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Green Synthesis, Characterization, and Assessment of Iron Oxide Nanoparticles' Antibacterial Activity Using Blumea lacera Root Extract

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ABSTRACT: Iron and copper nanoparticles produced through green methods underwent analysis using UV-visible absorption spectrophotometry, X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and EDS. Findings indicated that iron oxide nanoparticles displayed an irregular spherical shape, with sizes ranging from 15.73 nm to 32.37 nm. The antimicrobial efficacy of iron and copper oxide nanoparticles synthesized through green techniques was evaluated against four bacterial strains causing human diseases. Results showed that these nanoparticles exhibited the highest antibacterial activity at a concentration of 20 mm/disc, while their effectiveness was lowest at 13 mm/disc concentrations. This study underscores the potential of iron and copper oxide nanoparticles produced via environmentally friendly methods to serve as antibacterial agents.

Keywords: *Blumea lacera*, Iron and copper Oxide Nanoparticles, Antibacterial Activity, and Human Pathogens.

1. INTRODUCTION:

Atomic clusters that range in size from 1 to 100 nm are known as nanoparticles; the term "nano" stands for one billionth of a meter [1]. Both physical and chemical processes can be used to create nanoparticles, but they have drawbacks such as the need for poisonous substances, the creation of potentially dangerous byproducts, large energy requirements, and high costs. Due to its ecologically benign character and applicability for various applications such as medication administration, medical diagnostics, cancer therapy, agriculture, and environmental goals, green synthesis of nanoparticles using plants is a topic of significant investigation. Metal ions are reduced by plant extracts more quickly than by other green methods. Depending on the type of plant and the concentration of phytochemicals present, nanoparticles can be produced in a short amount of time, anywhere from minutes to hours [2]. Lettuce-Leaf Blumea is an annual plant that grows up to 2,000 meters above sea level in the plains of northwest India. It has a strong aroma [3–7]. Blumea is fiery, spicy, and bitter, according to Ayurvedic experts. It's also a great antipyretic and helps with blood disorders, fevers, burning sensations, bronchitis, and other conditions. It is believed that treating oral diseases could involve maintaining the root in the mouth. In India's Konkan region, the herb is utilized to ward against parasites like fleas [8–10]. In the homeopathic system, it is prescribed for headaches, enuresis, neuralgia, and coughing brought on by colds [11–14]. The current study looked into the environmentally friendly production and characterization of iron and copper oxide nanoparticles using root extract from Blumea lacera, and evaluated the antibacterial efficacy of these nanoparticles against bacterial infections in humans.

2. MATERIALS AND METHODS

2.1. Collection and identification of the plant

Fresh roots of Blumea lacera were collected from Kalyan Local Park. The India Biodiversity Portal documents Blumea Lacera with the taxonomic identifier 1768.

2.2. Preparation of *Blumea lacera* aqueous leaf extract

About 100 grams of fresh, vigorous roots from Blumea Lacera were air-dried under shade and subsequently pulverized into a fine powder using a household blender. The powdered leaves were immersed in 200 ml of double-distilled water and left to soak overnight at 4°C in a refrigerator, following which the mixtures underwent boiling for 10 minutes. Once cooled to room temperature, the extracts were filtered using Whatman filter paper (No.41).

2.3. Synthesis of iron oxide nanoparticles using *Blumea lacera* extract

The synthesis process of iron oxide nanoparticles commenced with dissolving $FeCl_3$ and $FeCl_2.5H_2O$ in a 1:2 molar ratio in 100 ml of double-distilled water within a 250 ml beaker. The solution was then gently heated to 80°C while being stirred using a magnetic stirrer under atmospheric pressure. Subsequently, after 10 minutes, 20 ml of Blumea lacera extract in aqueous form was introduced into the mixture, resulting in a rapid color change from light green to dark black. Following another 10 minutes, 20 ml of sodium hydroxide solution was slowly added to the mixture at a rate of 3 ml per minute, facilitating the uniform precipitation of iron oxide. Upon cooling the mixture to room temperature, the iron oxide nanoparticles were separated by decantation to obtain magnetite. The generated magnetites underwent three rinses with double-distilled water and three rinses with ethanol before being air-dried at ambient temperature.

