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Fabrication and Analysis of Selenium and Copper Nanoparticles Integrated with Biomaterial for Antibacterial Evaluation

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ABSTRACT

The plant contains different important phytochemicals that can be used as potential medicine for various ailments. This work describes a green synthesis approach for synthesizing copper oxide and selenium nanoparticles from *Punica granatum* leaves extract. The formation of CuNPs & SeNPs was monitored by UV-Visible, FT-IR, zeta, XRD and SEM techniques. The FT-IR spectra confirmed the presence of functional groups which are associated with the bioactive molecules, whereas the suspension solution confirmed the formation of SeNPs and CuNPs as done by UV analysis. X-ray diffraction (XRD) study exhibits the amorphous nature for both SeNPs and CuNPs. The morphology and crystalline phase of the metal nanoparticles were determined using scanning electron microscopy (SEM). The CuNPs showed potent antibacterial activity whereas SeNPs showed considerable activity against Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). Both SeNPs and CuNPs exhibit efficient rhodamine B dye degradation in the presence of UV or sunlight. However, CuNPs have better catalytic degradation ability for rhodamine B dye as compared to SeNPs.

KEYWORDS

Copper oxide, Selenium, Nanoparticles, *Punica granatum*, Selenium, Copper, Nanomaterials, Antibacterial activity, Photocatalytic activity.

INTRODUCTION

Among the scientific disciplines which is now making the most strides is nanotechnology. Nanomaterials are produced using processing at the nanoscale scale and a surface to volume ratio to regulate their size and form. Traditionally, manufacturing techniques are considered to be nanoscale when the size is smaller than 100 nm [1]. To synthesize nanomaterials, two processes are employed. Usually, it deals with very small particles. In the first technique, bigger pieces of the material are used to develop extremely tiny components. Nanostructures are created using the bottom-up approach by treating them atom by atom or molecule by molecule. Through the introduction of several previously unexplored disease experiments and treatment possibilities, the growth of nanotechnology over the last three decades has altered how people see medication research and development. For medical agents and drug delivery

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vehicles, a number of nanomaterials have been employed, comprising polymers, dendrites, metal nanoparticles (Au, Ce, Cu, Fe, Se, Ti, Y, *etc*.), silicon and carbon-based materials [2].

The nanomaterials and carbon nanotube nanocomposite are some of the important materials that are extremely marketable in both research domains and markets. One of the most intriguing materials with enormous promise is nanomaterials. It assists in removing barriers in a variety of industries, including corrosion, medicine delivery, electronics and other fields [3]. The development of materials for drug delivery applications, heat, light, as well as other specialized devices to detect cells such as cancer cells is one excellent use of nanomaterials in the medical area. As a result of the produced particles' ability to identify specific sick cells, individual cells may now be treated directly. In this technique, the kind of protein produced by diseased kidneys is attached to by gold nanorods. The colour of the nanostructures changes as protein builds up on them. The test is primarily intended for issue early identification and it is affordable for problem early detection. Utilizing quantum dots to treat illnesses with antibiotic resistance is a major area of study [4].

Nanomaterials are seen to be the most potential solution for this problem. The most effective antibacterial treatments against Gram-positive and Gram-negative bacteria are silver nanoparticles. Due to their chemical stability, catalytic activity, ability to heal wounds, high conductivity and surface plasma resonance, the materials have attracted a lot of attention. It has been found that they often have stronger antibacterial and antimicrobial activities than bulk materials. However, several medical uses are hampered by AgNP's limited stability [5], therefore it is crucial to look into how long silver nanoparticles can be stored under various storage conditions. Many approaches used today often involve complicated processes that employ a lot of energy or harmful reducing chemicals. Since less harmful or even non-toxic compounds may be used to create simpler processes, several studies have promoted this. In this instance, several papers have reported employing *Punica granatum* peel extraction, a substance that is neither inexpensive nor readily accessible, to create selenium and copper nanoparticles using a variety of plant extracts [6].

Several key features including size, shape, atomic arrangements, nanostructures and surface charge, are dominant in the application of nanoparticles. In this work, the selenium and copper nanoparticles were synthesized using peel extract of *Punica granatum* which acts as a good reducing agent. The use of peel extract has the advantage of being both cost-effective and eco-friendly since the unfertilized extract is readily available and relatively inexpensive [7].

