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FRUIT EXTRACT-BASED SYNTHESIS, CHARACTERIZATION, AND ANTIMICROBIAL STUDY OF CuO@Pd NANOCOMPOSITE

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ABSTRACT

In order to treat bacterial and fungal infections, metal-based nanoparticles have been utilised increasingly alongside the use of antibiotics. The plant-material-based green synthesis of these nanoparticles has received a lot of attention for its cost-effectiveness, safety, and efficiency. In this study, CuO and Pd nanoparticles were synthesized via a fruit extract of *Bombax ceiba*. CuO@Pd-based nanocomposite was synthesized under ultrasonic conditions using green-synthesized CuO and Pd nanoparticles. With the help of various analytical methods, CuO@Pd has been characterized and used as an antibacterial agent against different kinds of bacterial and fungal species. The maximum zones of inhibition against *B. subtilis*, *E. coli*, and *S. aureus* was found to be 15.7 ± 0.13, 6.7 ± 0.45, and 18.4 ± 0.21 mm. In case of *A. niger* and *C. parapsilosis*, the zones of inhibition were observed to be 9.1 ± 0.11 and 10.3 ± 0.23 mm, respectively.

Keywords: *Bombax ceiba* Fruit Extract, CuO@Pd, Synthesis, Characterization, Antimicrobial Activity.
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INTRODUCTION

In the recent past, the fields of agriculture, food, medicine, energy storage, wastewater treatment, and catalysis have all benefited significantly from the use of nanomaterials. Advanced thermal, mechanical, electrical, optical, and catalytic capabilities have been demonstrated by these materials.[1] The adjustable features, along with the improved performance of nanoparticles (NPs), render them extremely important in the fields of science and technology.[2] NPs are small particles with sizes less than 100 nm, and they may differ in their shape (0 to 3D). Their size has an enormous effect on a material's physicochemical characteristics.[3] Metal-based NPs have played a crucial role for over a century.[4] These NPs have potentially been used in medical, water remediation, electronics, sensors, food sectors, and information technology [5,6]. Common metal-based NPs are silver (Ag), platinum (Pt), gold (Au), palladium (Pd), zinc oxide (ZnO), cupric oxide (CuO), iron oxides, cerium oxide (CeO_2), titania (TiO_2), manganese oxide (MnO_2), calcium oxide (CaO), and magnesium oxide (MgO).[6,7] The development of novel antimicrobial agents, such as metal-based NPs, has been encouraged by an increase in antibiotic resistance.[8] Strong antimicrobial activity renders metal-based NPs one of the most promising in medical, biotechnology, and pharmaceuticals.[9] In order to enhance surface area, surface activities, and efficiency, transition metal oxide NPs can be doped with or mixed with other transition metal NPs to create metal oxide/metal-based nanocomposites.[10] A variety of physical and chemical methods are available for the synthesis of metal-based NPs, and the selection of a suitable method is based on some facts such as efficiency, low cost, eco-friendliness, and feasibility for the creation of NPs with the desired shape as well as size.[11] Green synthesis is an affordable, environmentally friendly, and sustainable substitute for conventional techniques in the fabrication of metal-based nanoparticles. The use of plant-based extracts minimizes the need for costly and energy-intensive processes as well as potentially hazardous chemicals. The plant

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extracts contain different biologically active components that act as stabilising and reducing agents. These biologically active components can also be helpful in the functionalisation of NPs.[12,13] Cupric oxide NPs (CuO NPs) are promising nanomaterials due to their strong antimicrobial, anticancer, catalytic, and coating properties. CuO NPs are cost-effective and p-type semiconductors that show thermal and chemical stability.[14] These NPs have been used in sensors, antimicrobial agents, wastewater treatment, electronics, catalysis, and different biomedical applications.[15] Palladium NPs (Pd NPs) have different advanced physicochemical properties, such as excellent chemical and thermal stability, good electronic, and plasmonic properties. Pd NPs can also be synthesised cost-effectively and used in catalysis, fuel cells, sensors, and hydrogen storage.[7] *Bombax ceiba* (*B. ceiba*) is also known as the Kapok tree or Moca. This plant is a common member of the Bombacaceae family. *B. ceiba* is used in Indian traditional medicine. The fruits contain fibres, and these fibres are used in life jackets, pillows, and mattresses. The fruits also have important medicinal values and are used in respiratory and digestive problems, skin treatments, and wound healing.[16-18] In this work, CuO and Pd NPs were synthesised using the fruit extract of *B. ceiba*. After that, we mixed these green-synthesised NPs in an appropriate ratio and treated them with ultrasonics to produce the CuO@Pd nanocomposite. CuO@Pd was then used as an antimicrobial agent and examined using a variety of advanced analytical techniques.

