

A Systematic Investigation on Elaeocarpus Sylvestris Leaf Extract Capped CuO Nanoparticles as Reducing agent and their Antioxidant activity

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Abstract

Recently, the role of plant-based nanoparticles as sustainable catalysts has emerged as a new field in material science. As a result of their wide applicability, bio-designed nanocatalysts have dominated the present study. At this juncture, this study gives the rapid and systematic procedure for the formation of an aqueous *Elaeocarpus Sylvestris* plant leaf extract capped Copper Oxide nanoparticles (ESCuO NPs). A benign method has been established for the fabrication of ESCuO nanoparticles that which serves as an innocuous, renewable, and mild reducing agent. Vivid spectrochemical and optical experimental investigation supported the formation of the ESCuO particles. Based on TEM investigation, the produced ESCuO NPs had spherical shape and an average size of 53 nm. The phytochemicals were used as a reducing agent internally without the use of harmful chemicals or extremely high temperatures. The derived ESCuO NPs have been employed as a powerful catalyst to reduce 4-nitrophenol. Further, rate constants for different doses were calculated. The antioxidant efficiency of synthesised ESCuO NPs was determined. This study illustrates the way for cutting-edge synthesis of CuO NPs with numerous applications.

Introduction

As a result of the industrial revolution and the quick development of technology, all types of Industries, in often discharge massive amounts of dangerous chemicals into the air, water, and land, wreaking havoc on the ecosystem. Emerging contaminants are being released into water bodies as a result of the industrial revolution. These new toxins pose dangers to ecosystems and human health, necessitating creative treatment strategies. More specifically, 4-nitrophenol is a straightforward organic aromatic molecule that is widely employed in the production of numerous goods needed in current society, including colours, pesticides, medications, and so forth [1]. However, toxic chemicals like 4-nitrophenol, which are not only harmful but also anthropogenic and hindering in nature, so its reduction is a crucial challenge.

Additionally, 4-nitrophenol and its by-products are utilised to create pesticides, insecticides, and herbicides that pose a harm to both the environment and people [2].

In this connection, the significant challenge of reduction of 4-nitrophenol has been performed with the application of nanotechnology with their specific characteristic properties recently [3]. There is a plethora of effective research on the use of nanoparticles to reduce 4-nitrophenol. Metal nanoparticles have many uses in the biological, electrochemical, and medicinal domains because of their distinctive features, such as substantial surface area relative to volume ratios and significant surface energies related to their bulk molecules[4–7]. There are various types of metal oxide nanoparticles, but copper oxide nanoparticles are of particular interest due to their potential for usage in both science and industry. Due to their biocidal qualities, CuO have drawn attention and may be used effectively in a variety of biomedical applications, including illness treatment, drug delivery, cellular delivery, and biomedical imaging [8]. CuO NPs are employed as heterogeneous catalysts in the pharmaceutical industry as drug delivery, antioxidant,

anticancer, and therapeutic agents [9]. The extremely unique crystal shapes and large surface areas of copper oxide NPs make them extremely excellent antibacterial particles [10].

Like other nanoparticles, CuO NPs can be produced through chemical and physical methods, however current research has begun to recognise green CuO nanoparticles due to the green revolution in all fields. To prevent hazardous chemicals and an abrasive environment when using chemical methods and a laborious procedure while using physical methods, a green protocol for the production of CuO NPs has been established. As a result, the biological technique for producing CuO-NPs is receiving greater attention because it is simple to use, easy to handle, does not require cell culture, and is environmentally benign [11]. Thus, using plant extracts for the synthesis of CuO NPs has already been reported for *Gloriosa superba* [12], *Aloe barbadensis* [13], *Malva sylvestris* [14], and *Carica papaya* [15].

In the current study, *Elaeocarpus Sylvestris* plant leaf extract is used as a green source for producing copper oxide nanoparticles in a sustainable manner. The *Elaeocarpus Sylvestris* plant has uttermost priority both in the spiritual purpose as well as scientific research. This plant belongs to the *Elaeocarpaceae* (Rudraksha) family. These are subtropical and long-leaved evergreen shrubs and trees. The research on this plant reveals that phytochemicals like alkaloids, terpenoids, and flavonoids are present, as reported by Amit Dadhich [16]. The antioxidant activity of this plant was reported by Amalia Indah Prihantini et al. [17]. This shows its action towards depression, stress, pain in the nerve, anxiety, palpitation, migraine, asthma, hypertension, and alleviations related to the liver and arthritis traditionally [18–22]. CuO-NPs have been synthesised employing plant leaf extract from *Elaeocarpus Sylvestris* for the first time. The current study investigates the environmentally friendly synthesis of CuO-NPs, and characterises them using various microscopic and spectroscopic methods, and tested its reduction activity of 4-nitrophenol and antioxidant activities.

Experimental methods

Reagents

The chemicals utilized in this research were of superior quality, conforming to analytical grade specifications. $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, NaOH, 4-nitrophenol, NaBH_4 , and ethanol were obtained from Merk and employed without any refinement. The *Elaeocarpus Sylvestris* tree leaves were piled up near the Lord Shiva temple, Bommuru, Rajahmundry. Double-distilled water was utilised throughout all synthetic procedures.

Green protocol description of ESCuO Nanoparticles:

The fabrication of nanoparticles takes place through a variety of methods and approaches. A facile green protocol through the coprecipitation method has substantially emerged recently. Natural (plant biomolecule) capping, stabilising, and reducing agents are being employed to accomplish CuO NPs by avoiding perilous reagents [23, 24]. Irrespective of all plant particles, leaves are a good extract yielding part through the co-precipitation method.