EXPERIMENTAL

All of the chemicals utilized in the synthesis process, including cupric nitrate and selenium acid, were of analytical purity standard and were purchased from Sigma-Aldrich, USA.

Plant extract preparation: *Punica granatum* leaves were obtained from a plant which is readily available. The good ones are taken for extract preparation, they are all uniform in size, appearance. They were washed and used for the preparation of extract. They were dried in air and 50 g of leaves were added in 100 mL of water. Then they were kept in a stirrer for heating at 60 ºC. water turns in colour and the extract was collected for further analysis. The leaves of the *Punica granatum* were used for the present study. The aqueous extract of the leaves were prepared.

Green synthesis of CuONP's: The copper oxide nanoparticles were synthesized in powder form by microwave irradiation method. *P. granatum* leaves were added to 100 mL of water and kept for boiling and after 30 min ode boiling the extract was taken for further use. *P. granatum* leaf extract is added to cupric nitrate solution. Initially the cupric nitrate was blue in colour and after the addition of the *P. granatum* leaf extract the colour changes from blue colour cupric nitrate to green colour. The solution were kept for drying.

Green synthesis of selenium nanoparticles: Even though this cutting-edge platform provides special benefits, the idea of nanomedicine has arisen as a new rising star in the field of pharmaceuticals. The complicated problems connected with traditional medication forms and their dose forms are taken into account by nanomedicine-based techniques as possible solutions. The reliability of nanomedicine is a well acknowledged benefit. The usage of Se in the form of nanoparticles has significantly allayed the toxicological worries related to Se, which was the main barrier from laboratory to clinic translation in the case of Se due to the short therapeutic window and little margin of dosage error. SeNPs may be created using a variety of techniques, such as biological or synthetic ones. The focus of the current study does not include a full discussion of the numerous techniques utilized to synthesize SeNPs, which may be found in previously reported studies. A selenium acid solution is infused with *P. granatum* leaf extract. Ruby red colour replaces the formerly colourless selenious acid. By using a stirring technique, selenium nanoparticles were created in powder form. Utilizing various approaches such UV-visible spectroscopy, FT-IR, field emission scanning electron microscopy, ZETA size and XRD, synthesized SeNPs are evaluated to determine their antibacterial and photocatalytic activities.

RESULTS AND DISCUSSION

Characterization of green synthesized nanoparticles

UV-Visible spectroscopy: CuO & SeNPs production was studied using UV-Visible spectroscopy. When the extraction was added to a cupric nitrate mixture, the colour changed from blue to green. This happens as a result of surface plasmon vibrations being excited by copper oxide nanoparticles, which is confirmed by a peak at 340 nm. Selenium nanoparticles were synthesized, as shown by the absorbance at 320 nm with regard to the reaction's duration. When the reaction liquid turned from translucent to ruby red over time, it was clear that se nanoparticles were forming. After 24 h of incubating, no further colour change was visible. These findings showed that biomolecules from *P. granatum* leaves totally produced Se nanoparticles [8].

FT-IR (Fourier transform infrared spectroscopy) analysis: Fig. 1a shows the outcome of an FT-IR investigation of artificial CuO nanoparticles. O-H stretch alcohols and phenols are shown by a wide peak at 3328 cm^{-1}. C-H stretch