EXPERIMENTAL

Materials Used

All chemicals were of AR grade. *B. ceiba*, double-distilled water (DDW), copper acetate [$Cu(CH_3COO)_2$], sodium hydroxide (NaOH), palladium chloride ($PdCl_2$), Mueller–Hinton agar (MHA), Ciprofloxacin, Amphotericin B, and Sabouraud dextrose agar (SDA) were used in this work.

Preparation of Fruit Extract

The collected fruits of *B. ceiba* were carefully washed, dried, and crushed into small pieces. 5 g of dried fruit has been mixed with 100 mL of DDW and placed on a temperature-regulated magnetic stirrer for 40 min at 70 °C. 1 mL of ethanol was mixed into this mixture and boiled again for 35 min. Cool the mixture at room temperature and then filter. The filtrate was used as a fruit extract.

Synthesis and Characterization of CuO@Pd

After adding 10 mL of fruit extract to 100 mL of 0.2 M $Cu(CH_3COO)_2$, the mixture was shaken at 80^0C for 100 min. Thereafter, the mixture was cooled to room temperature, and immediately after that, a few drops of 0.1 M NaOH were introduced into it and shaken for 45 min. The obtained precipitate was separated by centrifugation and washed with DDW. After drying, the precipitate (CuO NPs) was calcined at 400^0C. A mixture of 100 mg of $PdCl_2$ and 100 mL of DDW was shaken for 30 min. This mixture was then agitated for 30 min at 60^0C after 10 mL of the extract had been added. Once it has cooled down to room temperature, add a few drops of 0.1 M NaOH. Stir it for another 30 min, and then the residue was collected and dried in hot air after being centrifuged and completely rinsed with DDW. The Pd NPs powder was calcined for 2 h at 350°C. Both non-calcined and calcined CuO and Pd NPs have been mixed in a ratio of 15:1 and ground for 1 h. These mixtures were added to DDW and then placed in a sonicator for 3 h, and then completely dried. The dried non-calcined and calcined CuO@Pd were preserved for characterization and antimicrobial studies. The characterization of CuO@Pd has been done via different analytical techniques, such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared (FTIR), energy-dispersive X-ray spectroscopy (EDX), and X-ray diffraction (XRD) techniques.

Antimicrobial Activity

The disc diffusion technique was utilised to investigate the antibacterial properties of both non-calcined and calcined CuO@Pd against different bacterial strains, such as *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus*). The disc diffusion method is very common in clinical microbiology. It is effective, low-cost, and easy to use. The bacterial culture was applied over the MHA plates, and each disc was filled with 10 mL of each concentration. Ciprofloxacin (10 mg) was used as a positive control. The petri plates were incubated at 37 °C for 24 h. Following the incubation time, the discs' surrounding clear zones of inhibition (ZOI) were examined. Similarly, the

antifungal study has been conducted against *Aspergillus niger* (*A. niger*) and *Candida parapsilosis* (*C. parapsilosis*). For this study, fungal culture was put on the SDA plates for inoculation. Each disc was filled with 10 ml of each concentration. 50 mg of Amphotericin B has been used as the positive control. The plates were incubated for 48 h at 37 °C. The ZOI was measured around the discs after the incubation period.