Elaeocarpus Sylvestris leaf extract preparation

The fabrication of ESCuO NPs was executed by using *Elaeocarpus Sylvestris* leaf extract as a green source operator. *Elaeocarpus Sylvestris* tree leaves were washed to remove the dust particles. To dry these leaves, they were kept in the shade for 15 days. Subsequently, a fine green powder of the dried leaves was obtained by using Ultra Household Mixy. In order to create the 1% aqueous leaf extract, 100 ml of distilled water were combined with one gram of finely powdered leaf. For 20–25 minutes, the aqueous mixture was heated to 60°C while being constantly stirred. Then, to obtain a clear solution free of any particles, the brown aqueous leaf extract was filtered through Whatman No. 1 filter paper. The filtrate was then kept at 4°C.

Elaeocarpus Sylvestris functionalised CuO Nanoparticles synthesis:

By using an environmentally friendly source, such as an aqueous solution of *Elaeocarpus sylvestris* leaf extract, the Coprecipitation process was used to create *Elaeocarpus Sylvestris* functionalized CuO nanoparticles. A typical synthesis technique involved dissolving 1.87 g of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$ in 90 mL of distilled water. At room temperature, the solution is a clear blue colour after being thoroughly mixed for 20 to 25 minutes. The reaction mixture volume was then increased to 100 mL by adding 10 mL of aqueous leaf extract, which caused it to turn dark green. Then it was stirred for 15 minutes to get sonicated well with the mixture. Later, the temperature was raised to 80°C and maintained for up to 1 hour, and an intense green colour was observed. Accordingly, 1M 10 mL NaOH was added dropwise, and soon after addition, the mixture turned dark brown, which indicated that the formation of *Elaeocarpus Sylvestris* functionalised CuO NPs as per literature [25]. The workup procedure included washing 2–3 times using distilled water and later two times with ethanol. The prepared CuO NPs precipitate was kept in the oven for drying overnight.

Reduction of 4-nitrophenol by ESCuo NPs

Here, normally used procedure has been employed as per literature [26]. Commonly, 25 mL of an aqueous solution of 4-nitrophenol was taken in an RB flask for 10 minutes while stirring to ensure good mixing. 10 mg of synthesised NPs made from copper oxide were then added to the reactant at 2.5 mM. Then, to decrease 4-nitrophenol at room temperature, 0.25 M NaBH_4 that had been dissolved in a 25 mL aqueous solution was mixed into the reaction solution. The mixture was a deep yellow colour, and it was allowed to mix thoroughly through stirring till it became colourless. The colourless reaction mixture was indicated the complete reduction of 4-nitrophenol. Frequently obtained samples of the reaction mixture, then a UV-visible spectrophotometer was utilised to check if the reaction had finished. Additionally, the outcomes were analysed with and without NaBH_4 .

Antioxidant Potentiality

The "DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging test" is a technique used to investigate the synergistic effect of antioxidant molecules on capped CuO NPs as well as their effectiveness as antioxidants. The ESCuO NPs have been employed for conducting the experiment. It was calculated

using the DPPH free radical scavenging potential method. Using the equation given here, the findings were estimated as an amount of scavenging of a control blank with the absorption value of the investigated materials calculated at 517 nm.

$$\text{Radical Scavenging activity (\%)} = \frac{A_{\text{control blank}} - A_{\text{sample}}}{A_{\text{control blank}}} \times 100$$

Elaeocarpus Sylvestris functionalised CuO Nanoparticles Instrumentation

The nanoparticle synthesis would be established by vivid instrumental techniques from various angles, like dimensions, which can be corroborated through microscopic, composition through elemental, functional groups through spectral, morphology through FESEM, and other optical techniques. Here, a variety of instruments and analytical methods, including UV-visible spectroscopy as well as FTIR, X-RD, SEM, EDAX, and TEM-SAED instruments, are used to examine the bio-orchestrated CuO NPs.

Results and Discussion

The hydroxyl and carboxyl groups found in *Elaeocarpus Sylvestris* leaves are the best green sources to act as reducing, stabilising, and capped operators for CuO NPs, according to the remarkable results achieved by several instrumental approaches. The biosynthesised ESCuO NPs are apt for further catalytic applications as their metabolites constitute amino, hydroxyl, and carboxyl functional groups.

UV-Visible Spectroscopy

Figure 1 depicts the comparative UV-Vis spectrum of both an aqueous solution of *Elaeocarpus sylvestris* leaf extract and the synthesised ESCuO NPs. In Fig. 1(a), the absorption of the specified signal at 232 nm was observed for the aqueous leaf extract of *Elaeocarpus Sylvestris*. Whereas the absorbance at 232 nm is related to the benzoyl ring system of $\pi - \pi^*$ or $n - \pi^*$ transitions, these are demonstrating that polyphenols are some of the constituent phytochemicals in the *Elaeocarpus Sylvestris* leaf extract [27]. Figure 1(b) gives the UV - Visible spectrum of ESCuO NPs. Now, the drawn spectrum revealed the absorption peaks at 225 and 262 nm. The peak at 225 nm is attributed to the plant constituents [28], and the peak at 262 indicates the formation of ESCuO nanoparticles [27 & 29].

