SYNTHESIS OF $LaAlO_3:Dy^{3+}:Bi^{3+}$ NANOMATERIALS TO OPTICALLY MIMIC THE BIOLUMINESCENCE OF *PHOTINUS SCINTILLANS* (FIREFLY) AND ANTIBACTERIAL APPLICATIONS

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Abstract. *Photinus scintillans* is one of the species of fireflies, emits yellowish-green light with a peak wavelength of 575 nm. $LaAlO_3:Dy^{3+}:Bi^{3+}$ nanomaterials prepared through sono-chemical process can serve as material for photo luminescence. The wavelength of light emitted by *Photinus scintillans* can also be obtained by optically exciting the synthesized sample with 353 nm wavelength. Dipole transition $^4F_{9/2} \rightarrow {}^6H_{13/2}$ gives photon of wavelength 576 nm, which is exactly same as that emitted by *Photinus scintillans*. The color chromaticity shows the yellowish green emission region of nanoparticles. This material can be used for the preparation of LEDs to emit a wavelength of 576 nm. When LEDs are prepared from this material, they can mimic the bioluminescence of fireflies. Thus, prepared nanoparticles can mimic the bioluminescence of *Photinus scintillans* fireflies. Both Dy doped and Bi co-doped samples at higher concentrations showed zone of clearance for Gram positive bacteria. Bi co-doped at higher concentrations had substantial inhibition across gram positive and negative bacterial colonies.

Key words: *Photinus scintillans* (fireflies), luminescence, nanoparticle, antibacterial activity.

INTRODUCTION

Bioluminescence is a method in which living organisms change chemical energy into light energy. Organisms that produce light are said to be bioluminescent. This may be due to the survival, adaptation and finding a suitable mate. Fireflies are common organisms exhibiting this process in finding the mate. Species of bioluminescence can be found on the entire earth region, but likely to be in the depth of ocean, and these species help in photosynthesis in completely

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dark atmospheres and can emit light by different biochemistry processes for various purposes [1]. Fireflies being the wonderful individual to study, meanwhile, they use bioluminescence to catch companions at night, however, there are some firefly species that use other means (*e.g*., pheromones) to catch companions and fly throughout the day and night [11]. The luciferase enzyme interacts with its substrate, luciferin, in the presence of oxygen and ATP to release a photon of light, thus, producing a light signal [8]. Fireflies' pattern of precise flash may be utilized for companion gratitude and select [8]. The light signal emitted ranges from yellow (579 nm) to green (554 nm). However, a few species can emit light down 400 nm or up to 700 nm [1]. Fireflies light signal color varies among the same species and between different species. Hence, offer an exceptional scheme in to study signal production and response in the perspective of signaling environments [10]. Various studies reveal that female fireflies select mates based on particular male flash nature and features. Developed male fireflies exhibit higher flash rates and enhanced flash intensity, which is found to be attractive to females in two altered firefly species. There are more than 2000 species of fireflies in nature, each with unique flash pattern. They vary the rhythm and intensity of light to make them identifiable to other members of the family. There is one group of species, *Photinus scintillans*, which emits 575 nm wavelengths [16]. These species belong to phylum: *Arthropoda*, class: *Insecta*, order: *Coleoptera*, family: *Lampyridae*and genus: *Photinus*.

In recent years, the development of LED (light emitting diode) technologies has made a revolution in the global market, replacing all the conventional household light sources. LEDs are available in most possible wavelength ranges starting from ultraviolet to visible to infrared wavelengths. By choosing suitable material for the production of LED, the wavelength of light emitted can be tuned. Flickering LEDs are widely used in ornamental purposes. Flickering LEDs with wavelength of 575 nm, can mimic fireflies. A series of this type of LEDs, when spread on a plant will be a visual treat as it gives a beautiful impression of presence of fireflies on the plant. This has a great commercial application. This makes it possible to mimic natural bioluminescence of fireflies. Finding the proper LED material that has the luminescence with 575 nm or near 575 nm wavelength is the big challenge. To serve this purpose, phosphor nanoparticles can be used, which has multiple applications in the field of material science.

