table 2. Zone of inhibition of La_2O_3 NPs against selected bacterial strains.

Concentration	Zone of Inhibition (mm in diameter)			
	Bacillus sp	*Enterobacter sp*	*Staphylococcuus aureus*	*Pseudomonas* sp
25µl	1.6	1.6	1.4	0.4
50µl	1.7	1.8	1.5	0.8
75µl	2.0	1.8	1.8	1.1
100µl	2.1	1.9	2.0	1.2

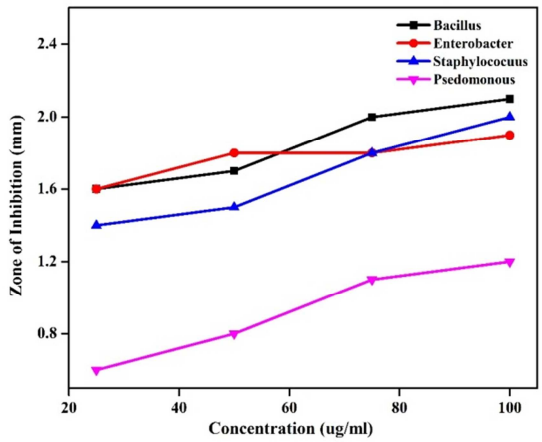

Fig. 10. Bar diagram La_2O_3 NPs against selected bacterial strains.

Conclusion

As the benefits of nanotechnology begin to move rapidly from laboratory to large-scale industrial production, the nanomaterials are used in all biomedical applications. The present method promotes the phase pure biosynthesis of lanthanum nanoparticle using the leaf extract of *Eclipta prostrata*. The synthesized La_2O_3 nanoparticles were completely characterized by using UV, FTIR, XRD, SEM, FL, PSA and TGA. This newer method provides the synthesis of La_2O_3 nano particles with faster and purest in a reliable shorter duration period. The synthesized nano particles were found to show excellent antimicrobial activity towards the tested microorganism provides an entrance for developing a new variety of antibacterial activity material. The bio reduction of metal will be boon for the progression of hygienic, harmless and ecologically satisfactory green method to yield metal nanoparticles. This could result in economic feasibility and environmentally friendly for drug delivery, treatment of various infectious diseases and cancer, commercial and sensors appliances and other electronic and medical applications.

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