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Enicostemma Littorale Plant Assisted Synthesis of HgO Nanoparticle shows remarkable Photo Physical character and Antibacterial Activity

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ABSTRACT: In materials science, "green" synthesis has garnered significant attention as a reliable, sustainable, and eco-friendly approach for producing an array of materials and nanomaterials, including metal and metal oxide nanomaterials, hybrid materials, and bioinspired materials. This study specifically investigates the green synthesis of HgO nanoparticles, utilizing a method that incorporates environmentally friendly practices. The synthesis was achieved through the reaction of mercury chloride with an extract from the Enicostemma littorale plant. The resulting HgO nanoparticles, which were annealed at 400 °C, appeared as brown crystalline structures with a yield of 94%. Various characterization techniques were employed to analyze these nanoparticles. An absorption peak at 228 nm was observed, indicative of the presence of mercuric oxide, confirming the successful synthesis of HgO. Additionally, scanning electron microscopy (SEM) provided insight into the nanoparticles' morphology, revealing their spherical shape and lack of aggregation, with an average size measuring approximately 26-32 nm. Furthermore, the antibacterial properties of the synthesized nanoparticles were explored. The results demonstrated significant antimicrobial activity at concentrations as low as 9 µg/mL, effective against a range of pathogenic bacteria, including Bacillus subtilis (BS), Bacillus cereus (BC), Staphylococcus albus (SA), Pseudomonas aeruginosa (PA), Escherichia coli (E. coli), and Klebsiella pneumoniae (KP). These findings highlight the potential applications of HgO nanoparticles in both medical and environmental fields, particularly for combating bacterial infections.

KEYWORDS: Enicostemma littorale, Nanoparticles, Mercury oxide Np, Absorption, Antibacterial study

I. INTRODUCTION

Nanotechnology is a crucial technology in translational research, especially because of its groundbreaking applications in creating metallic nanoparticles using environmentally friendly methods that incorporate biological materials [1-4]. This area focuses on particles that range from 1 to 100 nanometers in size, utilizing a variety of synthesis techniques and manipulations [5,6]. It merges several scientific fields, such as chemistry, physics, biological sciences, engineering, materials science, and computational sciences, to develop advanced nanostructures [7,8]. The unique properties of these nanostructures are impacted by factors like size, distribution, and morphology, leading to a wide range of applications across different sectors. Significant applications include the biomedical sector, catalysis, chemical industries, cosmetics, drug delivery systems, and electronics, along with energy science, food and feed, healthcare, mechanics, optics, and space industries [9]. In particular, metallic nanoparticles are recognized for their considerable potential. Nanoscale drug carriers function as cohesive units regarding their characteristics and transport abilities, usually exhibiting a narrow size distribution with at least one dimension between 1 and 10 nanometers. Terms like "nanopowders" and "nanocrystals" refer to clusters of ultrafine particles and crystalline forms at the nanoscale, respectively [10-14].

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There are two main strategies for creating nanomaterials: the top-down approach, which involves breaking larger structures into smaller particles via chemical, physical, or biological processes; and the bottom-up approach, where materials are built from the atomic level through various reactions to create larger nanostructures [15]. Biological and chemical methods are mainly utilized in the development of nanostructured carriers, while physical and chemical strategies are employed in the synthesis of nanoparticles. However, the use of hazardous chemicals in these processes can pose serious risks, including carcinogenicity and toxicity, impacting both the production of nanoparticles and their future applications, especially in clinical and biomedical settings [16-18]. Therefore, there is a significant demand for safe, clean, and environmentally sustainable techniques for the synthesis of nanoparticles. Biological synthesis presents a promising alternative, utilizing both multicellular and unicellular organisms such as bacteria [19-22]. The nanoparticles generated using biological means offer a vast potential for investigating their shape, size, composition, and physicochemical traits [23-30]. Biological entities can efficiently aid in the assembly and creation of materials at the nanoscale. This research article highlights the benefits of biological methods for producing metal and metal oxide nanoparticles, particularly focusing on the resistance certain organisms develop against specific metals, which may limit the choice of microbial sources [31-34]. Key microbial organisms used to synthesize commonly researched nanoparticles include algae, fungi, bacteria, viruses, and yeast. The metals and metal salts frequently studied in this domain encompass copper, silver, gold, cadmium, platinum, palladium, cadmium sulfide, titanium dioxide, and zinc oxide [35-37].