Phosphor nanoparticles show special luminescence properties by which it can transform the incident electromagnetic radiation of lesser wavelength to upper wavelength and vice versa. The conversion of lower wavelength radiation to higher wavelength radiation is of particular interest in the applications of LEDs. In LEDs, not all the energy is converted into visible light energy, some UV radiations also emerge. The UV radiations emitted can be converted into visible radiation by the use of a small coating of phosphor material, which increases the efficiency of LEDs.

In the present research work, lanthanum aluminate ($LaAlO_3$) nanoparticle doped with rare earth element dysprosium (Dy) and co-doped with bismuth (Bi) is synthesized by sono-chemical method. The reason for doping the material $LaAlO_3$ is to create defects in the material, which act as activators and sensitizers, and they increase the apparent to size portion during the synthesis process [22]. The presence of rare earth ions in the mass material enhances the diverseness of applications of phosphor materials. The choice of dopants shows a key part in the properties of phosphors. Here, trivalent Dy^{3+} RE ions produce transitions from $^4F_{9/2}$ level to the H- levels resulting in the emission of green wavelengths from the ion centers [17]. The method of preparation of the sample decides the morphology of the samples, like particle size, particle distribution and its accumulation, surface area. Nanoparticles can be prepared in many ways, but sono-chemical synthesis (SCS) is the most widely used efficient methods of preparation, because, of its simplicity, versatility and cost effectiveness. SCS gives high pure sample of desired composition at a rapid pace and highly suitable for commercial scaled up productions. This method also makes it possible to alter many parameters without affecting the repeatability of the properties [3, 5, 20]. The nature of the nanoparticles formed depends on the temperature, nature of reducing agent, ratio of reducing agent to oxidant material. In the present research work, $LaAlO_3$, Dy^{3+} activated and Bi^{3+}co-doped nanoparticle was prepared by sono-chemical method using sugar as reducing agent and size, constituent, luminescent properties have been studied to prepare a material in the manufacture of LED's which can mimic the fireflies. Bacterial infections are one of the major reasons for diseases. Many researchers have shown that, nanoparticle have antibacterial properties. In the present research the antibacterial activity of prepared samples has been studied and the results were shown graphically.

MATERIALS AND METHODS

SYNTHESIS OF $LaAlO_3:Dy^{3+}:Bi^{3+}$NANOPARTICLES

The chemicals $Al(NO_3)_3:9H_2O$, $La(NO_3)_3:6H_2O$, $Bi(NO_3)_3:H_2O$, $Dy(NO_3)_3:H_2O$ were of analytical grade, have been used to synthesize lanthanum aluminum oxide ($LaAlO_3$)using pure sugar (analytical grade) purchased from standard firms have been used as reducing agent in sono-chemical method [2, 21]. The ratio of oxidizing agent (O) to reducing agent was kept unity [5]. The stoichiometric ratio of precursors taken in a beaker were liquefied using purified water and by mixing with magnetic stirrer for about 10 min to get uniform solution at room temperature. Later dopant/co-dopant and reducing agent were mixed gently

into the solution and stirred using magnetic stirrer. The high intensity, pulse mode Titanium horn ultrasonic probe was presented in the resulting mixture for nearly 1 hour. Lastly, the precipitous is attained and divided by centrifugation carefully for a number of times by double distilled water. The resulting powder was dried at 80 °C for nearly 24 hours and calcined at nearly 900 °C for 3 hours in a muffle furnace.

ANTIBACTERIAL ACTIVITY BY DISC IMPREGNATION METHOD [15]

Preparation and dilution of nanoparticle for antibacterial activity: Various dilutions of the prepared nanoparticle, ranging from 5 mg/mL to 30 mg/mL, were added to dimethyl sulfoxide. From Whatman No. 1 filter paper, 6.0 mm diameter discs were produced and saturated with various nanoparticle dilutions.

BACTERIAL STRAINS USED

By using the disc diffusion method, the antibacterial activity of nanoparticles was evaluated against four micro-organisms. *Staphylococcus aureus* and *Bacillus subtilis* are two gramme positive bacteria. Escherichia coli and *Pseudomonas aeruginosa* are two gramme negative bacteria. The bacterial strains for the study were procured from Azymes Biosciences Pvt. Ltd.

