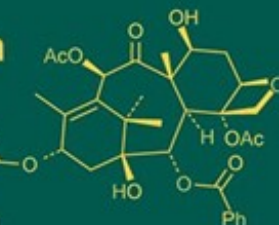
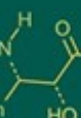
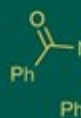
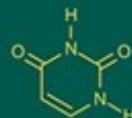
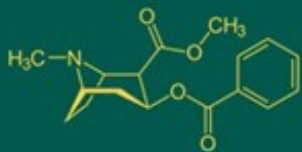


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## Green fabrication of copper oxide nanoparticles using aqueous extract of *Nigella Sativa* L. seeds: A physicochemical characterization

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**ABSTRACT**

The synthesis of nanoparticles through physical and chemical methods requires advanced equipment and skilled personnel, and poses a risk to health due to associated toxic effects. To combat this, a simple, cost-effective, less toxic and environment friendly method, Green nanotechnology, has grabbed the attention of researchers. Copper nanoparticles have broad applications across diverse fields due to their low cost, easy availability and therapeutic properties. Different plant parts, e.g. root, seeds, leaf, etc., are a good source of phytochemicals which help in detoxification of heavy metals, bioreduction of metallic ions, and environmental problems. In this study, copper nanoparticles were synthesised and characterised through green route using the aqueous extract of *Nigella sativa* seeds. The extracts acted as an efficient stabilising and reducing agent. A visible colour change indicated the presence of copper oxide nanoparticles, which appeared as a brownish solution when copper sulphate solution was added. The formation of the fabricated nanoparticles were analyzed using various physicochemical characterization techniques. The UV-Visible absorption peak at 375.3 nm confirmed the characteristic surface plasmon resonance. The X-ray diffraction pattern confirmed the crystalline nature of the synthesised Copper Nanoparticles. Fourier Transform Infrared spectroscopy analysis showed peaks below 700 cm<sup>-1</sup>. The synthesized nanoparticles have an almost spherical shape with an average size of 51 nm, as revealed by the Field Emission Scanning Electron Microscopy image. Thus, the results demonstrate that this eco-friendly synthesis route yields nanoparticles that may be a crucial and promising approach for future applications in different sectors due to its less toxicity in therapeutic applications.

**Keywords:** Green nanotechnology, Copper nanoparticles, *Nigella sativa*, Aqueous extract, Absorption peak, Physicochemical, Etc.

**Introduction**

In recent decades, nanotechnology has been considered as a rapidly growing field all over the globe. This technology focuses on the synthesis of nanomaterials, formulating material to achieve properties that can be employed in nanomedicines against several kinds of diseases, including cancer. Nanoparticles for cancer applications can be synthesized to have different kinds of consists of both primary and secondary metabolites including flavonoids, which hold promise for the redox reaction in green synthesis of AgNPs

Nanotechnology is a multidisciplinary field that integrates principles from physics, chemistry, biology, materials science, and medicine and has been a rapidly growing field globally. Nanoparticles are the building blocks of nanotechnology, and there are several physical and chemical methods of synthesising metal nanoparticles. Chemical methods are ecologically hazardous, hindering its applications in the field of medicine and biology. Eco-friendly methods are economically effective, feasible commercially, and thus provide a sustainable route for the synthesis of metal NPs, especially noble metals such as silver, copper, gold, platinum, etc. and their alloys, which are developed for nanomaterial synthesis (Ranjan *et al.*, 2013; Nagar *et al.*, 2018) [1, 2]. Amongst them, due to its easy availability, low cost and similar properties to other metallic nanoparticles, copper nanoparticles are of keen interest (Nagar *et al.*, 2018; Yan *et al.*, 2020) [2, 3]. Recently, copper nanoparticles have been used for the treatment of a wide range of disorders, including parasitic, viral, bacterial, and fungal diseases (Yan *et al.*, 2020) [3]. Nanotechnology focuses on nanomaterial synthesis that can be further used for preparing materials that can be used in nanomedicines against several types of diseases, including cancer. Several bioactive compounds from medicinal plants have been utilised in nanoscience, including synthesising nanoparticles using plants, plant parts or their derivatives by the green synthesis method (Rohini *et al.*, 2019) [4].

