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Bio-Synthesis of Bismuth Oxide Nanoparticles (Bi_2O_3) using Oldenlandia Umbellata and their Biological Studies

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ABSTRACT: Nanotechnology is an emerging field that is impacting all aspects of human life. The manufacturing of nanomaterials is one of the most demanding tasks in the field of nanotechnology. The properties of materials at the Nano level scale differ from their same-sized bulk counterparts due to significant quantum size effects. These unique properties have wide-ranging applications in chemistry, physics, material science, and biomedical science. Green synthesis of nanoparticles is achieved through a literature procedure. In one study, Bi_2O_3 nanoparticles were synthesized by reacting bismuth (III) chloride and *Oldenlandia umbellata* plant extract. The Bi_2O_3 nanoparticles were obtained as brownish-green crystals with a yield of 92%. An absorption peak was observed at 313 nm, characteristic of bismuth. The antibacterial activity of bismuth oxide nanoparticles exhibited antibiotic characteristics against Bacillus subtilis (BS), Bacillus cereus (BC), Staphylococcus albus (SA), and Pseudomonas aeruginosa (PA) bacteria. The results indicated that these nanoparticles exhibited significant antibiotic activity, notably at 7 μg/mL deficient concentrations.

KEYWORDS: Nanotechnology, Bi_2O_3 nanoparticles, Oldenlandia umbellate, Antibacterial, Green Synthesis

I. INTRODUCTION

Nanotechnology is widely used in translational research. One important area of focus is the development of metallic nanoparticles using biological materials in an eco-friendly manner [1]. Nanotechnology deals with particles ranging in size from 1 to 100 nm, including their synthesis and manipulation [2]. This field combines natural sciences such as chemistry, physics, biological sciences, engineering, materials science, and computational sciences for the formulation of nanostructures [3]. Nanostructures have various applications based on their size, distribution, and morphology, leading to new or enhanced properties [4]. Nanotechnology has wide-ranging applications in fields such as biomedical research, catalysis, chemical industries, cosmetics, drug delivery, electronics, environmental science, energy, food and feed, healthcare, mechanics, optics, space industries, non-linear optical devices, single-electron transistors, and photoelectrochemical applications [5-8]. Metallic nanoparticles are particularly promising for these applications. Nano-scale drug carriers function as single units with specific properties and transport capabilities [9-12]. These nanoclusters have a narrow size distribution and at least one dimension between 1 and 10 nanometers. Agglomerates of ultrafine particles, nano-clusters, or nanoparticles, are known as nano-powders, while nanocrystals are crystals of nanoparticle size [13-15]. Synthesis of nanomaterials typically involves two general strategies: the top-down approach [16], where a larger structure is broken down into smaller pieces using chemical [17], physical, and biological energy; and the bottom-up approach, where the material is synthesized from the atomic level using various chemical, physical, or biological reactions to build a large nanostructure [18-21]. Chemical and biological methods are primarily used to construct nanostructured carriers (NC), while physical and chemical strategies are employed to synthesize nanoparticles [22-25]. Although toxic chemicals could pose potential hazards such as carcinogenicity and toxicity, nanoparticles have various factors affecting their synthesis, and possible mechanisms are employed, along with potential applications, for the nanoparticles formed using biological factories [26,27].

Researchers have received great attention from Bismuth nanoparticles because of their unique applications in various sectors such as photocatalytic, antimicrobial activity, and electrochemical sensing [28]. Consequently, several recent research studies have been published on plant extract-based green synthesis approaches. Among these methods, the plant extract-based green route has been considered a valuable substitute for other methods for nanoparticle synthesis since it is an inexpensive, biocompatible method, and easy to scale up, by avoiding the requirement of additional stabilizing agents during nanoparticle synthesis. Plant extracts contain various key phytochemicals such as phenols, alkaloids, terpenoids, and tannins including various vitamins in significant concentrations. These phytochemicals act as reducing, capping, and stabilizing agents during the synthesis of metal nanoparticles from their respective precursors. Due to these unique features of phytochemicals, cobalt nanoparticles have been effectively produced by the green synthesis approaches using extracts of plant parts such as roots, bark leaves, etc. This review summarizes the new findings in the synthesis of cobalt nanoparticles using extracts of different plants and their parts. The required characterization tools for the analysis of synthesized cobalt nanoparticles are also discussed. Further, the advantages of cobalt nanoparticles in various applications such as photocatalytic environmental applications for remediation of water pollution, electrochemical sensing, and antimicrobial activity are specially focused in this review.