FT-IR

The important constituents responsible for the capping, stabilising and reducing of synthesised ESCuO nanoparticles are corroborated through FTIR. In this context, the functional group particulars of the plant and of synthesized ESCuO nanoparticles were investigated and depicted in Fig. 2. The FTIR spectrum of the employed biosource are shown in Fig. 2(a). The obtained spectra expressed the key functional groups through its characteristic ranges corresponding to the values of 3323.90, 2107.53, 1634.43, and 531.44 cm^{-1} respectively. The spectrum shows value at 3323.9 cm^{-1} was confirmed the -OH bond stretching

vibration robustly. However, plant literature reveals that there is no compound with N-H functional group. Actually, the employed biosource contains ellagic acid, gallic acid, methyl gallate as major constituents with -OH & -COOH groups [30]. 1634.43 cm^{-1} is given to the bending vibration of group -OH explicitly. Another intense peak at 2106.33 cm^{-1} is ascertained to C = O in biomolecules. The single intensify peak below 900 is 531.55 cm^{-1} is due to the bending vibrations of C-C aromatic ring.

Moreover, the FTIR analysis of green synthesized ESCuO NPs in Fig. 2**(b)** exhibited a shift in the transmittance intensity values compared to the spectra of biosource employed. Besides, it confirms the surface functionalisation of bio biosource of *Elaeocarpus Sylvestris* on the green-synthesised CuO NPs. The FTIR image depicts the peak values at 3795.67 , 2801.95 , 2661.75 , 990 , and 583 cm^{-1} respectively. One of the prominent peaks, called OH stretching, shifted purposefully from 3323.9 cm^{-1} to 3795.67 cm^{-1} , indicating that the phenolic -OH of the biosource is tightly bound to the CuO NPs. The stretching frequencies of the C-C and aromatic groups were assigned values of 2801.95 and 2661.75 cm^{-1} , respectively, while the C-O bond of the plant's components was given a value of 990 cm^{-1} . It's interesting to note that the Cu-O bond's stretching vibrations are represented by the other band at 583 cm^{-1} . Therefore, the above results confirmed the typical role of biocomponents of plant such as substituted carboxylic acids, reducing sugars, polyphenols and proteins are acted as best in functionalization and stabilization of ESCuO NPs.

XRD

The crystal structure of ESCuO NPs was confirmed by XRD. Here, diffraction patterns of ESCuO NPs were obtained and presented in Fig. 3. Green CuO NPs have been shown to have prominent diffraction peaks that demonstrated their crystalline nature at various peak positions. The observed peak positions at $2\theta = 32.59^\circ, 35.61^\circ, 38.78^\circ, 48.82^\circ, 53.54^\circ, 58.37^\circ, 61.60^\circ, 66.31^\circ, 68.15^\circ, 72.46^\circ$, and 75.30° were ascertained to corresponding hkl planes (110), (111), (200), (202), (020), (202), (113), (311), (220), and (400) [31]. These are highly compatible with the cupric oxide nanoparticle JCPDS Standard No. 01-080-0076. Finally, it validates the monoclinic phase, face-centered cubic structure of CuO NPs. As per previous reports, the presence of diffraction peaks between $35\text{--}39^\circ$ designated the formation of CuO NPs. 53 nm is the average size of CuO NPs as measured by Debye-Scherrer's formula [32].

$$D = k\lambda/\beta \cos \theta$$

SEM

The interpretation of surface texture of orchestrated ESCuO NPs was carried out through the SEM microscopic technique. Figure 4**(a-d)** displays SEM images of ESCuO NPs. Moreover, the SEM pictures represented in Fig. 4 reveals the defined spherical morphology of the orchestrated CuO NPs. It is demonstrated that the ESCuO NPs yields relatively tiny, spherical particles that are almost uniform in size. Small nuclear particles actively self-aggregate and oriented in a way to form larger spheres explicitly. In addition to this, the SEM micrographs were like shattered pieces (agglomerated particles) on a rough

surface. Figure 4(e) displays the chemical composition of the produced NPs being examined by the EDX method. Only two powerful signals, Cu and O, contribute significantly to the spectrum, with weight percentages of 69.43 and 27.53, respectively. Additionally, the presence of significant concentrations of Cu and O with sharp peaks points to the stability and quality of the synthesised ESCuO NPs. Sodium was another signal that was seen and had a very low intensity (3.04%). The findings showed that the ESCuO NPs were functionalized by the *Elaeocarpus Sylvestris* leaf extract biomolecules in a significant way.

TEM

Figure 5 (a-e) depicts the micrographs of the synthesised ESCuO NPs. The spherical morphological distribution of orchestrated ESCuO NPs with sizes in various ranges between 9–25 nm is explicitly depicted in Fig. 5. The TEM micrographs illustrate that the particles are scattered, and some appear to be well coated with the leaf extract of *Elaeocarpus Sylvestris*. The different magnifications of TEM images declare that very small particles below 25 nm are observed. The mean particle size was characterised by "image J software (National Institute of Health, Bethesda, MD, USA)," and it was 53.99 nm. Correspondingly, the obtained value is in accordance with the crystallite size attained by the Debye-Sherrer equation using XRD. The bright diffraction rings are observed in the "Selected Area Electron Diffraction (SAED)" phenomenon of orchestrated ESCuO NPs in Fig. 5(f). This showed that the CuO NPs are polycrystalline in nature. It is further confirmed that the particles are finely crystallized. The SAED image's diffused ring dot pattern fits the XRD pattern, demonstrating the monoclinic structure of the synthesised ESCuO nanoparticles.

Reduction of 4-nitrophenol using ESCuO NPs

4-nitrophenol can be reduced by different metal, metal oxide, and bimetallic nanoparticles like Au, Ag, Pt, Pd, and Au-Ag. Eventually, all the aforementioned metals and metal oxides are cost-effective, and some are harmful, but the literature says green-synthesised CuO nanoparticles are also used as reducing agents in organic reactions [33]. Nevertheless, CuO NPs are eco-friendly, bioactive, and commercially viable. Here, ESCuO NPs act as blistering agents and an efficient reducing agents for removal of hazardous 4-nitrophenol. Figure 6(a) denotes the a schematic representation of conversion of para nitrophenol to para aminophenol by ESCuO NPs and NaBH₄.