II. MATERIALS AND METHODS

2.1 Materials. Mercury acetate was purchased from Merck India. Acetone, Ethanol, N, N-dimethyl formamide, Hydrochloric Acid, and Acetic Acid (AR, Merck) were used as received. Double-distilled water is obtained by distilling distilled water over alkaline potassium permanganate.

2.2 Selection and Collection of Plants

Medicinal plants have been crucial for treating human diseases for centuries, with around 1.42 billion people relying on traditional medicine, especially plant-based remedies. Herbal medicines offer advantages over synthetic drugs, as they generally have minimal side effects and are considered safer. Accurate knowledge of these crude drugs is essential for their preparation and efficacy. Standardization ensures consistent therapeutic effects in herbal products through assessments like extractive values and active components.

Figure.1. Structure of E. littorale

E. littorale, commonly referred to as "Vellarugu" in Tamil, is a perennial herb belonging to the Gentianaceae family. This plant is found throughout India and parts of Sri Lanka. It has a long history of traditional use for inflammatory conditions, managing diabetes, and regulating bowel functions [38]. E. littorale is noted for its anti-inflammatory, anti-diabetic, antioxidant, and hepatomodulatory properties. However, awareness and standardization of its usage are primarily confined to tribal regions. Therefore, the physicochemical and phytochemical standardization of E. littorale is crucial for its wider application in modern medicine.

This plant is classified under the Kingdom: Plantae, Subdivision: Angiospermae, Class: Dicotyledonae, Subclass: Gamopetalae, Series: Bicarpellatae, Order: Gentianales, Family: Gentianaceae, Genus: Enicostemma, and Species:

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littorale. The name "Enicostemma" likely derives from the combination of the words “en,” meaning inside, “icos,” meaning twenty, and “stemma,” meaning wreath or circle, due to the arrangement of numerous flowers in circles at the leaf axils along the stem. This tropical genus is widely distributed across South America, Africa, and Asia, with E. littorale thriving in various habitats, including savannas, grasslands, and beaches, and it can endure both wet and arid conditions, even in highly saline environments. The Indian herbal plant Enicostemma littorale was collected from our college campus. The dry and waste parts of the plant were removed [39]. The collected specimens were washed with tap water, then cut into small pieces and air-dried thoroughly in the shade at room temperature for 14 days to prevent the loss of phytoconstituents due to direct sunlight exposure. Following this, the shade-dried materials were ground using a pulverizer and sieved to an 80-mesh size. The resulting fine powder was then homogenized and stored in an airtight container for further analysis.

2.3. UV-Visible Absorption Spectrum.

The electronic spectra were recorded in the 200-900 nm regions on Deep Vision UV/VIS spectrophotometer using a cuvette with a 1 cm path length. The concentration of ligand and metal complexes was kept at 1.00 x 10^{-5} mol L^{-1}, at 310 K [40].

2.4. Solubility test

About 0.1 gm of substance was taken in a clean 10 ml test tube this 1 ml of low polar solvent like benzene and petroleum ether was added and shaken well [41]. If the substance remains insoluble the same procedure was repeated with increasing order of polarity in solvents like Aceton, Acetonitrile, Tolune, ethanol, etc. Finally, the substance solubility was analyzed with high-polar double distilled water. After confirming the solubility an additional portion of 0.1 g of the NP was added until saturation limit was reached. From this inference, the saturation/solubility limit of our NPs will be tested.

2.5. Antibacterial Activity

Disc diffusion methodology was used to determine the antibacterial potential of plant-mediated metal NPs using various bacterial strains like Bacillus subtilis (BS), Bacillus cereus (BC), Staphylococcus albus (SA), Pseudomonas aeruginosa (PA), E. coli, and Klebsiella pneumoniae (KP). Before the activities were conducted, bacteria were sub-cultured overnight in nutrient broth media and were kept in an incubator at 37 ^{o}C for 24 hr. To confirm the antibacterial potency of NPs, an overnight culture of bacterial strains was spread on pre-prepared agar media and allowed to dry for 5 min. Furthermore, the filter disc loaded with different concentrations of NPs (2–10 µg/ml) was dried and kept on the surface of the plates. The plates were kept incubator and were observed for the ZOI. The Streptomycin antibiotic was used as a positive control and DMSO as a negative control.