INOCULUMPREPARATION

The microbial strains were grown on nutrient agar medium and kept at 37 °C for 18 to 24 hours as a protective measure. The inoculum was supplied with nutrient broth; the microbial strains were grown in the broth for 18 hours at 37 °C and then stored at 4 ^{o}C for later use. The bacteria culture's density was set at 0.5 Mc Farland standards. The observation was conducted by adhering to the prescribed standard protocol [24]. 20 mL of nutritional agar media were dispensed into sterile petri plates and allowed to freeze. Approximately 100 µL of inoculums were discharged and routinely cleaned using a cotton swab. Agar plates were placed with nanoparticle impregnated discs (0, 5, 10, 20 and 300 g/mL) on them, and they were then incubated at 37 ^{o}C for 24 hours. Ciproflaxacin (1 mg/mL) and dimethyl sulfoxide (DMSO) were employed as the positive and negative controls, respectively. Here, DMSO is used because it is an important polar solvent that dissolves both polar and non-polar compounds and is miscible in a wide range of organic solvents as well as water. By measuring the diameter of the clear zone of inhibition surrounding the discs, antibacterial activity was calculated. The assessment was conducted three times. The mean zone of inhibition diameters (mm) formed by the leaf extract was used to measure antibacterial activity.

TECHNIQUES OF CHARACTERIZATION

The powder X-ray diffraction (PXRD) spectra using copper K_α radiation (1.5418 Å) were taken to know the size of the material. Here, powder X-ray diffraction method was used because it can identify unknown crystalline materials and helps to determine the crystallite sizes also. The optical characteristics of the material were investigated using a photoluminescence spectrofluorometer.

RESULTS

SIZE OF THE $LAALO_3$ NANOPARTICLE

Crystallite sizes of the synthesized nanoparticle were examined using PXRD spectra which were shown in Fig. 1. Rhombohedral structure with space group R 3m and lattice parameter a = 5.35700 Å was confirmed by the diffraction pattern. The diffraction peak was well matched with the file no. 85-1071 when compared with Joint Committee on Powder Diffraction Standards (JCPDS). JCPDS is an organization which maintains the powder X-ray diffraction pattern and characteristics files of most of the crystals. It forms a standard record to compare the synthesized compounds with the file and confirmation prepared sample can be made. Scherer formula (Eq. 1) was used to find the average crystal size (D).

$$D = \frac{k\lambda}{\beta\cos\theta} \tag{1}$$

where k is the Scherer constant, λ is the wavelength of copper alpha radiation, β is the full width at half maxima and θ is the half Bragg angle. The calculated crystallites were between 24-26 nm. The reduction in size for the doped/co-doped sample was due to deficiencies that arise in the nanoparticle, because of smaller ionic radius of Dy^{3+} (91 pm) in contrast to lanthanum ionic radius (122 pm). Addition of Dy^{3+} to the host matrix replaces La ions in the structure and creates defects which origins orbital reduction leads to improved density of orbital levels.

Also, the lattice strain dependent size of the nanoparticle can be verified with (Eq. 2) [13] by William-Hall method as shown in Fig. 2.

$$\beta\cos\theta = \frac{k\lambda}{D} + 4\varepsilon\sin \tag{2}$$

The linear fit of the plot between β cos θ and 4 sin θ showed the incline of the line which is the measure of strain (ε) and the intercept [(0.9λ)/D] gives the crystallite size. The result nearly resembles with the size of the crystal obtained by Scherer's method.

Photoluminescence (PL) study of $LaAlO_3:Dy^{3+}$ and $LaAlO_3:Dy^{3+}:Bi^{3+}$ nanophosphors: This method clearly confirms the exact wavelengths the synthesized material is going to emit upon excitation with some known wavelength. A standard material is excited, and the wavelengths emitted will be shown on the material under study.