Oldenlandia umbellata L. (Rubiaceae) is a natural dye-yielding plant. It is a small, prostrate, profusely branched perennial herb. The plant is native to the Indian subcontinent but distributed in the forests of Myanmar, Sri Lanka, Cambodia, Indonesia, India, Pakistan and Africa [29]. It is commonly known as the Madder plant or Chay root and the red dye extracted from the roots used in calico printing and coloring of wool and silk fabrics for centuries [30]. All parts of O. umbellata are traditionally used by the tribal people and ethnic communities of India and China for the treatment of asthma, bronchitis, and bronchial catarrh [31,32]. This plant produces some important bioactive compounds including anthraquinone derivatives, saponins, tannins, terpenoids, ursolic acid, kaempferol-3-O-rutinoside, oledicoumarin, hedyotiscone, cedrelopsin, pheophorbide, etc. [34]. The natural population of this plant has been depleted due to over-exploitation of O. umbellata for its roots (for red dye) and medicinal values [35]. This plant is propagated by seeds only, but the plants are harvested before flowering and fruiting therefore, naturally grown plants cannot fulfill the ever-increasing demand. The tissue culture protocols were developed by various research groups as an alternate method of propagation to conserve this important forest plant [36]. Though in vitro propagation techniques attained immense importance in recent years, the hidden drawbacks rely on the loss of tissue culture-raised plants during field transfer. The presence of growth regulators, sucrose, constant temperature, and low CO2 concentrations under in vitro conditions lead to the formation of abnormal anatomical structures (variations in stomatal distribution and pattern, venation pattern and vein density, trichomes, raphides, and crystals) affect the survival of plantlets under harsh natural conditions. The foliar micromorphological studies might be extremely useful in acclimation research as the internal structures of the leaves are highly responsive to environmental changes which leads to improved survival percentage of tissue culture-raised plants under natural habitats [37]. Understanding foliar micromorphological developments of in vitro and field-grown plantlets could help to overcome the difficulties in the successful establishment of plants under field conditions. Moreover, foliar micromorphological changes can also serve as one of the potential markers to study the changes during the acclimatization of micropropagated plants under natural conditions.

II. EXPERIMENTAL METHODS

Typically, the reduction of plant extract involves mixing an aqueous extract with a solution of the appropriate metal salt. Nanoparticle synthesis takes place at room temperature and completes within a few minutes.

2.1. UV-Vis Absorption Spectrum

The electronic spectra were recorded in the 200-900 nm regions using a Deep Vision UV/VIS spectrophotometer. A cuvette with a 1 cm path length was utilized. The concentration of the solution was maintained at 1.00 x 10-5 mol L^{-1} at a temperature of 310 K.

2.2. Selection and collection of plants

We collected Indian herbal plants such as Oldenlandia umbellate from our college campus. We separated the dry and waste parts of the plant and washed the collected plants with tap water. Then, we cut the plants into small pieces and air-dried them thoroughly under shade (at room temperature) for 14 days to prevent direct loss of phytoconstituents from sunlight. Subsequently, we powdered the shade-dried materials using a pulverizer and sieved them up to 80 meshes. The powdered material was then homogenized to a fine powder and stored in an air-tight container for further analysis.

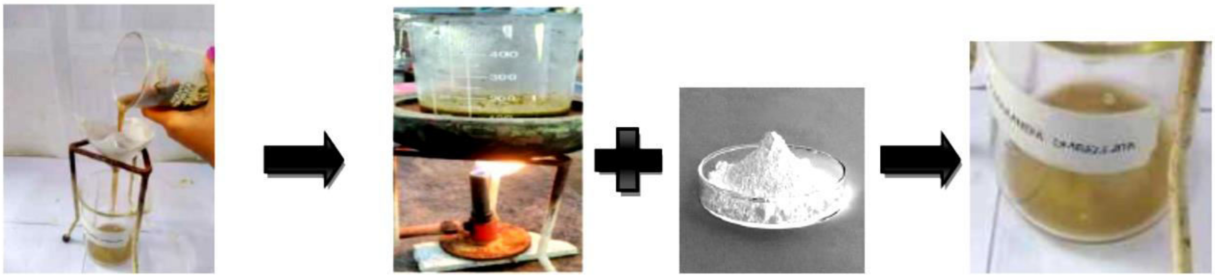
Figure 1: Image showing the plant *Oldenlandia umbellata L*

2.3. Preparation of plant extract

The plant extract was prepared by mixing 10 g of freshly dried plant powder with 200 ml of distilled water separately. The mixture (plant powder + distilled water) was heated for 2 hours at 80˚C with continuous stirring. The solution was cooled to room temperature and filtered three times using Whatman filter papers. The filtered extract was then stored at 4°C for future use.