2.6. Synthesis of HgO NPs using the green extract of E.littorale

Separately place about 5 g of whole plant powder of the E. littorale, in an accurately weighed moisture disc. For estimation of loss on drying, it was dried at 65°C for 5 hours in an oven, cooled in a desiccator for 30 minutes, and weighed without delay. The loss of weight was calculated as the content of in mg per g of air-dried material.

Figure 2. E. littorale dry plant

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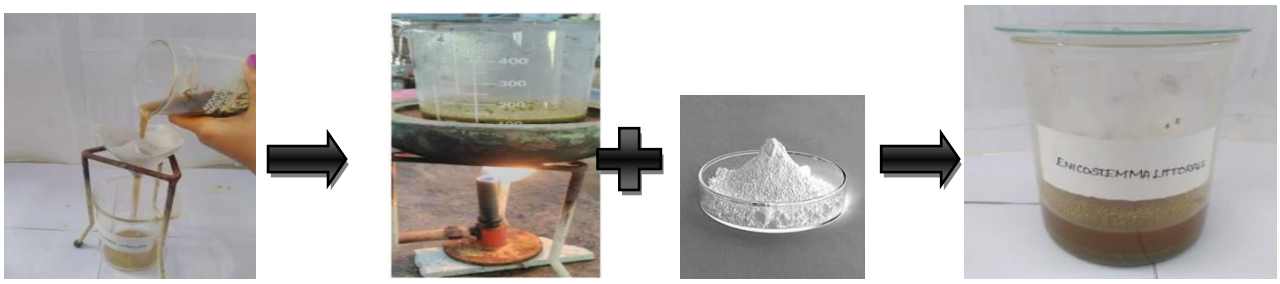
2.7. Green Synthesis of Mercuric Oxide Nanoparticle

Mercuric oxide nanoparticle was synthesized using mercuric chloride ($HgCl_2$) as a precursor and green extract of E.littorale as a dispersing solvent. To 50 mL of green extract of E.littorale, 10 g of $HgCl_2$ was added and kept under continuous stirring for 1 h till complete dissolution of $HgCl_2$. NaOH (0.1 M) was added drop by drop under stirring until the pH reached 14. After the complete addition of NaOH, the reaction mixture was stirred for 2 h. The appearance of an orange color indicated the completion of the reaction. After washing the precipitate several times with distilled water and methanol, it dried at 100 ^oC for 8 h to afford conversion of $Hg(OH)_2$ to HgO. The nanoparticle was obtained as colorless crystals at 95 % yield.

About 0.1 gm of HgO NP was taken in a clean 10ml test tube to this 1ml of double distilled water was added and stirred until clear solution was obtained. This confirms the compound solubility in water solvent. While stirring an additional portion of 0.1 g of the HgO NP was added until saturation limit was reached. From this inference, the water solubility limit of our NPs was tested. The nanoparticle saturation limit is 0.8 gm in 1 ml of water. The reaction is depicted in scheme 1.



Scheme 1. Synthesis of E. littorale extract-based Mercuric oxide nanoparticle

2.8 UV-visible spectroscopy. It is confirmed that the presence of nanoparticles by reduction of mercury ions in the solution (Figure 3). The mercuric oxide nanoparticles were placed in a quartz cuvette and observed for wavelength scanning between 250 and 700 nm with water as a reference. The absorption peak was observed at 228 and 302 nm, characteristic of Mercuric oxide nanoparticles and the flavonoids present in plant extract.

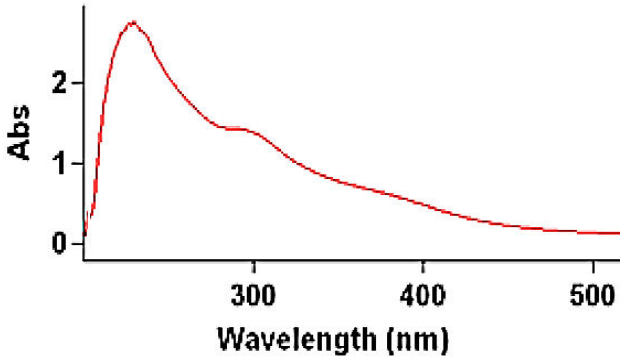


Figure 3. The UV-visible spectrum of Mercuric oxide nanoparticle

2.9 Surface morphological study

The surface morphology of the synthesized HgO nanoparticles was analyzed in detail using Scanning Electron Microscopy (SEM), which is a technique particularly effective for examining the microstructure of materials. The SEM images clearly indicated that the samples predominantly exhibited square shapes with average diameters of 29 nm. This finding demonstrates that the extract derived from E. littorale significantly influences the production of HgO nanoparticles with diameters ranging from 26 to 32 nanometers. In addition to their distinctive morphology, the HgO