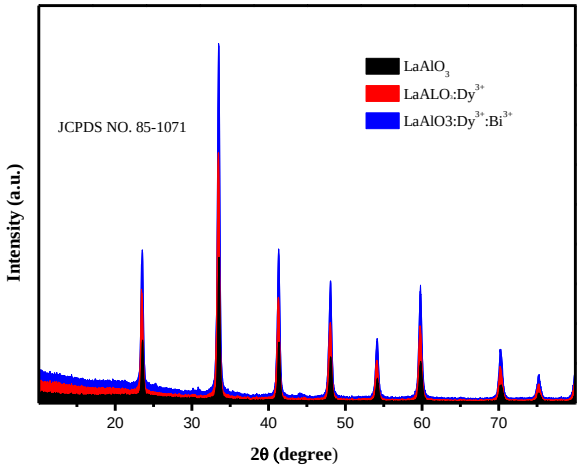


Fig. 1. PXRD diffraction pattern of lanthanum aluminate nanoparticles.

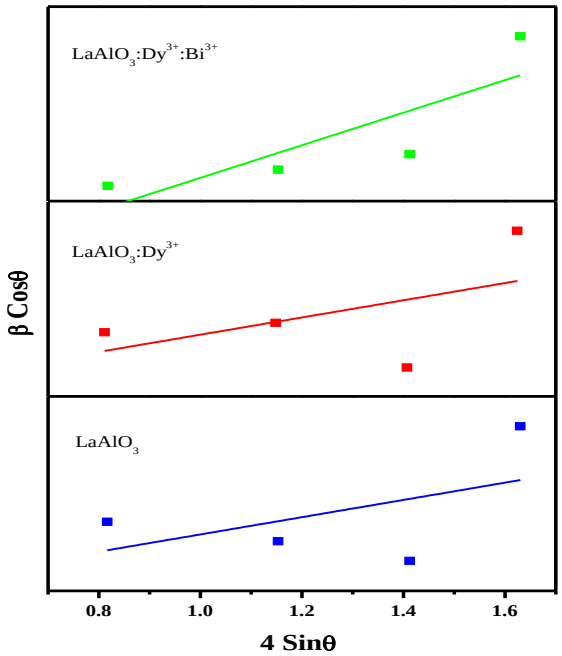


Fig. 2. W-H analysis of lanthanum aluminate nanoparticles.

Even though it has many wavelengths (Fig. 3), 322 nm, 353 nm, 367 nm, 367 nm, 389 nm and 428 nm, the highest intensity peak is 353 nm. The effect of this peak on both the samples (Dy doped and Bi co-doped) will be considerable, which results in the emission spectra.

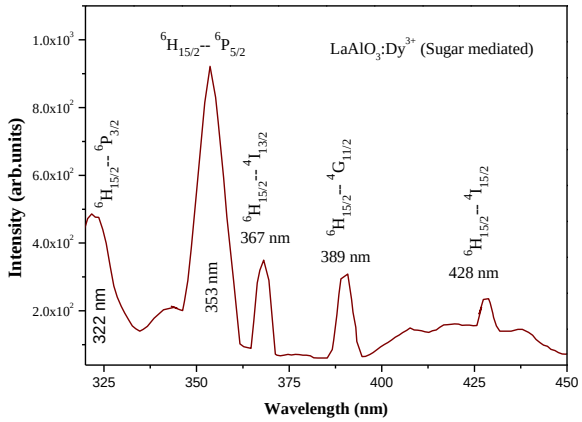


Fig. 3. Excitation spectra of $LaAlO_3:Dy^{3+}$nanophosphor for the emission wavelength of 576 nm.

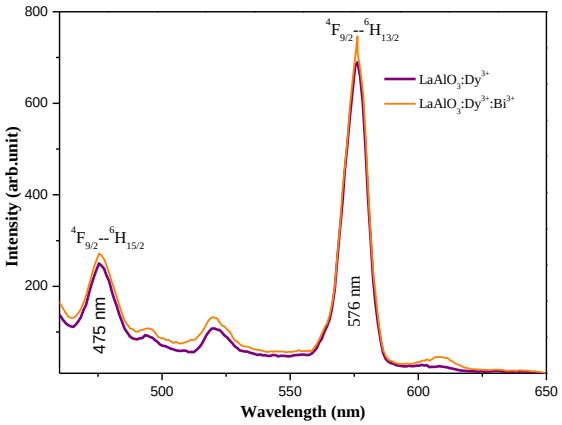


Fig. 4. Emission spectra of lanthanum aluminate nanophosphor for the excitation wavelength of 353 nm.