2.4. Green synthesis of Bismuth nanoparticles

In a 250 mL beaker, 0.5 g of Bismuth (III) chloride and 10 mL of freshly prepared *Oldenlandia Umbellata* plant extract were combined and kept under stirring for 30 minutes. The resulting solution was heated to 80˚C and stirred continuously for two hours using a magnetic stirrer. A known amount of 0.2 N NaOH solution was added until the solution became basic (pH 14). The final solution was then left undisturbed and incubated at 80˚C for 24 hours. The obtained crystal was calcined at 500 ºC in an autoclave for four hours. The Bi_2O_3 nanoparticle was obtained as brownish-green crystals at 92% yield.



Scheme 1. Synthesis of *Oldenlandia Umbellata* extract based Bismuth Nanoparticle

2.5. Solubility cum saturation test.

About 0.1 g of Bi_2O_3 NP was transferred to a clean 10 mL test tube, and then 1 mL of double-distilled water was added and stirred until a clear solution was obtained. This confirmed the compound's solubility in water. While stirring, 0.1 g of Bi_2O_3 NP was added until the saturation limit was reached. This allowed us to determine that the water solubility limit of our NPs was 0.6 g in 1 mL of water.

III. RESULT AND DISCUSSION

3.1. UV-Vis Absorption Spectrum

It is confirmed that the presence of nanoparticles is indicated by the reduction of bismuth ions in the solution (see Figure 2). The bismuth oxide nanoparticles were placed in a quartz cuvette and observed for wavelength scanning between 200 and 800 nm, with distilled water as a reference. An absorption peak was observed at 313 nm, characteristic of bismuth nanoparticles.

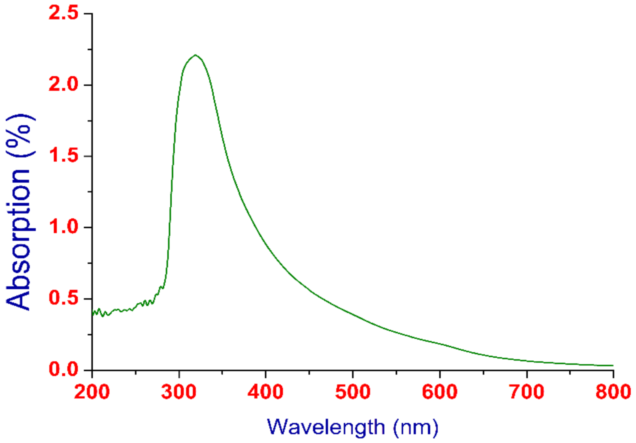


Figure 2. UV-VIS spectrum of bismuth oxide nanoparticle

3.2 Surface morphological study

The surface morphology of the nanoparticle was studied using SEM analysis. It demonstrates that the produced Bi_2O_3 nanoparticles are spherical and oval shapes of 50 to 100 nm sizes. The high colloidal dispersibility of the Bi_2O_3 NPs and the coating layer observed in the SEM image suggest that phytochemicals present in *Oldenlandia Umbellata* juice also act as capping agents. This accumulation occurred during drying as confirmed by the stable water dispersibility of Bi_2O_3 nanoparticles with an average hydrodynamic diameter (Dh) of 85 nm.