2.4. Synthesis of copper oxide nanoparticles using *Blumea lacera* extract

A 250 ml beaker was utilized to dissolve $CuCl_2.5H_2O$ in 100 ml of double-distilled water for the synthesis of copper oxide nanoparticles. Following this, the mixture was gradually heated to 80°C while being stirred gently with a magnetic stirrer under atmospheric pressure. Within minutes of adding 20 ml of Blumea lacera root extract aqueous solution to the mixture, the color transitioned from a pale green to a deep black (occurring 10 minutes later). To ensure uniform precipitation of copper oxide, 20 ml of an aqueous sodium hydroxide solution was gradually introduced into the mixture at a rate of 3 ml per minute, 10 minutes after the initial addition. Subsequently, magnetite was prepared by allowing the solution to cool to room temperature and then separating the copper oxide nanoparticles through decantation. The resultant magnetites underwent three washes with ethanol and two washes with double-distilled water before being air-dried at ambient temperature.

2.5. Characterization of iron and copper oxide nanoparticles

The absorbance spectra of the sample were examined utilizing a UV-Vis double-beam bio-spectrophotometer JASCO V650, covering the wavelength range from 250 to 700 nm. Laser diffractometry with a Nano Size Particle Analyzer was employed to determine the size of magnetic iron and copper oxide nanoparticles, ranging from 0.6 nm to 6.0 µm. Analysis of the nanoparticles' structure and crystalline size was conducted via X-ray diffraction (XRD) using a SHIMADZU XRD-6000 model. Furthermore, the functional groups of the nanoparticles were investigated using FT-IR spectroscopy (Brucker, IR Affinity 1, India) across a scan range of 4000 to 500 cm^{-1}, with a resolution of 4 cm^{-1}. Morphological analysis of magnetic iron and copper oxide nanoparticles was carried out using a FEI Nova Nano SEM 450 scanning electron microscope (SEM).

2.6. Screening of antibacterial activity

2.6.1. Bacterial strains

The clinical strains of different bacteria were obtained from the Konkan Gyanpeeth Rahul Dharkar College and Pharmacy and Research Institute, encompassing B. subtilis, E. coli, S. aureus, and P. aeruginosa.

2.6.2. Antibacterial analysis by disc diffusion method

The effectiveness of iron and copper oxide nanoparticles produced through environmentally friendly methods was evaluated using the disc diffusion technique. Sterile 6 mm discs (Hi-media) were impregnated with varying concentrations of iron and copper nanoparticles: 10 µg/disc (10 µg/µl), 15 µg/disc (15 µg/µl), 20 µg/disc (20 µg/µl), 25 µg/disc (25 µg/µl), and 30 µg/disc (30 µg/µl). Culture plates were prepared by pouring 20 mL of Mueller-Hinton agar (MHA) medium and subsequently inoculated with a bacterial suspension using a sterile cotton swab. The plates were left to stand for a few minutes before delicately applying the discs upside down and incubating them for 24 hours at 37°C. As a positive control, ciprofloxacin discs (20 µg/disc) were also placed on the MHA plates. After the incubation period, the susceptibility of the test organisms was determined by measuring the diameter of the inhibition zone using a Himedia zone scale, and the gathered data were compiled for analysis.

3. RESULTS AND DISCUSSION

3.1. Green synthesis of iron oxide nanoparticles using *Blumea lacera* extract

In this study, iron and copper oxide nanoparticles were synthesized using a more environmentally sustainable approach, which involved the utilization of an aqueous extract obtained from Blumea lacera. Various sizes and shapes of iron and copper oxide nanoparticles have been successfully produced through eco-friendly methods using plant extracts such as Musa ornate, Averrhoa bilimbi, Juglans regia, Platanus orientalis, and Asparagus racemosus. The formation of iron and copper oxide nanoparticles was confirmed by closely observing the color change during the reaction process. Initially, the aqueous extract of Blumea lacera exhibited a pale green color, but upon the addition of Fe^{+3} and Fe^{+2} ions along with NaOH solution acting as a reducing agent, it transformed into a dark black hue.