Table 1
The kinetic parameters determined during the conversion of para nitrophenol to para aminophenol by ESCuO NPs

S.No	CuO NPs in (mg)	rate constant (min ⁻¹)	R ²	Time required for reduction of 4-nitrophenol (min)
1.	5	0.1477	0.9834	24
2.	10	0.1596	0.9718	14

The rate constant (k) of reduction of 4-nitrophenol using different doses of ESCuO NPs was determined (Fig. 6 (b)) (Table 1). The reason for the higher rate constants when there is a greater concentration of ESCuO nanoparticles is because there is a corresponding increase in the quantity of reactive sites available for the reduction of 4-nitrophenol. Fig. (c) exhibits 4-nitrophenol reduction by ESCuO NPs and NaBH₄. Fig. (d) depicts the kinetics of reduction followed.

Antioxidant activity

The antioxidant activity of ESCuO NPs was investigated by the DPPH assay method. ESCuO NPs were used as radical scavenger and DPPH was used as the radical source. The colour of the DPPH solution changed from deep violet to pale yellow in the presence of CuO NPs. The absorbance of DPPH is at 517 nm and gradually decreases with increases in concentrations of ESCuO NPs, which further confirms the free radical scavenging activity of ESCuO NPs.

Table 2
The antioxidant activity of ESCuO NPs

Sl.No	CuO NPs in (µg/mL)	DPPH (%) (mean ± S.D)
1.	100	59.45 ± 0.03
2	200	59.56 ± 0.01
3.	300	60.33 ± 0.02
4.	400	60.44 ± 0.02
5.	500	61.21 ± 0.03

These results show that the antioxidant efficiency of ESCuO NPs is dose-dependent (Table 2). All values represented in the tables are the average ± SD of the results of two separately conducted experiments. Figure 7 shows the free radical scavenging activity of ESCuO NPs, which exhibits up to 61.21 ± 0.02% in 500 µg/mL concentration of CuO NPs.

Conclusion

Finally, a novel, simple and green method is established in a promising and sustainable way for the production of CuO nanoparticles. To escape from regular chemical methods and their toxicity, an environmentally friendly substitute is the need of hour. This green protocol with *Elaeocarpus Sylvestris* leaf extract became successful in this way. The fabricated particles are corroborated with respective instrumental analysis. The findings revealed that the formed particles have a spherical shape with a 53 nm size and a face-centered cubic structure. In future perspective the study gives the new path way in a variety of applications, including biomedicine and catalysis. The ESCuO NPs shown efficient catalytic activity in the conversion of para nitrophenol to para aminophenol. Further, it proves ESCuO NPs have

good antioxidant potentiality. Therefore, ESCuo NPs act as a good catalyst for elimination of organic pollutants.

Declarations

Ethical Approval

This declaration is “not applicable”.

Competing interests

This declaration is “not applicable”.

Authors' contributions

Tejaswini wrote manuscript, Laxmi Mangamma prepared tables, Praveena prepared figures, Manga Raju and Meher Baba collected the data, Bhagya Lakshmi K reviewed the manuscript.