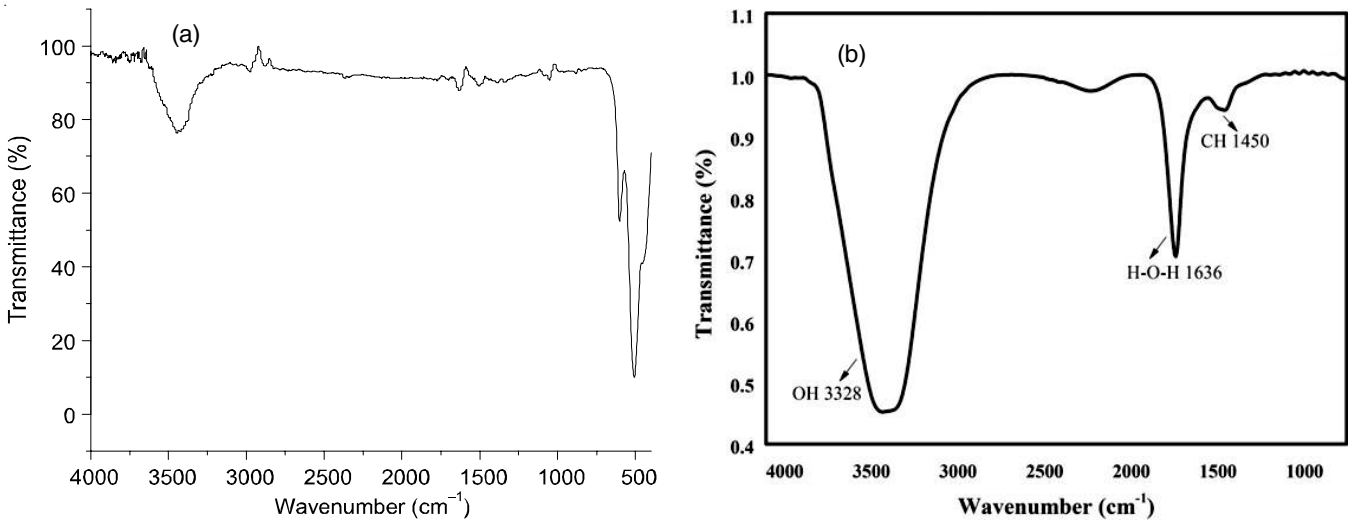

Fig. 1. FT-IR peak of (a) CuONPs and (b) selenium nanoparticle

aromatic hydrocarbons correlate to the absorption peak at 1636 cm^{-1}. Alkanes' C-H bending causes the strong band at 1636 cm^{-1}, or (CH_3 bend). Fig. 1b shows the results of an FT-IR investigation of produced selenium nanoparticles. O-H stretch alcohols and phenols correlate to a large peak seen at 3456 cm^{-1}. N-O asymmetric stretch nitro compounds are responsible for the adsorption peak at 1525 cm^{-1}. This finding suggests the existence of multiple functional groups in biomolecules, which may be in charge of simultaneous stability and reduction [9].

Dynamic light scattering (DLS analysis): The size of the prepared nanoparticles were analyzed by dynamic light scattering (DLS), this clearly reveals that the particles are evenly distributed. The optimized CuO nanoparticles shows the size rage of 55 nm, Se nanoparticles shows the size range of 48 nm and prepared *Punica granatum* shows the size range of 32 nm (Fig. 2). This size range clearly shows the prepared particles are mono dispersed and the particles are in nano size range. These nanoparticles can be further taken for various analysis and for various applications [10].

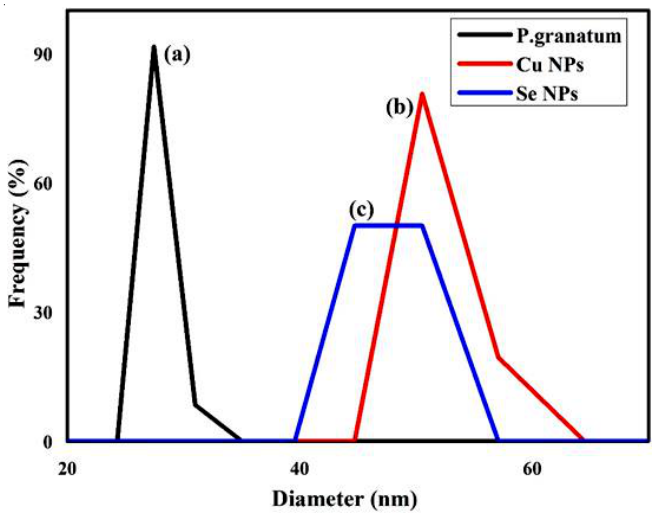

Fig. 2. DLS of *P. granatum*, CuNPs, SeNPs

XRD analysis: The nature of the crystalline phase and size of the reduced Se nanoparticles and CuO nanoparticles were determined by using X-ray diffraction pattern. The XRD patterns obtained shows the main peaks characteristics of crystalline Se and CuO nanoparticles. Fig. 3a shows the XRD patterns of CuO there the results shows the crystalline peaks at 2θ values of 23.3º, 39.4º, 50º, 59º, 70.2º, which corresponds to the crystal planes of (111), (200), (202) and (220) respectively.