3.2. UV-visible spectroscopy analysis

An effective technique for examining the structure of iron and copper oxide nanoparticles is UV-visible spectroscopy. Surface plasmon resonances are the main factor influencing the optical absorption spectra of metal nanoparticles [16]. Two absorption peaks in plant extracts were found at 268–270 nm and 328–339 nm, according to **Figure 1** analysis. The generated iron and copper oxide nanoparticles' surface plasma resonance causes the peak to appear at approximately 261 nm and 378 nm. The outcomes are comparable to the absorption maxima at 440 and 465 nm found in the UV-visible spectra of iron and copper oxide nanoparticles produced by Averrhoa bilimbi [17]. According to earlier research, iron and copper oxide nanoparticles made from plant extracts have absorption maxima in the UV-visible band between 400 and 500 nm [18].

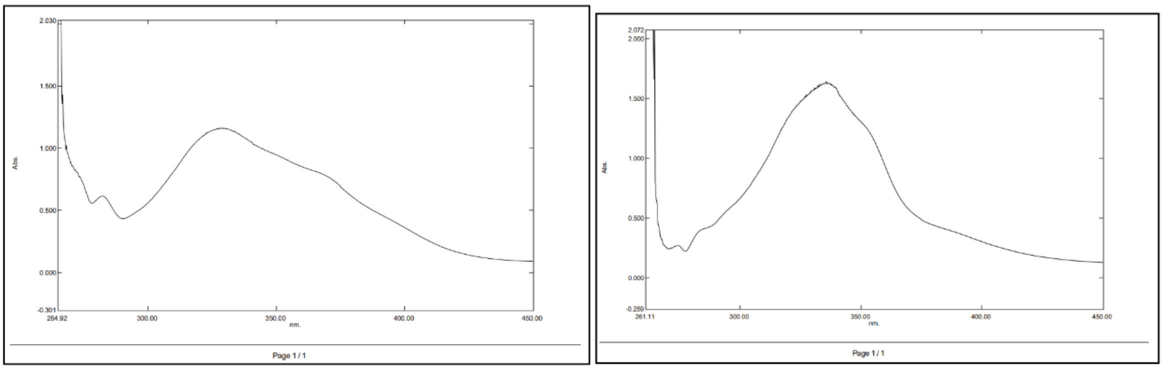

Figure 1. UV-vis spectra of Fe_2O_3-NPs and CuO-NPs

The phase, purity, and crystallite size of the iron and copper oxide nanoparticles were examined using X-ray diffraction (XRD). Figure 2 shows the X-ray diffraction pattern of iron and copper oxide nanoparticles produced by Blumea lacera. A rhombohedral crystal structure is indicated by the iron and copper oxide powder XRD pattern. The iron and copper oxide nanoparticles' conspicuous peaks lined up with their respective crystal faces, which are (016), (105), (110), (111), (331), (026), (111), (116), (211), and (300). According to Malarkodi et al., the peaks in the XRD pattern show that the iron and copper oxide nanoparticles made with the extract of Blumea lacera officinalis are crystalline and have a rhombohedral structure [19].