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nanoparticles display excellent colloidal dispersibility, a critical attribute for their potential applications across various fields. The distinct coating layer visible in the SEM imagery strongly suggests that the phytochemicals extracted from E. littorale function as effective capping agents. These phytochemicals are likely instrumental in stabilizing the nanoparticles during the synthesis process and in minimizing their agglomeration. Moreover, the stability of these nanoparticles during the drying process ensures that their size and shape are effectively preserved, thereby enhancing their functional properties. This is further corroborated by the hydrodynamic diameter (Dh) of the HgO nanoparticles, which averages 29 nanometers in aqueous dispersion (Figure 4). Such a small diameter implies a high surface-to-volume ratio, significantly enhancing their reactivity and increasing their suitability for incorporation into diverse applications, including catalysis, electronics, and drug delivery.

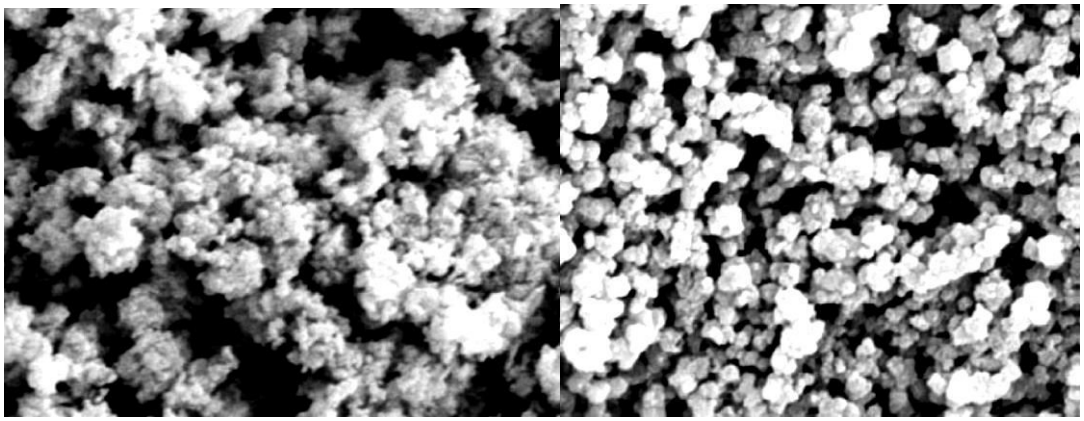

Figure 4. SEM images of HgO nanoparticle

2.10 Antibacterial activity. Antibacterial activity of Mercuric oxide nanoparticles was measured against Gram-negative and Gram-positive bacteria for different concentrations of the samples by determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and zone inhibition. A known volume of bacterial strains was added to each of the samples in physiological serum to give the count of 100,000 bacteria per mL and placed in an incubator at 37 °C. The positive control group and the negative control group are also considered. Disk diffusion method was done for measurement of zone inhibition by stained with different concentrations of samples at appropriate distances in agar medium. Finally, the discs were incubated in a 37 °C incubator for 24 h. The diameter of zone inhibition was measured by the ruler. According to the results, mercuric oxide nanoparticles are good candidates for antibacterial activity at the solution concentration of 9 µg/mL on Bacillus subtilis (BS), Bacillus cereus (BC), Staphylococcus Albus (SA), Pseudomonas aeruginosa (PA), E. coli (EC), and Klebsiella pneumoniae (KP) (Figure 5 and Table 1).

