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Original Article

AJWAIN SEEDS AS CAPPING AGENT FOR Bi_2O_3 NANOFLAKES: SYNTHESIS AND GROWTH INHIBITING EFFICIENCY OF BACTERIA

BHARANI DHARAN SETHURAMAN[a], LAKSHMI PRABHA CHANDRASEKAR[b], MANIKANDAN SUBRAMANI[c], ASWATHY KARANATH ANILKUMAR[d], MUNUSWAMY-RAMANUJAM GANESH[e], SIVAKAMI MOHANDOS[a*]

[a,b,c,a*]Department of Chemistry, SRM Institute of Science and Technology, Kattankulathur, India. [d,e]IIISM, SRM Institute of Science and Technology, Kattankulathur, India

*Corresponding author: Sivakami Mohandos; Email: sivakamm@srmist.edu.in

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ABSTRACT

Objective: This work is mainly focused to determine the antibacterial activity of the green synthesized Bi_2O_3 nanoparticles against the bacterial strains, *Staphylococcus aureus* and *Escherichia coli* using resazurin as indicator.

Methods: Bismuth oxide nanoparticles were synthesized from the precursor bismuth nitrate [Bi $(NO_3)_3.5H_20$] by using trachyspermum ammi (ajwain) seed extract. To carry out these works, the synthesized Bi_2O_3 NPs undergone characterizations and were confirmed by UV-Vis, FT-IR, XRD, SEM and EDAX, TGA-DTA and DLS. Biological activity was done using a well diffusion method.

Results: Bi_2O_3 NP's has been tested against bacteria (*S. aureus* and *E. coli*) in wells and shows blue colour, indicating bacterial growth inhibition in a dose-dependent manner for different concentrations.

Conclusion: The biological studies were done with one gram-positive and one gram-negative bacteria to show the inhibiting efficiency. The synthesized bismuth oxide nanoparticles showed good anti-bacterial activity (different concentrations) against S. aureus and E. coli.

Keywords: Green synthesis, Trachyspermum ammi, Bismuth oxide, Biological activity

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INTRODUCTION

In today's world, nanotechnology has gained a lot of attention, especially in areas such as medicine, materials science and engineering. Nanoparticles are small and have a large surface area, making them highly valuable [1]. There is a growing interest in metal oxide nanoparticles because it has attracted a great deal of attention in many research fields due to their unique physicochemical properties [2]. One of the methods is the production of metal NPs using biological systems such as microbes, fungi and several plant extracts. Among these organisms plants seem to be the best candidates and they are suitable for large-scale biosynthesis of NPs. NPs produced by plants are more stable and the rate of synthesis is faster in the case of microorganisms. Moreover, the NPs are more various in shape and size in comparison with those produced by other organisms [3]. By the way, here the bismuth oxide nanoparticles have been synthesized because the beneficial properties such as lower toxicity, good conductivity and large energy band gap. It is also an excellent photo catalyst [4].

As a relatively low-cost and easily accessible material, bismuth and its compounds plays a key role in a wide range of applications [5]. Several methods can be used to synthesize metal oxide nanoparticles. Biodegradation of metal ions by plant extracts usually leads to the formation of nanoparticles. There is evidence that plant metabolites such as total and reduced sugars, terpenoids, polyphenols, alkaloids, phenolic acids, and macromolecules (proteins) can contribute significantly to reduce the metal ions and convert them into NPs, resulting in their stability [6].

Green synthesis is a one-step synthesis, eco-friendly and budget-friendly option. Since the plant extract contains various biomolecules, it is capable of acting as both a reducing and capping agent. Bismuth oxide nanoparticles perform both photocatalytic activity as well as biological activities, including antibacterial properties.

In this study, Bismuth oxide nanoparticles were synthesized by using Trachyspermum ammi (ajwain) seed extract. Trachyspermum ammi, is also known as "Ajwain" in scientific terms. Omam is the Tamil word for it and is a native of Egypt and also cultivated in north India. The colour varies from slightly green to brown in color and gives a pungent, bitter taste. These plants are used to treat a variety of ailments. The seed has anti-inflammatory effects and also prevents coughing and improves airflow. It also cures cold, diabetes and cholera. It helps in weight loss and helps in getting rid of alcohol. They have nutrients like copper, calcium, phosphorus, fibre, carbohydrates, energy, magnesium and iron. Ajwain leaves improve the health of the skin as it helps to maintain the hormonal balance in our body.