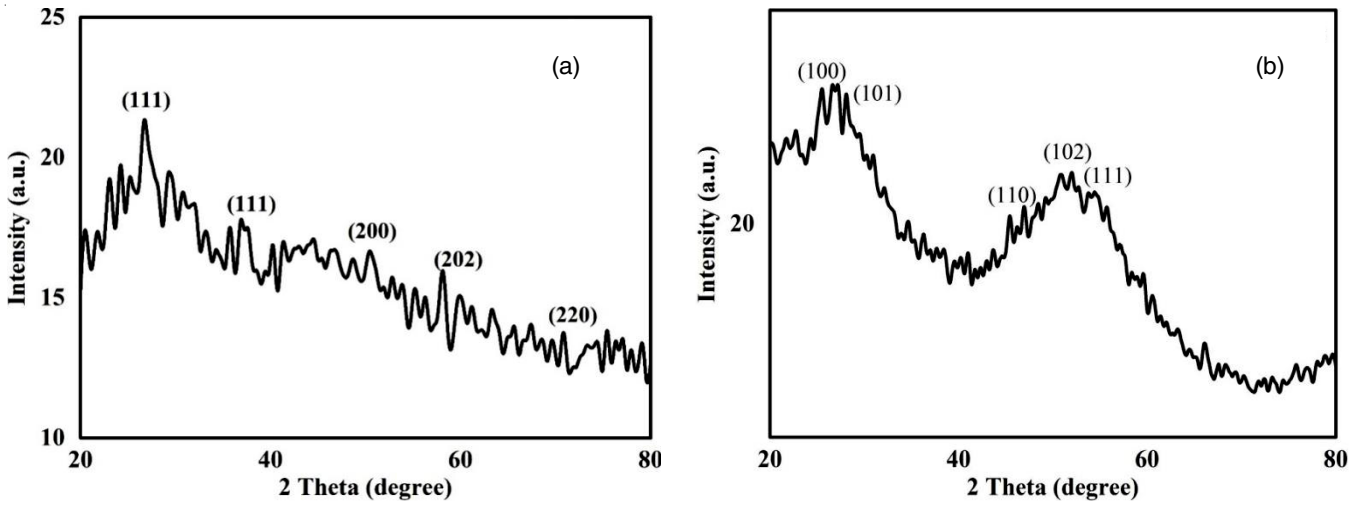

Fig. 3. X-ray diffraction of (a) CuONPs and (b) SeNPs

Fig. 3b shows the XRD patterns of selenium nanoparticles, there the results shows the crystalline peaks of 23.5º, 29.2º, 43.4º, 45º,50º, which corresponds to the crystal planes of (100), (101), (110), (102), (111). The results clearly reveals the crystalline nature of the nanoparticles and it shows the presence of CuONPs and SeNPs [11].

FE-SEM studies: The form of the CuO and SeNPs prepared by the green approach employing *P. granatum* leaves aqueous extraction is examined using scanning electron microscopy. The FE-SEM images of CuO and SeNPs quantities of the encapsulated spherical nanoparticles with diameters between 45 and 90 nm are shown in (Figs. 4 and 5). Because of the even distribution of these aggregated particles, it has been hypothesized that nanoparticle aggregation predominates over the reduction mechanism and initial nucleation of reduced atoms. This could be because more functional groups (found in the aqueous extract of *P. granatum* leaves) bind and nucleate cupric nitrate and selenious acid ions. It seems that fewer nucleation events engage the most accessible metal ions, which causes the metal to aggregate. An earlier study revealed that agglomeration NPs had increased biological activation. CuO and SeNPs mediated by the synthetic *P. granatum* leaves aqueous extraction may have useful uses in pharmacology [12].

Antimicrobial activity: On several harmful bacterial strains–*Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus* sp.–the antibacterial activity of CuO & SeNPs was tested. Ampicillin, an antibacterial medication, was a common medication. *Versus Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus* sp., CuONPs shown strong action. Against *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus* sp., SeNPs shown notable efficacy. However there was no action against the bacterium *Escherichia coli*. CuO & SeNPs have found a greater reduction zone in *Bacillus subtilis*, which suggests that it may have antibacterial properties (Table-1). The usage of green metal nanoparticle production will be of paramount relevance in the medical area [13,14].