RESULTS AND DISCUSSION

Characterization of CuO@Pd

Plant extract-based metal/metal oxide nanoparticles can be fabricated very effectively. This method avoids the use of harmful chemicals and is very low-energy-consuming. The fruit extract-based CuO@Pd has been characterized using the following methods:

FTIR

Figure-1 depicts the FTIR spectra of non-calcined CuO or CuO (NC), calcined CuO or CuO (C), non-calcined CuO@Pd (NC), and calcined CuO@Pd or CuO@Pd (C), respectively. The FTIR peaks for CuO (NC) are assigned at 3386, 2886, 2831, and 1584 cm^{-1} and indicate the presence of O-H (str), C-H (-CH_2 or CH_3 of hydrocarbon chains), and O-H (bending). The other peaks are assigned at 1413, 1012, 603, and 502 cm^{-1}, which confirms C=C, C-O or C-H (bending), and Cu-O bonds. Similarly, for CuO@Pd (NC), the peaks have been assigned at 3372, 2883, 2818, 1580, 1012, 648, and 502 cm^{-1}, which reveals the presence of O-H (str), C-H (-CH_2 or CH_3 of hydrocarbon chains), O-H (bending), C=C, C-O or C-H (bending), Cu-O, Pd-O, etc. bonds. The characteristic FTIR peaks of CuO (C) are assigned at 3420, 2882, 2802, 1671, and 701 cm^{-1}. These peaks indicate the presence of O-H, C-H, C=O (absorbed CO_2), and Cu-O bonds. The FTIR peaks such as 3440, 2882, 2824, 1435, 1352, 879, 703, and 595 cm^{-1} have been assigned for CuO@Pd (C). The peaks at 3440, 2882, and 2824 cm^{-1} reveal the presence of O-H and C-H bonds. The peak at 1435 cm^{-1} indicated the scissoring of -CH_2, while 879, 703, and 595 indicated the presence of Cu-O, Pd-O, Cu-Pd, etc. bonds.[19-25]

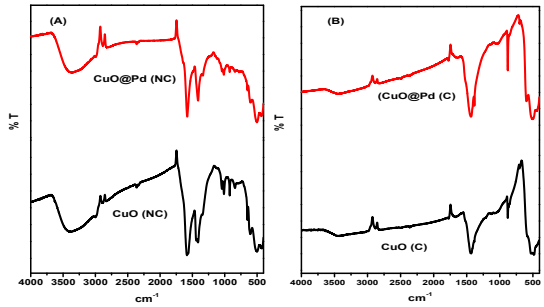

Fig.-1: FTIR Spectra of (A) Non-Calcined CuO & CuO@Pd and (B) Calcined CuO & CuO@Pd

XRD

The XRD patterns of CuO and CuO@Pd are presented in Fig.-2. These patterns reveal that the crystallinity of both components increases after the calcination. The major common peaks of CuO@Pd (NC) and CuO@Pd (C) were assigned at 2 θ = 32.6^0, 35.6^0, 38.8^0, 46.4^0, 49.3^0, 53.5^0, 58.3^0, 61.6^0, 66.6^0, 68.1^0, 72.2^0, and 75.4^0. These peaks are related to 110, 002, 111, 200, 202, 020, 202, 113, 022, 220, 220, 312, and 203 planes, respectively.[13,26-30]

SEM, EDX, and TEM

Figure-3 presents the SEM images of CuO (NC), CuO (C), CuO@Pd (NC), and CuO@Pd (C). Figure-3 (A and B) reveals a flower-like morphology of CuO (NC) and a sphere-shaped morphology of CuO@Pd (NC). Agglomerated irregular-shaped particles of CuO (C) and CuO@Pd (C) appear in the SEM images (Fig.-3C and D). The EDX spectra and composition of CuO@Pd (C) are also presented in Fig.-4. TEM image of the final composite CuO@Pd (C) also reveals the irregular morphology (Fig.-5).

Antimicrobial Activity of CuO@Pd

The development of novel antimicrobial agents is concerned with antimicrobial resistance. Metal-based nanomaterials are a growing concern, leading to the development of new antimicrobial agents. Their advanced physico-chemical properties can differentiate microbial and mammalian cells.