Bi_2O_3 nanostructure materials can be synthesized by numerous physical and chemical methods, i.e. pulsed laser deposition (PLD) [7], Epitaxial growth [8], thermal plasma [9], magnetron sputtering [10], chemical precipitation [11], metal-organic chemical vapour deposition (MOCVD) [12] and hydrothermal [13], etc.,

One of the well-popular transition metal oxide is Bi_2O_3 [14]. Therefore, the purpose of this study is to synthesize bismuth oxide nanoparticles using trachyspermumammi seed extract and to examine the anti-bacterial activity of bismuth oxide nanoparticles is reported against one gram-positive and one gram-negative bacterium.

MATERIALS AND METHODS

Chemicals

All of the compounds were of analytical quality. In this experiment, distilled water, Conc. HNO_3, [$Bi(NO_3)_3.5H_2O$] were employed.

Extracts and its preparation

The seeds of Trachyspermum ammi (ajwain) were collected and then the seeds were washed, dried and then powdered and processed into an aqueous extract. The extract was made by mixing 20g of powdered ajwain seed with 100 ml of distilled water and it is allowed to stir and boil for about 2 h at ambident temperature. The solution was filtered through the whatmann filter paper and allowed to cool. Thus, the extract was prepared.

Green synthesis of Bi_2O_3 nanoparticles

The image of the Trachyspermum ammi's seed and the synthesis procedure was given in the (fig. 1) and (fig. 2) respectively.

Biological activity

The antibacterial activity of the green synthesized bismuth oxide nanoparticles was measured against the bacterial strains, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) using Resazurin as indicator. This was carried out by resazurin assay method. This method is simple, sensitive, rapid and reliable, and could be used successfully to assess the antibacterial properties of compounds. S. *aureus* (Gram-positive) and *E. coli* (Gram-Negative) was used for determining the MIC in the current study. To isolate distinct colonies, bacterial cultures were cultivated on nutrient agar using the streak plate technique. Individual colonies were selected and injected in the nutrient broth after 48 h. After 24 h at a wavelength of 600 nm, both cultures were checked for growth using a UV-1800 spectrophotometer (Shimadzu, Japan) to get a final OD of 1. Resazurin solution was prepared by dissolving a 13.5 mg of resarzurin in 2.0 ml of sterile distilled water. A solution was vortexed for 1 min to get a well-dissolved and homogenous solution. A sterile 96-well plate was inoculated with bacterial culture along with, either antibiotic (Chloramphenicol 1µg/10 µl–positive control) or compounds and indicator.

Fig. 1: Image of the trachyspermum ammi's seed

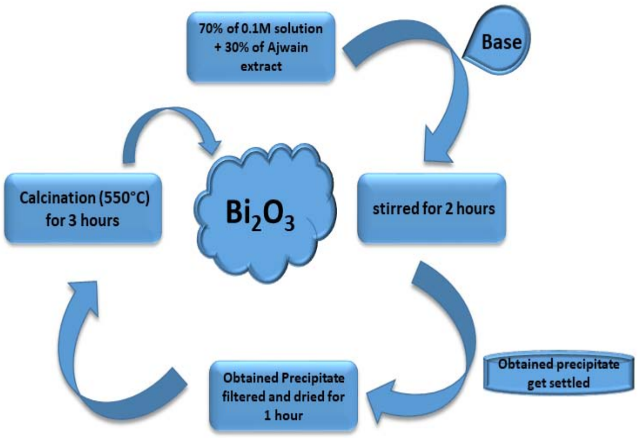

Fig. 2: Synthesis procedure of Bi_2O_3 NPs

Characterizations

UV-VIS

UV-Vis spectroscopic analysis was carried out to determine the optical properties of the synthesized bismuth oxide nanoparticles. Phytochemicals present in the seeds of ajwain plant play a role in the reduction and stabilization of the nanoparticles of bismuth oxide. Using distilled water as a reference, bismuth oxide nanoparticles were scanned in a quartz cuvette between 200 and 800 nm of wavelength. The λ_{max} at 282 nm (fig. 3) shows the presence of bismuth oxide, which almost matches the previous report [15].