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Figures

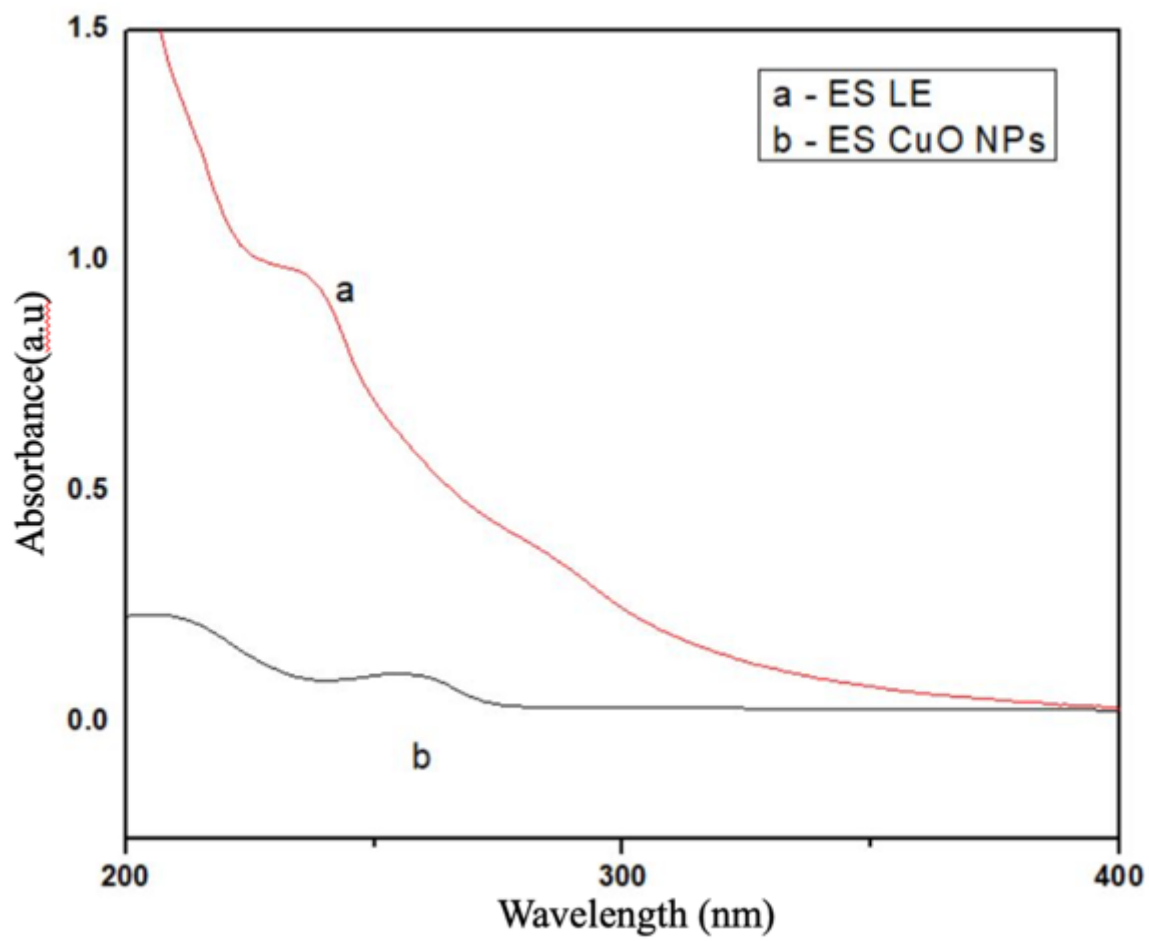


Figure 1

UV-Visible Spectrum of (a) ES Leaf extract and (b) ESCuO NPs