Figure 4 shows, emission spectra of $LaAlO_3:Dy^{3+}$ and $LaAlO_3:Dy^{3+}:Bi^{3+}$ phosphor. Sharp characteristic peaks between the ranges 450-490 nm and 540-580 nm correspond to blue ($^4F_{9/2} \rightarrow {}^6H_{15/2}$) and yellow ($^4F_{9/2} \rightarrow {}^6H_{13/2}$) excitations of Dy^{3+} ion respectively. Because Dy^{3+} has two high intense transition levels from $^4F_{9/2} \rightarrow {}^6H_{15/2}$ and $^4F_{9/2} \rightarrow {}^6H_{13/2}$ levels [11]. The weak transition $^4F_{9/2} \rightarrow {}^6H_{15/2}$ was due to magnetic dipolar transition emitting blue wavelength at 475 nm [18, 19, 20]. The high intense peak for the transition $^4F_{9/2} \rightarrow {}^6H_{13/2}$ was due to electric dipole-transition

emitting yellowish green wavelength at 576 nm. Even though, the spectra confirm the presence of both electric and magnetic dipole transitions, the latter is weaker. It was also evidenced that intensity of emission is more for Bi^{3+} co-doped material.

DETERMINATION OF COLOR CHROMATICITY

The actual emission chromaticity of the luminescent material can be calculated using *Commission International de l'Eclairage* (CIE – The International Commission on Illumination) software [4, 24]. CIE is the international authority on light, illumination, color, and color spaces. It was established in 1913. According to the CIE, every color can be expressed as three coordinates in space (RGB). These color coordinates recognize that human visual system uses three primary colors: red, green, and blue. The CIE chromaticity coordinates (*X*, *Y*) were calculated using photoluminescence spectroscopy (PL) data using the equations (3) and (4) and plotted in Fig. 5. In PL, a substance under study is excited by irradiating with laser light of known wavelength and the intensity and wavelength of radiation emitted by the substance during de-excitation is studied.

$$x = \frac{X}{X+Y+Z} \tag{3}$$

$$y = \frac{Y}{X+Y+Z} \tag{4}$$

The color coordinates of the synthesized $La_{0.99}Dy_{0.01}$ AlO_3 and $La_{0.98}$ $Dy_{0.01}$ $Bi_{0.01}$ AlO_3 nanoparticle confirmed the green-yellow emission phosphor, shown in Fig. 3. This suggests that the synthesized material is suitable to make LEDs which can emit 576 nm wavelengths.

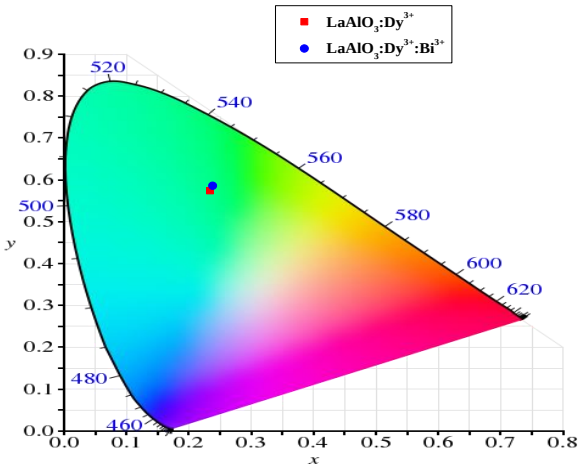


Fig. 5. CIE chromaticity plot of $LaAlO_3:Dy^{3+}$and$LaAlO_3:Dy^{3+}:Bi^{3+}$nanophosphor [13].

ANTIBACTERIAL STUDIES

The results were interpreted depending on the clearance zone seen in each of the petri plates. Current analysis demonstrated excellent inhibition by Bi co-doped in comparison to Dy doped. However, higher concentration of both the samples showed zone of clearance for Gram Positive bacteria. Bi co-doped at higher concentration had substantial inhibition across gram positive and negative bacterial colonies as shown in Table1 and in Figs 6, 7(a), and 7(b). However, present study also highlights the use of Bi co-doped (30 mg/mL) as an effective bactericidal agent against gram negative bacteria, as many of them are pathogens [24, 21].