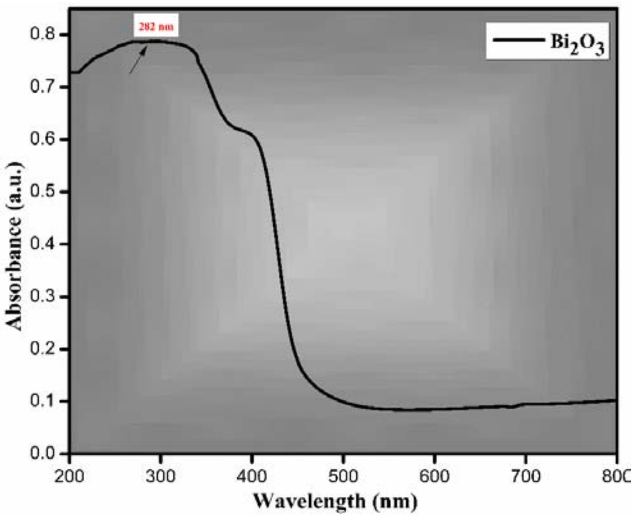

Fig. 3: UV-vis spectrum of Bi_2O_3 NPs

FT-IR

A vibration-based spectroscopy such as FT-IR (fig. 4) can be used to identify the bioactive compounds involved in the biosynthesis of bismuth oxide nanoparticles. The various bending and stretching frequencies of Bismuth metal oxides was observed between 400 and 4000 cm^{-1}. A sharp intense peak shows at 438 cm^{-1}, 512 cm^{-1} and 621 cm^{-1} is due to the stretching vibration of Bi-O bond. A large intense peak around 3430 cm^{-1} indicates the confirmation of–OH group which is present in thymol compound of ajwain. The stretching vibration of C=C shows a less intense peak at 2026 cm^{-1} which is present in the seed extract and–C-H asymmetric stretching shows a large peak at 2920 cm^{-1} which is due to the presence of organic moiety in the seed extract. The peak around 1120 cm-^{1}, which is very sharp and it is attributing to C-C stretching vibration. This data reflects the involvement of the bioactive compounds in the biosynthesis of Bi_2O_3 NPs. The finding of FT-IR study was in good agreement with previous studies [16].

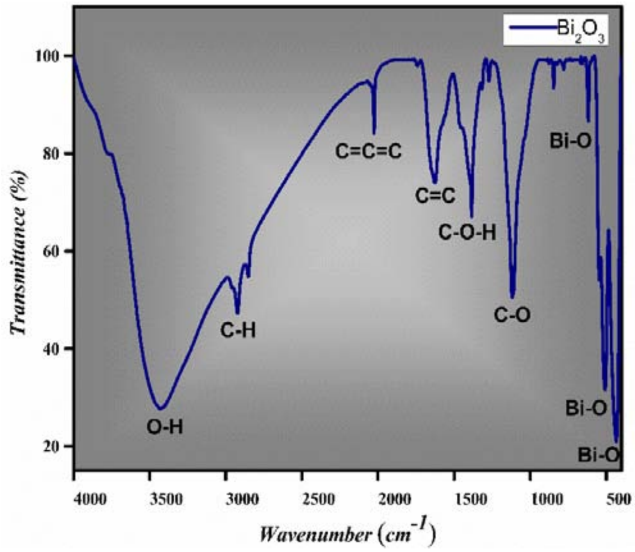

Fig. 4: FT-IR spectrum of Bi_2O_3 NPs

XRD

X-ray powder diffraction analysis was used to determine the crystalline structure and phase identification of the nanoparticles. The synthesized nanoparticles are extremely crystalline and precisely consistent with the conventional values for the monoclinic Bi_2O_3 phase, as shown by the intense and sharp peaks in the XRD analysis (fig. 5). After decomposition at 550 °C, bismuth oxide nanoparticles were analysed by X-ray diffraction in the 2θ range of 5° to 90° to determine their crystal structure. The intensity peaks are observed at 23.95°, 25.84°, 27.44°, 29.51°, 30.40°, 32.77°, 33.36°, 35.21°, 35.40°, and 37.70°. [JCPDS card no: 03-065-2366], which are indexed to the preferential orientations along (102), (002), (120), (121), (114), (202), (212), (031) and (112) planes respectively. Our study's diffraction pattern is very similar to the others [17-19]. The particle size of the synthesized bismuth oxide nanoparticles was determined by the debye–scherer equation.

$$d = \frac{k\lambda}{\beta \cos\theta},$$

Where k = (0.9), which is the typical value of the shape factor. β = FWHM (full-width half maximum) of diffraction peaks. The Bragg's angle =θ. λ= wavelength (0.154 nm). The average crystalline size was calculated and found to be 79.80 nm.