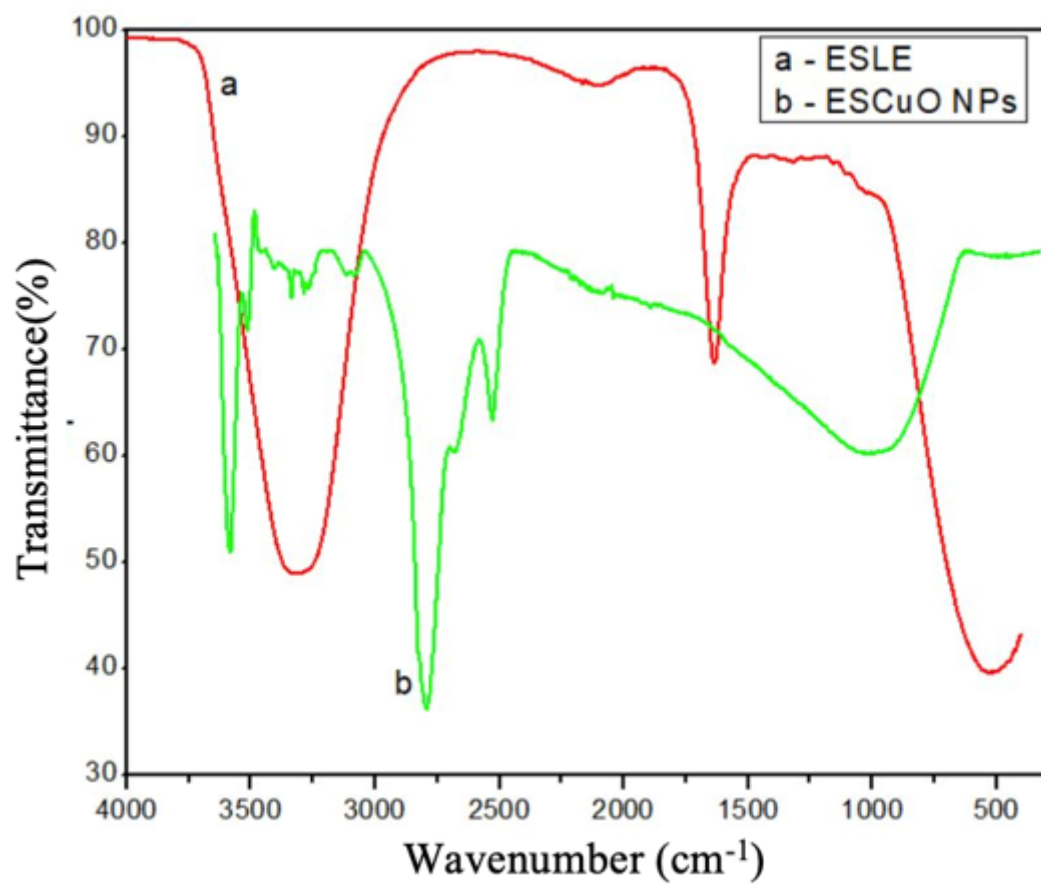


Figure 2

FT – IR spectra of ESCuO NPs

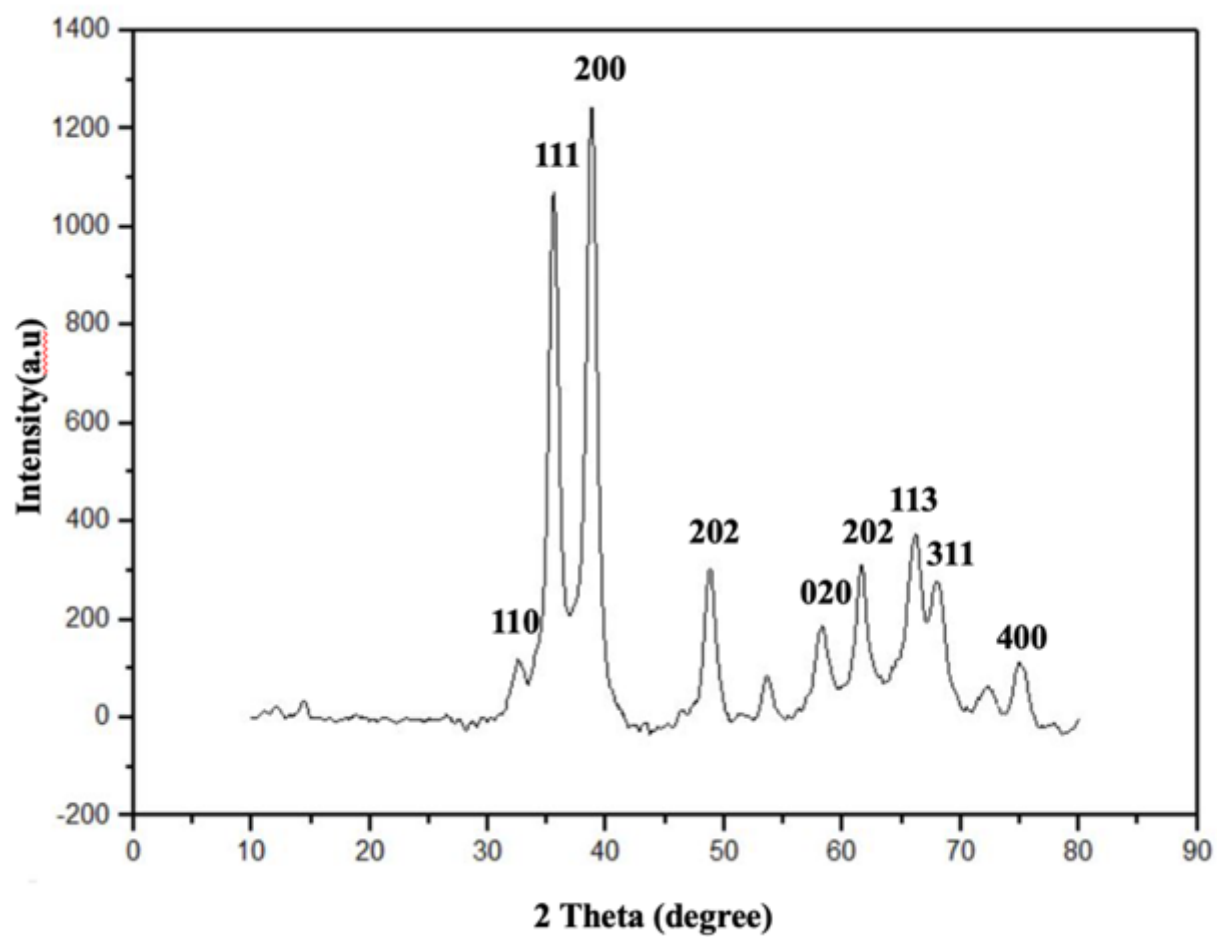


Figure 3

XRD pattern of ESCuO NPs

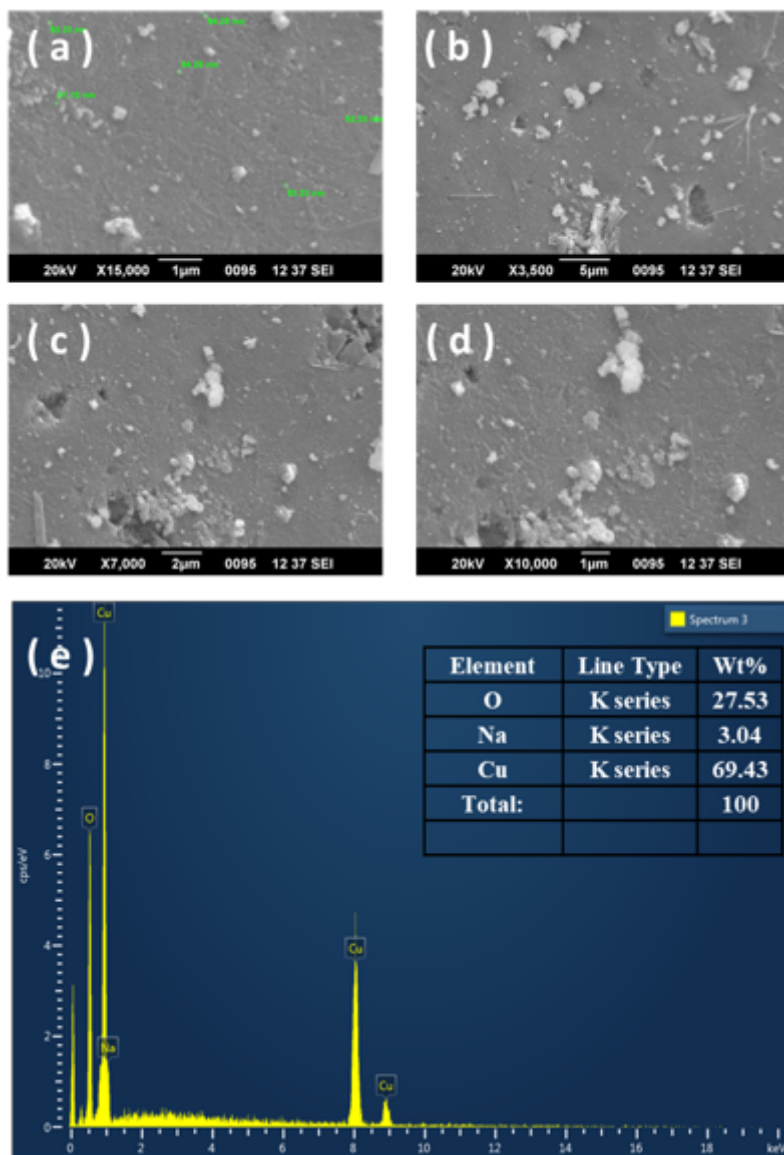