In this study, we observed a better bacteriostatic potency of both nanoparticle at 20 and 30 mg/mL by blocking the growth of communalistic pathogen. Even though Bi co-doped, 20 and 30 mg/mL was having superior inhibition compared to Dy doped, which also exhibited good activity at higher concentration, which can be even considered. This trend of growth inhibition seen by zone of clearance can be strongly correlated to death of the colonies mediated by nanoparticle.

Table 1

Antibacterial activity of different human pathogens against two nanoparticles used in the study, $LaAlO_3$: Dy and $LaAlO_3$:Dy:Bi. Data represented $^{*}p < 0.05$ *vs*. 30 mg/mL of $LaAlO_3$:Dy and $^{\#\#}p < 0.01$ *vs*. 20 mg/mL of $LaAlO_3$:Dy

Anti-bacterial activity for $LaAlO_3$:Dy						
Bacterial strains used in the study	**Concentration (mg/mL)**					
	5	**10**	**20**	**30**	**Positive**	**Negative**
B. subtilis	0.00	0.00	06.83 ± 0.29	11.33 ± 0.58	21.33 ± 0.58	0.00
S. aureus	0.00	0.00	06.67 ± 0.58	08.33 ± 0.58	25.33 ± 0.58	0.00
E. coli	0.00	0.00	06.50 ± 0.70	07.33 ± 0.57	23.67 ± 1.15	0.00
P. aeruginosa	0.00	0.00	10.66 ± 0.58	17.33 ± 0.58	29.33 ± 0.57	0.00
Anti-bacterial activity for $LaAlO_3$:Dy:Bi						
Bacterial strains used in the study	**Concentration (mg/mL)**					
	5	**10**	**20**	**30**	**Positive**	**Negative**
B. subtilis	0.00	0.00	16.33 ± 0.58$^{\#\#}$	18.67 ± 1.15*	27.33 ± 0.58	0.00
S. aureus	0.00	0.00	07.50 ± 0.70	17.66 ± 0.58*	27.33 ± 1.15	0.00
E. coli	0.00	0.00	06.67 ± 0.58	13.67 ± 0.58	22.33 ± 1.53	0.00
P. aeruginosa	0.00	0.00	14.33 ± 0.58	19.66 ± 0.58	26.33 ± 1.52	0.00

Many studies have revealed NP-mediated bacterial inhibition may be due to leaky cell wall, damaged genetic material, malfunctioning of cell organelles, lipid peroxidation and many more owed by free radical triggered by nanoparticle. Our

investigation reveals the use of green synthesized Dy doped and Bi co-doped nanoparticle as the replacement for antibiotics. Currently, most challenging situation in the medical field is treating deadly infectious diseases and nosocomial infections caused by several pathogenic microbes as they have greater tendency towards multidrug resistance. This report inspires us to open new window towards employing the nanoparticle as therapeutic prominence as they can be used in various fields like drug delivery, environmental uses, pharmaceuticals, agriculture and medicine as well [5, 6, 7, 8, 9, 12, 16, 23].