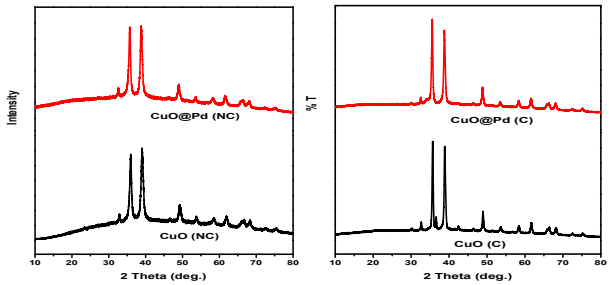

Fig.-2: XRD Patterns of (A) Non-calcined CuO & CuO@Pd and (B) Calcined CuO & CuO@Pd

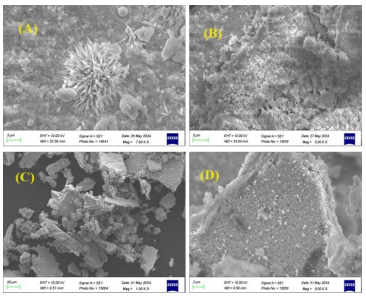

Fig.-3: SEM images of (A) Non-Calcined CuO, (B) Non-Calcined CuO@Pd, (C) Calcined CuO, and (D) Calcined CuO@Pd

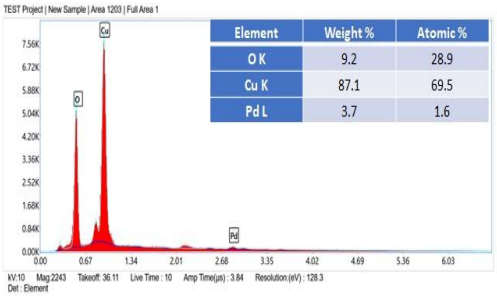

Fig.-4: EDX Spectrum and EDX Composition of Calcined CuO@Pd

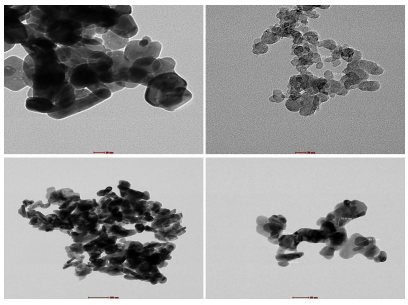

Fig.-5: TEM Images of Calcined CuO@Pd

Their smallest size has the strongest antimicrobial effect and is a promising candidate to challenge antimicrobial resistance. Metal-based nanomaterials show antimicrobial activity via different ways, such as the generation of reactive oxygen species (ROS), damage to DNA and protein, inhibition of biofilms, disruption of cell structure, enzyme malfunctioning, disturbances in nutrient uptake mechanisms, synergistic effects, and release of metal ions.[8, 31,32]

Antibacterial Activity

Metal-based nanomaterials have been used as excellent antibacterial agents. Excellent optical properties, multifaceted antibacterial activity, and application in photo-activated treatments such as photothermal and photodynamic therapy are among the desired physicochemical characteristics of these materials.[33] The

antibacterial activity of these materials takes place in three common ways: disturbing cellular metabolism, accelerating oxidative stress, and destroying bacterial membranes.[34] The antibacterial effect of CuO@Pd (NC) and CuO@Pd (C) is presented in Fig.-6. The minimum inhibitory concentrations (MICs) of CuO@Pd (NC) were found to be 12.5 mg/mL for *B. subtilis*, and 5 mg/mL for *E. coli* and *S. aureus*. The ZOI around the disc containing CuO@Pd (NC) was found to be 3.8 ± 0.19, 6.2 ± 0.13, and 7.2 ± 0.41 mm at 12.5 mg/mL against *B. subtilis*, *E. coli*, and *S. aureus* after the incubation period (Fig.-6A). This value increased to 7.6 ± 0.23, 7.9 ± 0.56, and 8.5 ± 0.73 mm at 50 mg/mL, and the maximum ZOI was observed to be 9.5 ± 0.21, 10.6 ± 0.42, and 9.9 ± 0.79 mm at 100 mg/mL. In the case of CuO@Pd (C), MICs are 5 mg/mL for *B. subtilis* and *S. aureus* and 25 mg/mL for *E. coli*. The ZOI was found to be 7.6 ± 0.49, 3.8 ± 0.71, and 8.6 ± 0.91 mm at 25 mg/mL against *B. subtilis*, *E. coli*, and *S. aureus,* and increased to 15.7 ± 0.13, 6.7 ± 0.45, and 18.4 ± 0.21 mm, respectively (Fig.-6B).