Figure 4

(a-d): SEM pictures of ESCuO NPs ; (e): EDX Spectra of ESCuO NPs

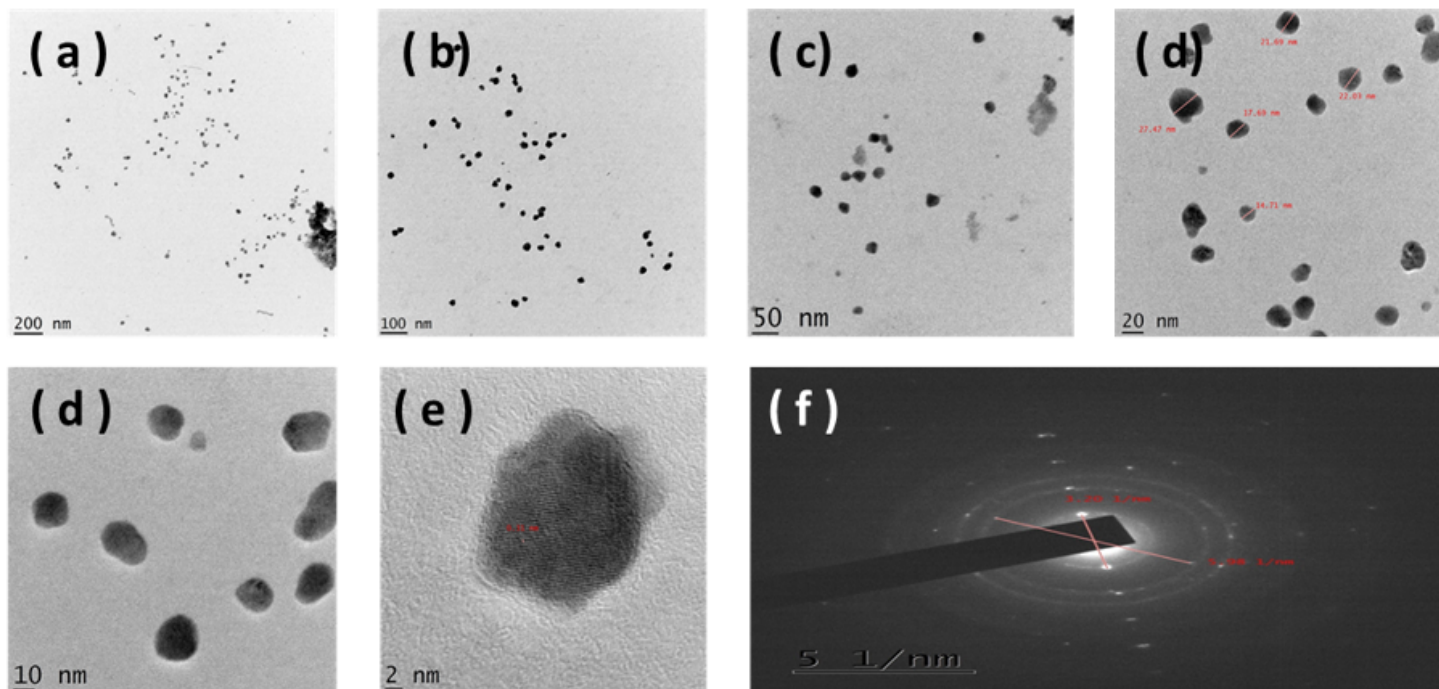


Figure 5

TEM images of ESCuO NPs; (f): SAED of ESCuO NPs;