Photocatalytic activity: Rhodamine B dye deterioration under ultraviolet light served as a test for the photocatalytic activity of the produced CuO & SeNPs. The progressive shift in hue from deep rose to light pink dye solution or colourless dye solution served as a visual indicator of dye degradation. The distinctive absorption peak for RhB was found at 554 nm (maximum). RhB dye degradation is observed in Fig. 6. In the presence of H_2O_2, which happens as a result of the generated hydroxyl radicals by absorbing photons, the frequency of

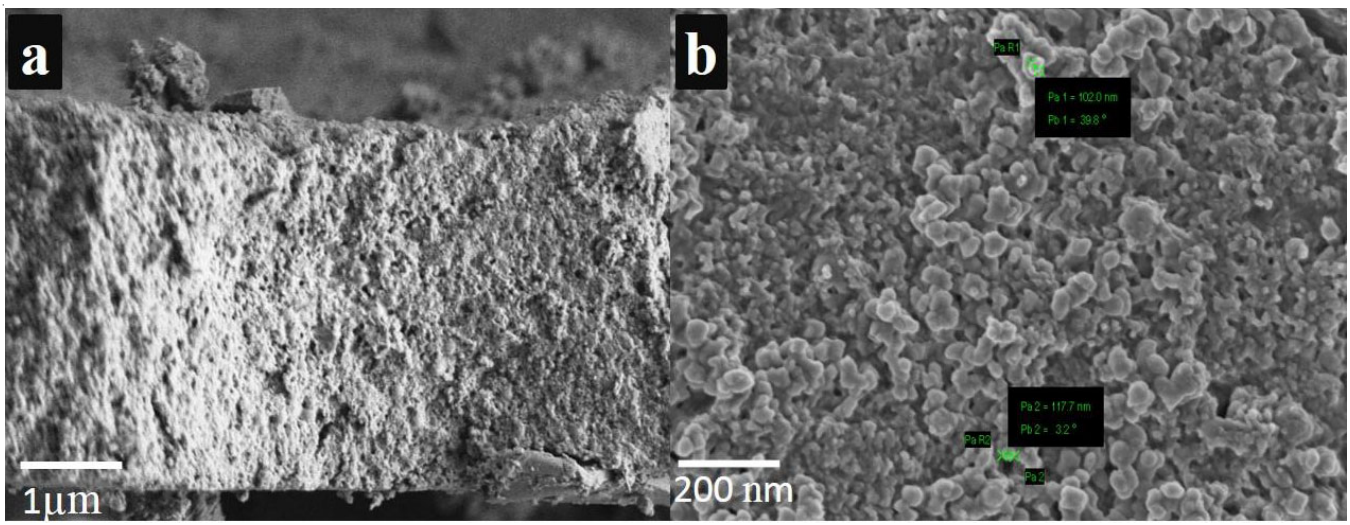

Fig. 4. (a) High magnification of FE-SEM image of CuONPs and (b) Low magnification of FE-SEM image of CuONPs