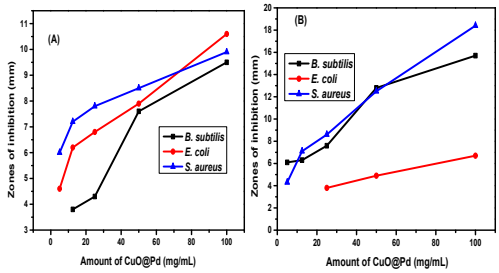

Fig.-6: Antibacterial Activity of (A) CuO@Pd (NC) and (B) CuO@Pd (C)

Antifungal Activity

The novel antifungal treatments are necessarily required because of the lack of available options and concerns about potential future resistance. Different antifungal agents, such as chemical products, plant extracts, essential oils, and metal-based nanomaterials, have been used to control fungal diseases. Metal or metal oxide-based nanomaterials can be good alternatives to the conventional antifungal agents. They can also be used after blending with conventional antifungal agents. These materials target fungal strains through ROS generation, biofilm inhibition, DNA damage, inhibition of fungal spore germination, cell membrane destruction, and release of toxic metal ions.[35,36] Figure-7 demonstrates the antifungal activity of CuO@Pd (NC) and CuO@Pd (C) against *A. niger* and *C. parapsilosis*. The MICs of CuO@Pd (NC) and CuO@Pd (C) against *A. niger* and *C. parapsilosis* were found to be 5 mg/mL. At this dosage, the ZOI for CuO@Pd (NC) was found to be 3.1 ± 0.61 and 4.2 ± 0.37 mm against *A. niger* and *C. parapsilosis*. The ZOI has been calculated to be 7.4 ± 0.13 and 8.1 ± 0.11 mm at 50 mg/mL, which increased to 9.1 ± 0.11 and 10.3 ± 0.23 mm at 100 mg/mL (Fig.-7A). In case of CuO@Pd (C), the ZOI was observed to be 6.8 ± 0.42 and 4.6 ± 0.73 mm against *A. niger* and *C. parapsilosis*; at maximum dosage, the ZOI was observed to be 8.6 ± 0.33 and 8.8 ± 0.23 mm (Fig.-7 B).

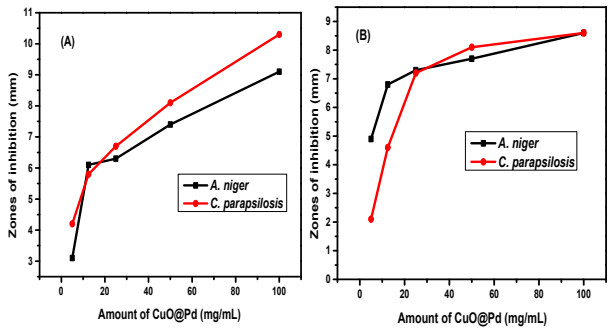

Fig.-7: Antifungal Activity of (A) CuO@Pd (NC) and (B) CuO@Pd (C)

CONCLUSION

The green synthesis utilizing plant extract is efficient and environmentally friendly. The metal-based nanoparticles can be fabricated effectively and utilized in different fields of science and technology. The antimicrobial capability of metal-based nanomaterials appears exciting. Additional research must focus on

the special mechanisms and their safety for exposure to humans and the environment. The fruit extract and ultrasonic-based synthesis of CuO@Pd were found to be efficient, eco-friendly, and cost-effective. The important physico-chemical characteristics of CuO@Pd were demonstrated using different analytical methods such as FTIR, XRD, SEM, TEM, and EDX. The CuO@Pd was found to be more effective against *S. aureus* and *A. niger* as compared to other bacterial and fungal strains.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest in the publication of this article.

AUTHOR CONTRIBUTIONS

This article is related to the *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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