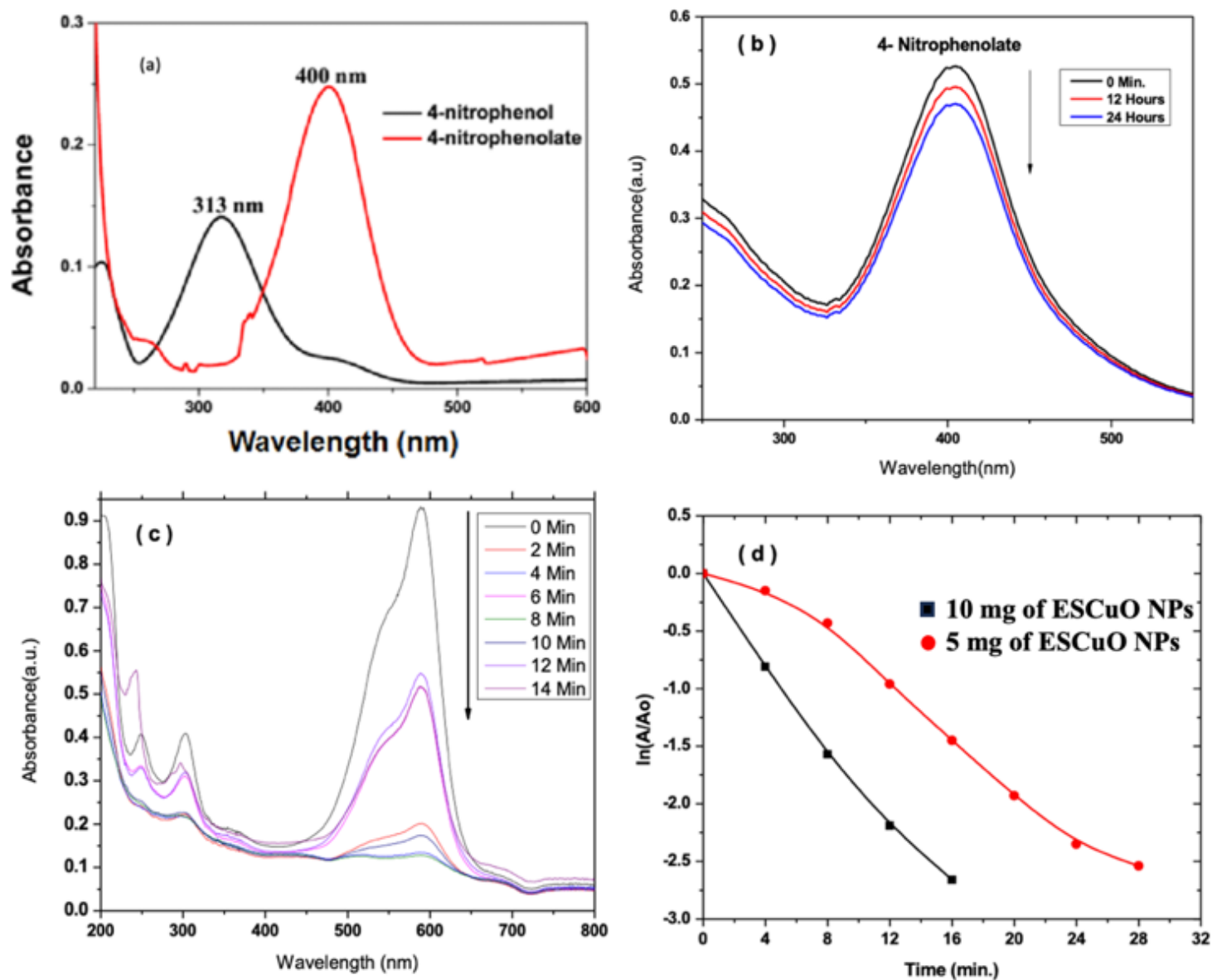


Figure 6

(a) A Schematic representation of conversion of para nitrophenol to para aminophenol by ESCuO NPs and NaBH_4 , (b) 4-nitrophenol reduction with ES leaf extract and NaBH_4 without ESCuO NPs (c) 4-nitrophenol reduction by ESCuO NPs and NaBH_4 (d) kinetics of reduction

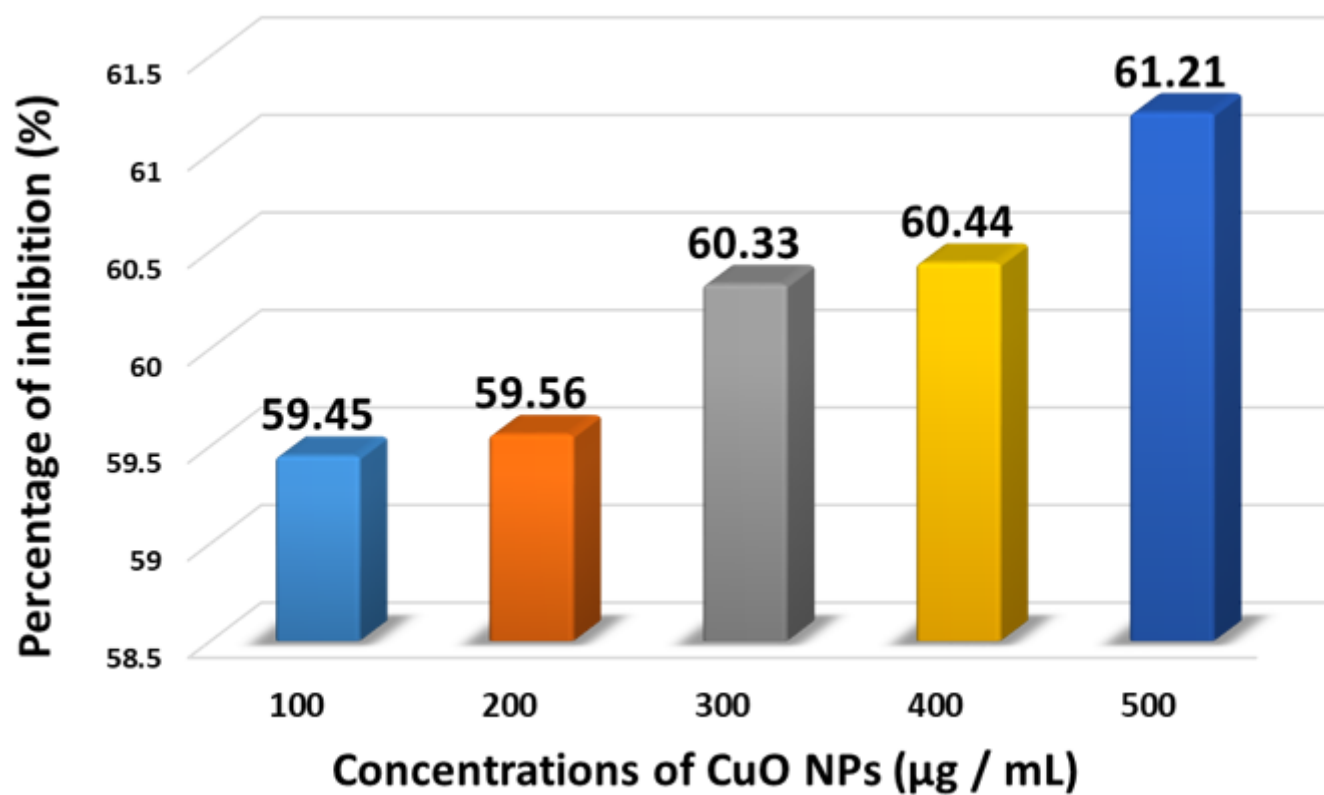


Figure 7

Free radical scavenging activity of ESCuO NPs