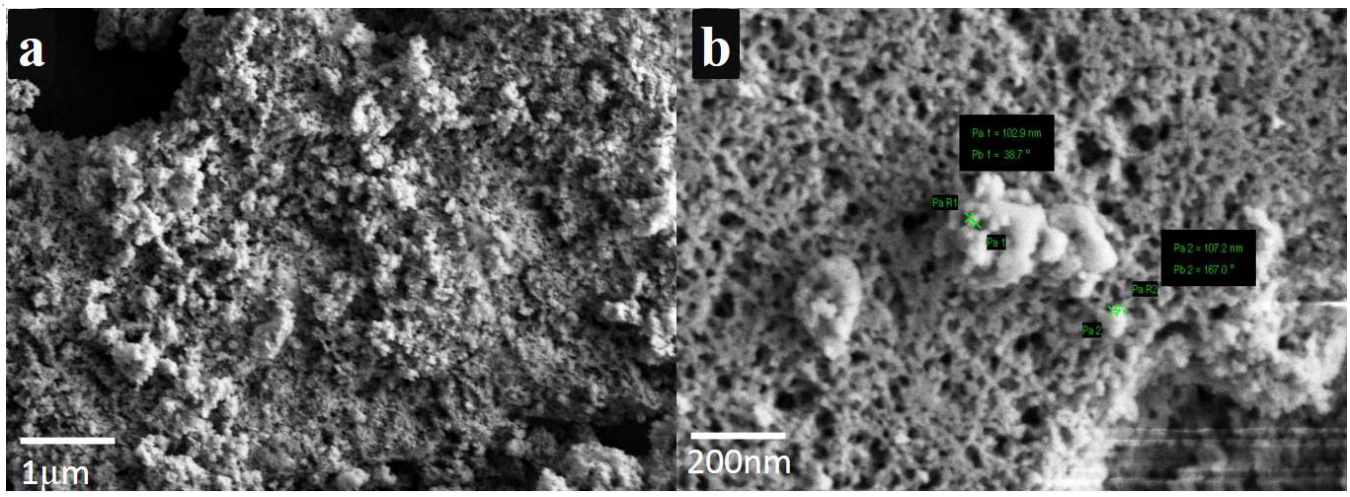

Fig. 5. (a) High magnification of FE-SEM image of SeNPs and (b) Low magnification of FE-SEM image of SeNPs

TABLE-1
ANTIMICROBIAL ACTIVITY USING
SYNTHESIZED NPs ZONE OF INHIBITION (mm)

Bacterial cultures	Zone of inhibition (mm)		
	Ampicillin (A9393-5G)	CuONPs	SeNPs
Bacillus subtilis	12	15	11
Staphylococcus aureus	10	12	5
Escherichia coli	25	12	0
Streptococcus sp.	0	12	10

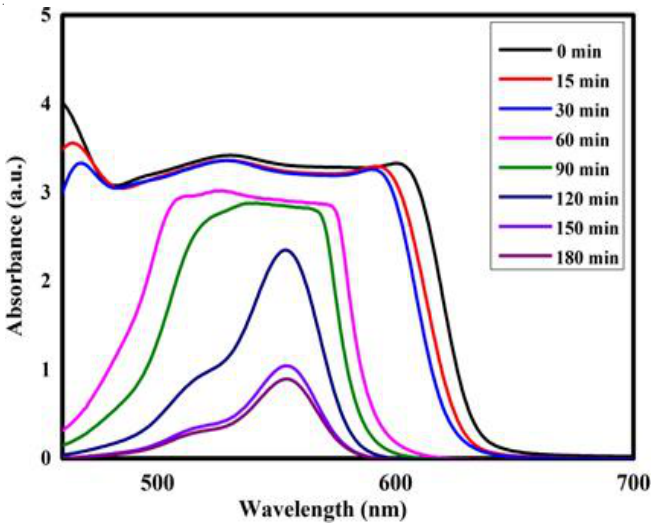

Fig. 6. Photocatalytic activity of SeNPs

photodegradation would rise. When it refers to photocatalytic performance, the dye degradation in the presence of synthesized CuO NPs performed extremely poorly in comparison to selenium nanoparticles. This was demonstrated by the fact that the peak intensity at 554 nm decreased during the course of a 30 min UV exposure. According to the photocatalytic investigation, SeNPs are effective at destroying RhB when exposed to UV light. As a result, they may be used in the textile and water treatment sectors. The UV spectrum of selenium nanoparticles has an intensity that varies from 4.3 to 0.4 times that of rhodamine B dye (Table-2) [15-17].

TABLE-2
PHOTOCATALYTIC ACTIVITY OF
SELENIUM NANOPARTICLES

Time (min)	Intensity
0	4.3
30	3.9
60	3.2
90	2.8
120	2.2
150	1.1
180	0.4

Conclusion

Punica granatum was generated using CuO and Se nanoparticles and its morphology was examined. In addition, the photocatalytic study found that the green synthesis of CuO and Se nanoparticles effectively degraded RhB. Those certain findings suggest that CuO and Se nanoparticles have considerable antioxidant activity and serve as a prospective antibacterial representative. They can be used in the water therapeutic and textile industrial applications. CuO and Se nanoparticles that were created have excellent antibacterial properties, while Se nanoparticles exhibit photocatalytic activity against RhB dye molecules. As a result, the interaction between phytocompounds and nanomaterials plays a significant role in its diverse characteristics. Compared to CuO NPs, SeNPs have even greater potential for photocatalytic performance.

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