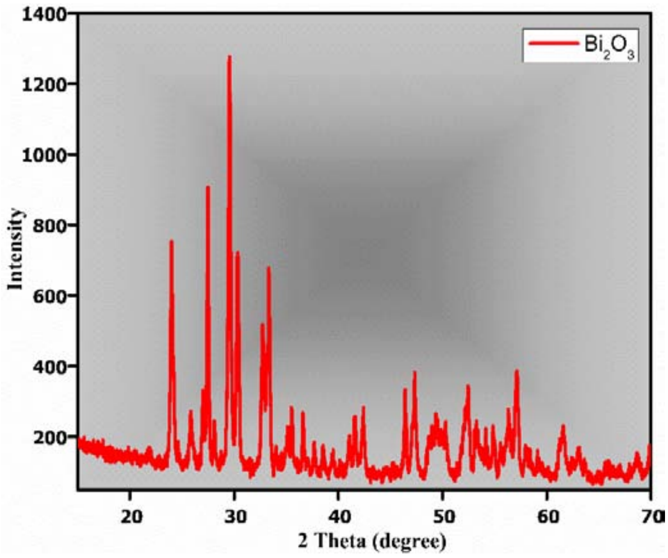

Fig. 5: XRD spectrum of Bi_2O_3 NPs

SEM

By using a scanning electron microscope, bismuth oxide nanoparticles were characterized morphologically and in terms of size. It was made known to obtain information about the surface's topography and composition. The SEM image of the synthesized bismuth oxide nanoparticles shows that it exhibited agglomerated morphology (fig. 6). It seems likely agglomerated clusters developed as a result of the accumulation of the small components of numerous bioactive reducing agents present in the plant extracts or this may be related to the seed extract's lesser capping capacity and bismuth-based nanoparticle's tendency of agglomeration due to its magnetic interactions. The particles appeared to be aggregated as a result of the H-bonds present in the bioactive molecules [20-22]. The mean area of the nanoparticles micro meter is found as 0.006 and the mean length of the nanoparticles micro meter is found as 1.114.

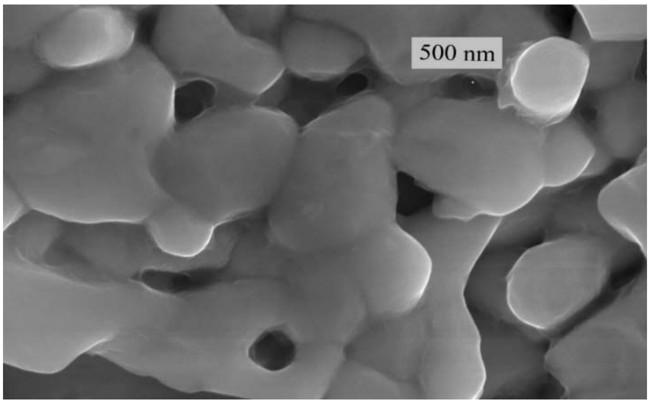

Fig. 6: SEM image of Bi_2O_3 NPs

EDAX

The highest percentage of elemental Bismuth (Bi) and oxygen (O) peaks was confirmed using EDAX analysis. The elemental structure and the percentage of each element in each atom of the synthesized bismuth oxide nanoparticles were examined using EDAX analysis. Peaks appearing in the EDAX spectrum (fig. 7a) and table 1 correspond to the elements Bismuth (Bi), Oxygen (O) and Aluminium (Al). The bismuth oxide nanoparticles are coated with aluminium for the purpose of conductivity. The peaks that appeared in (fig. 7b) and table 2 correspond to the elements Bismuth (Bi) and Oxygen (O) only. The atom's percentage is given in the tables.