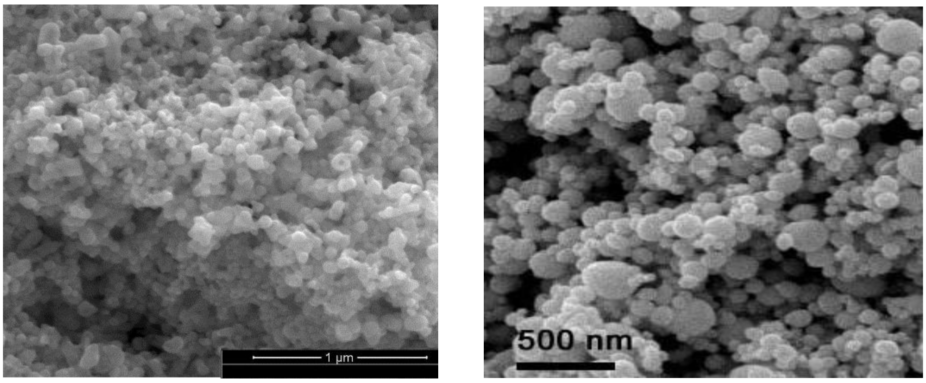


Figure 3. **SEM images of bismuth oxide nanoparticle**

3.3. Antibacterial activity

The antibacterial activity of bismuth oxide nanoparticles was evaluated against Gram-negative and Gram-positive bacteria using different concentrations of the samples. A specified volume of bacterial strains such as Bacillus subtilis (BS), Bacillus cereus (BC), Staphylococcus albus (SA), Pseudomonas aeruginosa (PA), E. coli, and Klebsiella pneumoniae (KP) was added to each sample in physiological serum to achieve a concentration of 100,000 bacteria per mL and then incubated at 37 °C. A positive control group and a negative control group were also included. The zone of inhibition was measured using the disk diffusion method, where the samples were applied at various concentrations on agar plates at specific distances. Following incubation at 37 °C for 24 hours, the diameter of the zone of inhibition was measured. The results indicated that these nanoparticles exhibited significant antibiotic activity, notably at very low concentrations of just 7 µg/mL. Also, the nanoparticle is a good candidate for exhibiting antibacterial activity against Pseudomonas aeruginosa (PA), E. coli, and Klebsiella pneumoniae (KP). Also, the nanoparticle shows little effect on Bacillus subtilis (BS), Bacillus cereus (BC), and Staphylococcus albus (SA) (Figure 4,5, and Table 1).

Table 1: Zone inhibition of bismuth oxide with Bacteria

S. No.	Bacteria	Bismuth Oxide nanoparticles @7 µg/mL	Control (STREPTOMYCIN)
1	Bacillus subtilis	8.8	22
2	Bacillus cereus	6.1	20
3	Pseudomonas aeruginosa	11.5	12
4	Staphylococcus albus	5.2	12
5	Klebsiella pneumoniae	8.6	13
6	E.coli	12.2	11

Figure 4. Antibacterial activity of bismuth oxide nanoparticle

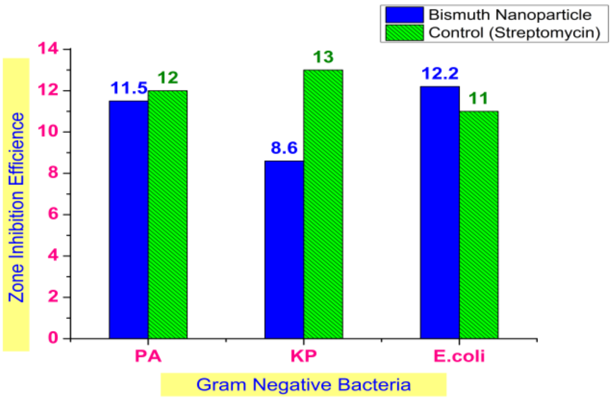


Figure 5. Antibacterial activity on Gram Negative bacteria

IV. FUTURE SCOPE

Medicinal plants containing secondary metabolites are used as an alternative treatment for infectious diseases [38]. Bioactive compounds exhibit antimicrobial activity through various mechanisms. Plants produce a range of bioactive compounds, primarily secondary metabolites, for protection against pathogens. These compounds include alkaloids, flavonoids, phytosterols, phenols, saponins, and tannins. The quantitative estimation of primary metabolites indicates that Oldenlandia Umbellata juice contains higher amounts of carbohydrates when compared to other metabolites. Extracting secondary metabolites with active principles was beneficial in preparing effective herbal drugs. Managing type 2 diabetes mellitus involves dietary and lifestyle modifications, as well as pharmaceutical treatment. Herbal drugs derived from Oldenlandia Umbellata plants can lower blood glucose levels without causing side effects.

The contrast of the image is the main criterion for defining the sensitivity of a diagnostic imaging method. In general, higher contrast efficacy is urgently needed to reduce the side effects caused by the high-dose administration of contrast agents in clinical imaging [39]. BiNPs have demonstrated high potential as a powerful polymer-based composite as a contrast agent for different imaging modalities and other manufacturers [23,24,40]. As a result, the Bi_2O_3 NPs can be employed for dual-modal and multi-modal imaging-guided therapies for precise diagnosis and monitoring of the treatment process. Bismuth oxides and related materials could be also combined with other species by producing a hybrid material by using ultrasmall silica-based bismuth and gadolinium nanoparticles for dual magnetic resonance and CT imaging. Also, conjugated Bi_2O_3 with iron oxides to improve the photothermal behavior leaving untouched the high bismuth radiopacity.