3.3. FT-IR analysis

The FT-IR spectra of iron and copper oxide nanoparticles made from Blumea lacera extract were examined in Figure 2. In the FT-IR spectra of the nanoparticles, the absorption peaks were mostly located around 3213–3268 cm^{-1}, 2188–2190 cm^{-1}, 2081-2107 cm^{-1}, 1990–2009 cm^{-1}, and 1631–1634 cm^{-1}. Strong stretching vibrations of the –OH bond are represented by the peak at 3213–3268 cm^{-1}, followed by C–N stretching vibrations at 2188–2190 cm^{-1}, aliphatic C–H stretching at 2081-2107 cm^{-1}, and 1990–2009 cm^{-1}. For conjugated carbonyl (–C=O) group stretching vibration and O-H bending, the frequency is 1631–1634 cm^{-1}. FT-IR measurement of various green-synthesized iron oxide nanoparticles was previously used to identify the functional groups [20–21].

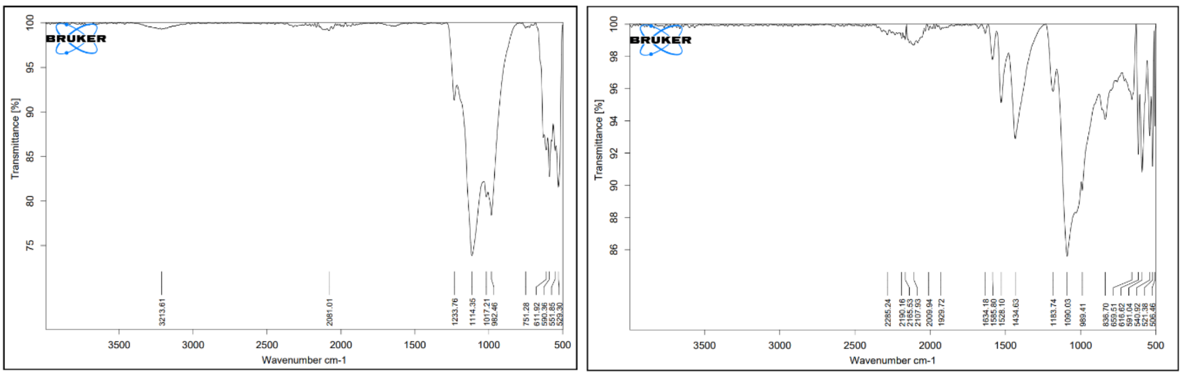

Figure 2: FT-IR spectra of Fe_2O_3 and CuO NPs

3.4. SEM analysis

SEM was used to analyze the morphology of the iron and copper oxide nanoparticles produced utilizing environmentally friendly processes. The quasi-spherical morphologies of the iron and copper oxide nanoparticles, which ranged in size from 15.73 nm to 574.3 nm, were visible in images captured with a scanning electron microscope. The findings are in line with those of Vijay Kumar et al. [21], who reported that α-Fe_2O_3 has a spherical shape and a constant size between 30 and 40 nm in diameter. Veeramanikandan et al. investigated [22] the shape of copper oxide and iron oxide nanoparticles made with environmentally friendly processes. The irregular rhombic shapes of the nanoparticles ranged in size from 117 µm to 1.29 mm.

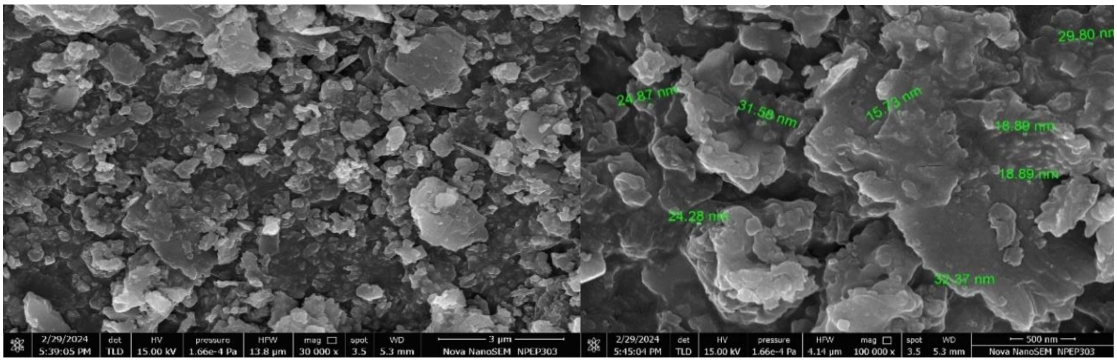

Figure 3: FE-SEM images of Fe_2O_3-NPs with a magnification (3µm and 500nm)