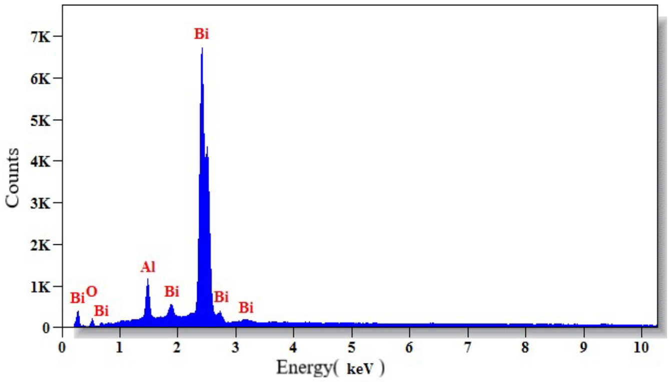

Fig. 7(a): EDAX analysis of Bi_2O_3 NPs coated with Al

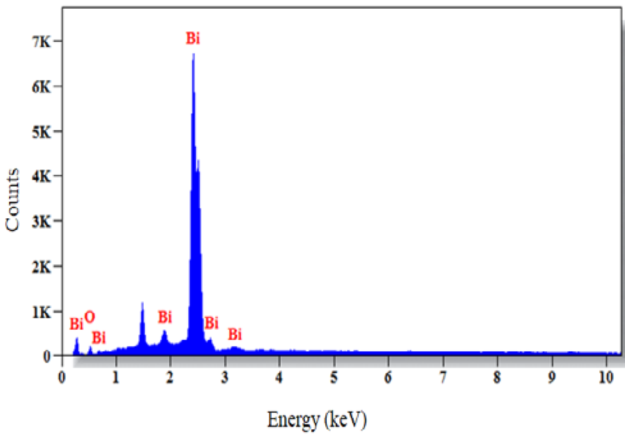

Fig. 7(b): EDAX analysis of Bi and O in Bi_2O_3 NPs

Table 1: % chart of fig. 7(a)

Element	Net counts	Weight %	Atom %
Bi	125396	93.59	60.19
O	786	2.31	19.39
Al	7554	4.10	20.43
Total		100.00	100.00

Table 2: % chart of fig. 7(b)

Element	Net counts	Weight %	Atom %
Bi	125796	97.60	75.72
O	786	2.40	24.28
Total		100.00	100.00

DLS

Dynamic light scattering (DLS) analysis is done to determine the average particle size and zeta potential of synthesized bismuth oxide nanoparticles. Based on the results of the study, the average particle size was found for the synthesized Bi_2O_3 NPs is size 279.04 nm. Due to the non-uniformity of size and shape of the green synthesized bismuth oxide nanoparticles, it confirmed the polydisperse particles (PDI=0.0085) and-31.0 mV is measured as the average zeta potential value (fig. 8). The results confirmed that the synthesized bismuth oxide nanoparticles exposed the good colloidal stability. The acidity of the phytochemicals present in the seed extract may be responsible for the higher negative value, which confirms the higher stability for a long time [23].

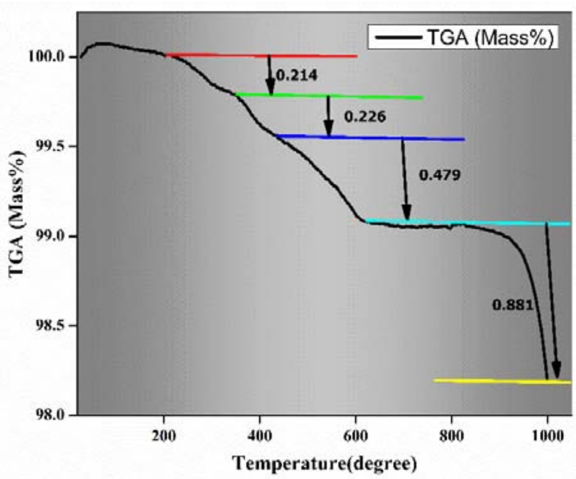

Fig. 8: Particle size of the Bi_2O_3 NPs

TGA-DTA

TGA/DTA studies help to identify the details of thermal behaviour and thermal stability data for the synthesized bismuth oxide nanoparticles. In the temperature range of 20 °C to 1000 °C, TGA measurements were carried out at a heating rate of 20 k/min. Fig. 9(a) depicts the nanoparticles of bismuth oxide's TGA curve. It confirms that the metal oxide is stable up to 990 °C which is very highly stable. The weight loss percentage was calculated and found to be 1.8%. Therefore, the synthesized bismuth oxide nanoparticles are confirmed as highly stable. Fig. 9(b) depicts the bismuth oxide’s DTA curve.