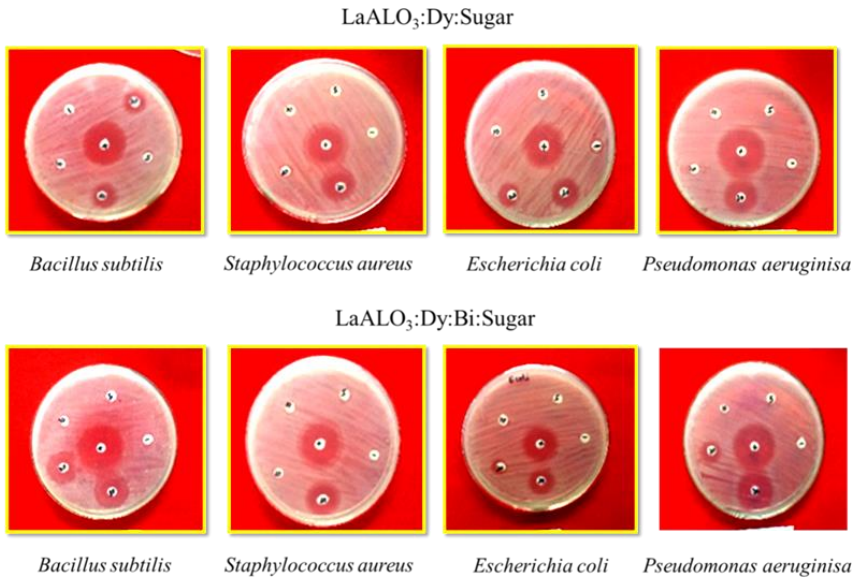
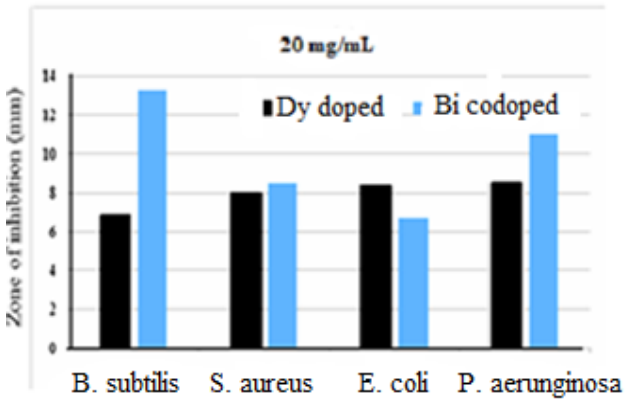
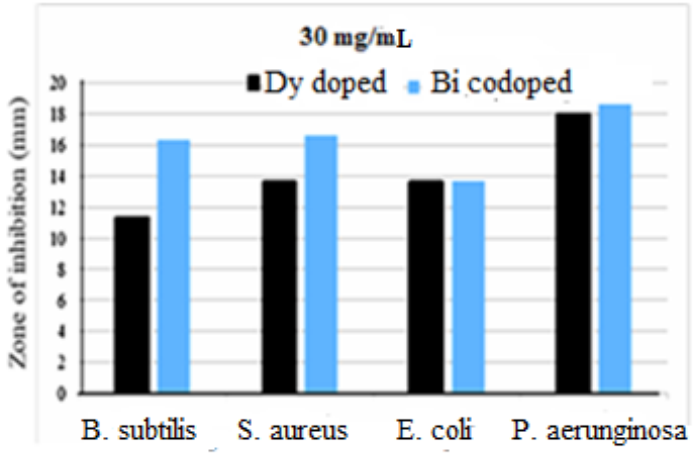


Fig. 6. Inhibition of bacterial activity of Dy doped and Bi co-doped nanoparticle using the disc impregnation method.



(a)



(b)

Fig. 7. (a) Zone inhibition for 20 mg/mL; (b) zone of inhibition for 30 mg/mL concentration for four bacteria.

CONCLUSIONS

Photinus scintillans is one of the species of fireflies, which emits yellowish-green light with a peak wavelength of 575 nm. $LaAlO_3:Dy^{3+}Bi^{3+}$ nanoparticle, which can produce similar bioluminescence as that of *Photinus scintillans* is synthesized by sono-chemical method. Optical studies reveal that, emission spectra of Dy^{3+} activated nanoparticle excited at 353 nm showed emission of two wavelengths 475 nm (less intense) and 576 nm (more intense), corresponding to $^4F_{9/2} \rightarrow {}^6H_{15/2}$ and $^4F_{9/2} \rightarrow {}^6H_{13/2}$ excitations respectively. Since the intensity of 475 nm is very weak, the prominent peak 576 nm will be the emission from the sample. The yellowish green emission area of nanoparticle is depicted using CIE color coordinates. This material can be used for the preparation of LED's, which can emit a wavelength of 576 nm. Since, *Photinus scintillans* species of fireflies emit 575 nm, when LEDs are prepared from this material can mimic the bioluminescence of fireflies. Thus, nanoparticle of Dy^{3+} doped and Bi^{3+} co-doped $LaAlO_3$ material when excited produce luminescence and can mimic firefly's flashes. Both Dy doped and Bi co-doped samples at higher concentrations showed zone of clearance for gram positive bacteria. Bi co-doped at higher concentration had substantial inhibition across gram positive and negative bacterial colonies. Amongst four bacteria used 30 mg/mL of Bi co-doped showed greater bactericidal activity for 30 mg/mL.

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