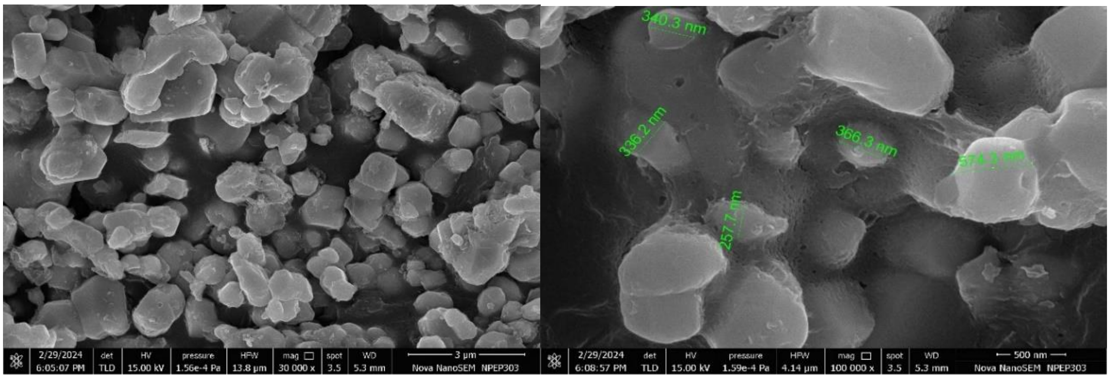

Figure 4: FE-SEM images of CuO-NPs with a magnification (3µm and 500nm)

3.5. Screening of antibacterial activity of iron and copper oxide nanoparticles synthesized by *Blumea lacera* aqueous extract

The antibacterial properties of green-produced iron and copper oxide nanoparticles were evaluated using the agar well diffusion technique against strains of both Gram-positive and Gram-negative bacteria. The green-produced iron and copper oxide nanoparticles have antimicrobial properties. The development of B. subtilis (20 mm) and E. coli (18 mm) among the bacterial strains under investigation was significantly reduced by iron oxide nanoparticles at a concentration of 25µg. At 25 µg, copper oxide nanoparticles demonstrated moderate growth inhibition of 15 mm for E. coli and 13 mm for S. aureus. The zone of inhibition formed by the commercial antibiotic ciprofloxacin (20 µg/disc) was larger than the ones produced by iron and copper oxide nanoparticles, measuring between 11 and 20 mm. The outcomes concur with those of Veeramanikandan et al., who found that Blumea lacera leaf extract may be used to produce iron and copper oxide nanoparticles that had strong antibacterial activity.

The antibacterial activity of the iron and copper oxide nanoparticles was shown to be highly controlled by their concentration. The antibacterial properties of Punica granatum peel extract against P. aeruginosa were demonstrated using concentration-dependent iron and copper oxide nanoparticles, according to research by Irshad et al. [23]. Iron nanoparticles were produced by Kiruba Daniel and associates using a leaf extract from the perennial shrub Dodonaea viscosa [24]. The resultant nanoparticles showed significant antibacterial activity against human infections, including B. subtilis, K. pneumonia, S. aureus, and E. coli.

CONCLUSION

In this investigation, iron and copper oxide nanoparticles were produced using an aqueous extract derived from Blumea lacera. Analysis conducted through XRD, SEM, and TEM affirmed the nanoparticles' varied shapes and sizes. The research outcomes suggest that iron and copper oxide nanoparticles synthesized via environmentally friendly approaches exhibit notable antibacterial effectiveness against multiple bacterial infections. These nanoparticles hold promise as potential alternatives to conventional antibacterial medications for combating bacterial infections.

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