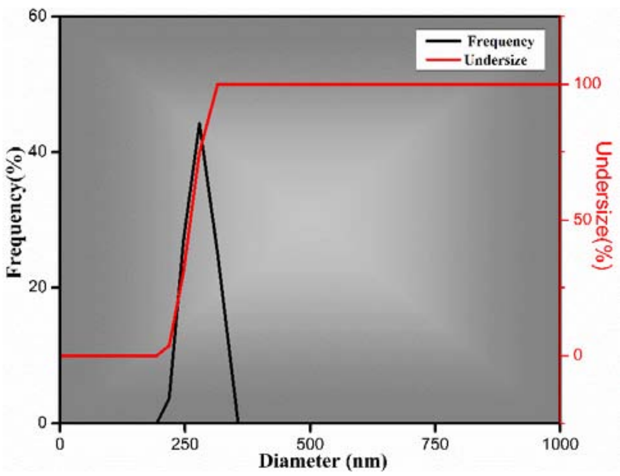

Fig. 9(a): TGA analysis of Bi_2O_3 NPs

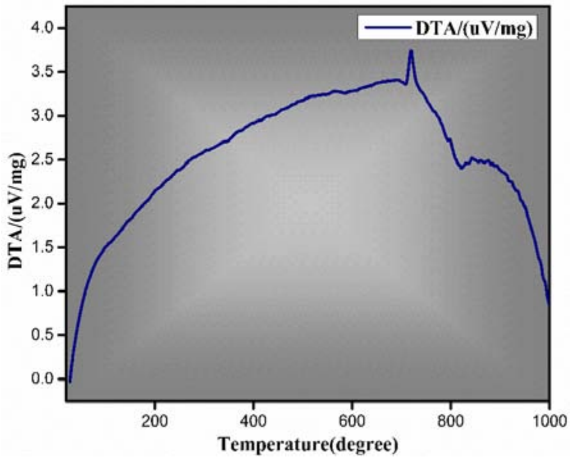

Fig. 9(b): DTA analysis of Bi_2O_3 NPs

Anti-bacterial activity

Synthesized bismuth nanoparticles were tested for broad-spectrum antibacterial activity against two different bacterial species (*E. coli*–gram-negative, *S. aureus*–gram-positive). As mentioned in earlier work, the antibacterial assays were carried out in a well assays format using Resazurin as an indicator [24]. In this antibacterial assay, the presence of blue colour indicates bacterial growth inhibition (fig. S1). The compounds with appropriate controls where incubated with the NPs and the values measured at the end (16h) of incubation and % inhibition calculated. Table 3 shows % inhibition of bacterial growth on the gram+ve and-ve bacteria species in the presence of various concentrations (0.03–1 mg/ml) of bismuth oxide nanoparticles. The values show a significant concentration-dependent inhibitory activity of bismuth oxide nanoparticles against both *E. coli* (61.54–94.83 %) (fig. S2) and *S. aureus* (63.18–94.07 %) (fig. S3), indicating a broad spectrum antibacterial activity for the nanoparticles. Fig. S1, fig. S2 and fig. S3 are given in the supplementary file.

Table 3: The concentrations and dead percentages in the anti-bacterial activity of Bi_2O_3NPs

Compound	Concentration (mg/ml)	*S. aureus* (% dead)	*E. coli* (% dead)
Bi_2O_3 NP	1	94.07	94.83
	0.5	92.18	94.70
	0.25	85.50	83.73
	0.125	80.33	79.45
	0.0625	72.13	72.13
	0.03125	63.18	61.54

CONCLUSION

Bismuth oxide nanoparticles were synthesized by using the trachyspermum ammi's extract and are confirmed by the characterizations such as UV-Vis, FT-IR, XRD, SEM with EDAX, TGA-DTA, DLS. It showed good results in the biological activity (broad spectrum anti-bacterial activity). From the results, it is confirmed that the synthesized metal oxide Bi_2O_3 nanoparticles are useful and have good benefits in inhibiting both the gram-positive and gram-negative bacteria with good dead percentages at different concentrations.

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AUTHORS CONTRIBUTIONS

Bharani Dharan Sethuraman: Methodology, Software-original draft, Visualization, Investigation. Lakshmi Prabha Chandrasekarand Manikandan Subramani-Formal Analysis. Munuswamy-Ramanujam Ganesh, Aswathy Karanath Anilkumar-Data curation of Biological studies. Sivakami Mohandos-Conceptualization, Writing,-Review and Editing, Supervision.

CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interest.

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