V. CONCLUSION

Medicinal plants have been a significant source of treatments for human diseases for a very long time. In this work, we have successfully demonstrated the simple, cost-effective, and eco-friendly synthesis of nanoparticles using plants. The nanocrystals' structure and appearance were analyzed using UV-visible spectroscopy which confirmed the identified phases. Additionally, the antimicrobial properties were tested against bacteria. The results showed that the synthesis method we used increased the antibacterial activity due to the small particle size, production of reactive oxygen species (ROS), and large surface area. These properties could lead to many benefits in the future with less harm and toxicity to human health and increased safety. As metals and compounds can interact with different stages of the pathogen's life cycle, they can be used to create new drugs.

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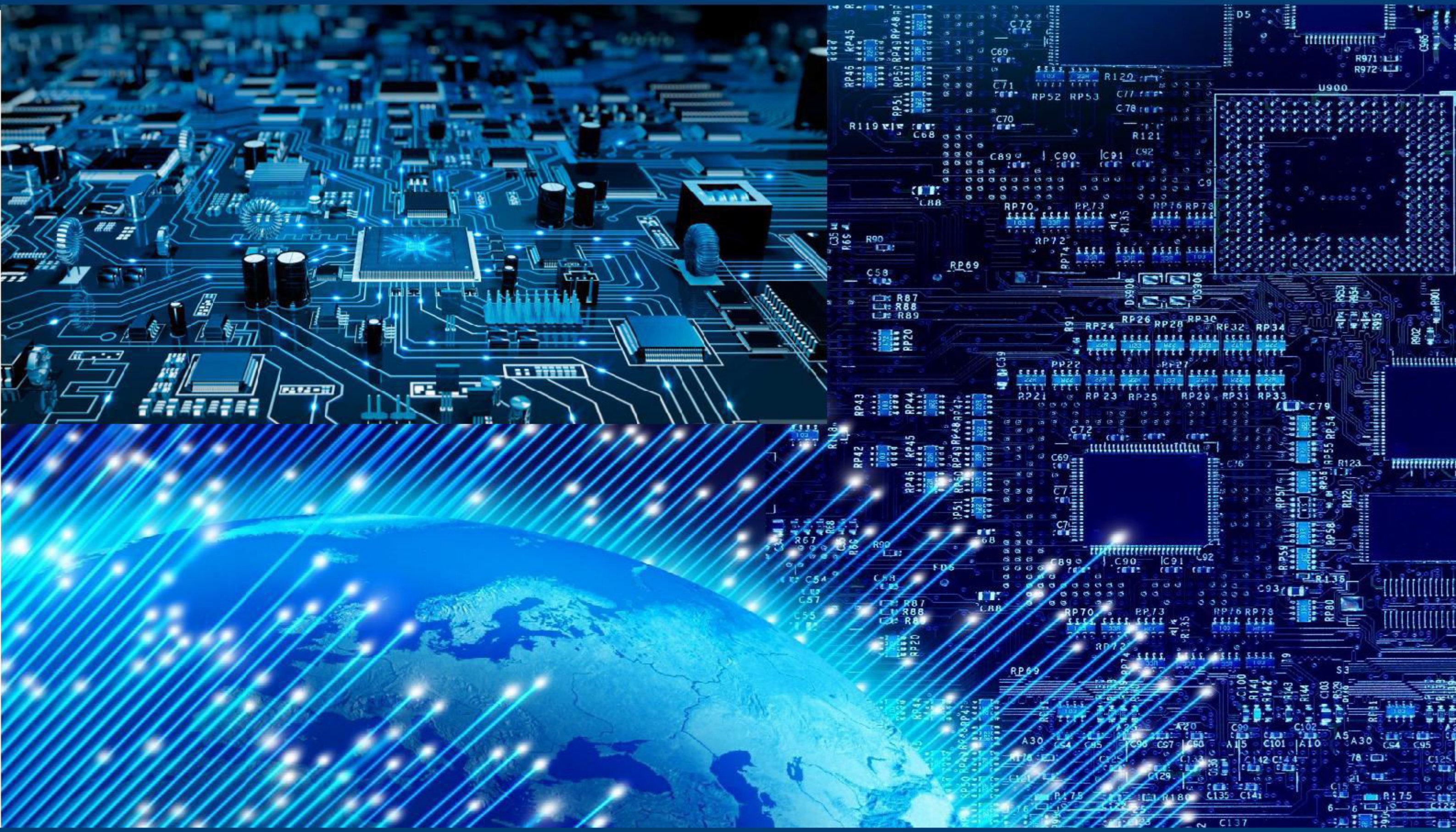

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