Table 1 shows the results of zone inhibition of Mercuric oxide with Bacteria

S. No.	Bacteria	Mercuric Oxide 9 µg/mL	Control (STREPTOMYCIN)
1.	**BS**	19	22
2.	**BC**	18	20
3.	**PA**	17	12
4.	**SA**	14	12
5.	**KP**	14	13
6.	**E.coli**	14.8	11

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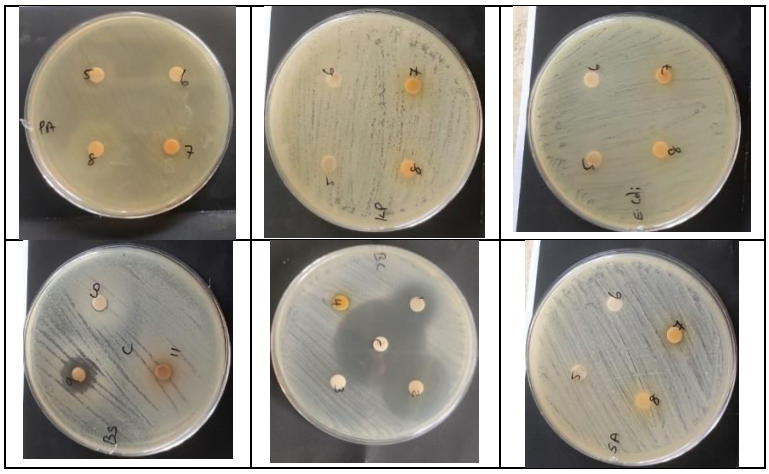

Figure 5. Antibacterial activity of mercuric oxide nanoparticle

III. SUMMARY OF THE RESULTS

Mercuric oxide nanoparticle was synthesized by co-precipitation method using mercuric chloride ($HgCl_2$) as precursor using green extract of E. littorale The nanoparticle was obtained as colorless crystals at 95 % yield. The mercuric oxide nanoparticles were placed in a quartz cuvette and observed for wavelength scanning between 250 and 700 nm with distilled water as a reference. The absorption peak was observed at 228 and 302 nm, characteristic of Mercuric oxide nanoparticles and the flavonoids present in plant extract.

Mercuric chloride ($HgCl_2$) is extremely hazardous because of its volatility in the metal state and ability to form numerous toxic volatile organic compounds under the action of bacteria present. Accumulation of trace metals, especially heavy metals, like mercury, in the soil has the potential to restrict the soil's function, cause toxicity to plants, and contaminate the food chain. Thus when disinfectants are used, they need to be used as directed for them to be effective and eco-friendly. Mercuric oxide nanoparticle has strong sterilization efficiency and are toxic not only to microbes but also to other superior organisms. E. littorale is traditionally used in Indian stomachic, bitter-tonic carminative to reduce fever and as a tonic for appetite loss in Indian ayurvedic medicine, E. littorale is taken in combination with other herbs, especially for diabetes. E. littorale is administered in ayurvedic pill form for treating type 2 diabetes since it plays a major role in reducing blood glucose increasing serum insulin level and significantly improving kidney function, lipid profile systolic and diastolic blood pressure, and pulse rate. E. littorale has demonstrated its anti-inflammatory activity, and tumor inhibition in rats. E. littorals has also been used by traditional healers for the treatment of dyspepsia and malaria. The formed nanoparticle made of HgO has traces of pharmaceutical compounds from the E. littorale plant. Due to this, the nanoparticle shows better antibacterial activity on bacteria like Bacillus subtilis (BS), Bacillus cereus (BC), Staphylococcus Albus (SA), Pseudomonas aeruginosa (PA), E. coli (E.C), and Klebsiella pneumoniae (KP) bacteria. Further, the nanoparticle is subjected to antifungal and antidiabetic studies.

IV. CONCLUSIONS

The green synthesis of mercuric oxide nanoparticles confidently demonstrates an absorption peak at 228 nm, confirming the successful formation of these remarkable nanoparticles. Scanning electron microscopy (SEM) offers clear insights into their morphology, revealing a distinct spherical shape and an impressive lack of aggregation, with an average size of approximately 26 to 32 nm. These nanoparticles exhibit exceptional sterilization efficiency, showcasing their toxicity not only toward microbes but also to higher organisms. The synthesized mercuric oxide nanoparticles are enriched with traces of pharmaceutical compounds from the E. littorale plant, contributing to their efficacy. Notably, these nanoparticles exhibit significant antimicrobial activity at concentrations as low as 9 µg/mL, effectively targeting a range of pathogenic bacteria. Their superior antibacterial action against Bacillus subtilis, Bacillus cereus, Staphylococcus albus, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella pneumoniae confirms their potential

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as a powerful antimicrobial agent. Ongoing research will further explore their antifungal and antidiabetic properties, affirming their broad spectrum of therapeutic applications.

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