Since ancient times, medicinal plants have been considered a remedy for treating human diseases. The use of medicinal plants has seen a notable increase in recent years compared to chemical drugs, driven by several factors, including their affordability, easy accessibility without a prescription, the perception that natural products have fewer side effects, and the lack of necessity to seek medical advice (Alkhalaf *et al.*, 2020) [5]. Plant extracts include a variety of bioactive metabolites, including flavonoids, phenols, proteins, terpenoids, and tannins, which function as reducing and stabilizing agents, transforming metallic ions into nanoparticles. The plant extract produces electrons, which cause copper salts to get reduced. Copper oxide nanoparticles are formed when phytochemicals react with copper ions, resulting in reduction (Chakraborty *et al.*, 2022) [6]. Copper nanoparticles are used in cosmetology, textiles, biotechnology, etc. Due to their unique system, high surface area, excellent thermal and mechanical stability, and ideal optical, magnetic, and catalytic properties, copper nanoparticles are widely used. Plant extracts act as reducing and capping agents for the synthesis of nanoparticles and can be used for large-scale synthesis (Prakash *et al.*, 2023) [7].

*Nigella sativa* (NS), also known as black cumin seeds or kalonji, is an annual flowering plant from the family Ranunculaceae. It grows to 20–30 cm (7.9–11.8 inches) tall and has linear lanceolate leaves. The delicate flowers have 5–10 petals, and the colors are usually yellow, white, pink, pale blue or pale purple. The fruit of the plant is large and inflated capsule composed of 3–7 united follicles, each of which has numerous seeds. The black-colored seeds are flattened, oblong and angular, funnel-shaped, with a length of 0.2 cm and 0.1 cm wide (Forouzanfar *et al.*, 2014) [8]. It is a therapeutic plant that grows in different places all over the world, especially Saudi Arabia, Syria, Pakistan, northern Africa and Turkey. It is considered an enriched source of nutritionally vital constituents, and its oil contains polyunsaturated fatty acids (PUFA), as well as several other phytochemicals that exhibit strong antioxidant properties and cause a lowering effect on glucose (Rohini *et al.*, 2019; Alkhalaf *et al.*, 2020; Forouzanfar *et*

*al.*, 2014; Alaghemand *et al.*, 2018) [4, 5, 8, 9]. The seeds of *Nigella sativa* have been explored in several traditional medicines; they possess diuretic, antihypertensive, antidiabetic, antimicrobial, anti-inflammatory, and anticancer properties. Nanoparticles synthesized from *Nigella sativa* have shown better efficacy against different types of cancers (Rohini *et al.*, 2019) [4]. Black cumin or kalonji is widely used whole or in crushed form for various usages. Essential oils and oleoresins derived by appropriate techniques to obtain value-added products and seeds are used in folk medicine for the treatment of a number of diseases, including diarrhoea, jaundice, amenorrhea, helminthiasis, ophthalmic, paralysis and asthma (Alaghemand *et al.*, 2018) [9]. In the present study, a completely green, cost effective, less toxic and sustainable approach for the synthesis of copper oxide nanoparticles using aqueous seed extract of *Nigella sativa* was done and characterized using UV, FTIR, XRD and FE-SEM analysis.

## 2. Materials and methods preparation of plant extracts

### 2.2.1. Plant sample collection

The seeds of *Nigella sativa* were purchased from the local market of Prayagraj city, India. The seeds were cleaned manually and then ground in a mortar pestle. The grounded seeds were dissolved in distilled water in a 1:10 ratio. The samples were filtered and stored in an Erlenmeyer flask and kept at room temperature away from light for further analysis (Kumar *et al.*, 2023) [10].

### 2.2.2. Preparation of aqueous extract of *Nigella sativa* seeds

The leaves were cleaned with tap water, then rinsed with distilled water and left to dry at room temperature. After drying, mortar pestle was used to grind the leaves into powder form and saved in sealed plastic bags until use. One gram of prepared powder was suspended in 10 ml of ionic distilled water and boiled for 15 min, then filtered through filter papers Whatman (no. 1), and stored in glass tube at 4°C. (Figure 1)



**Figure 1.** Filtered extract of *Nigella sativa* seeds (aqueous)

### 3. Green synthesis of various nanoparticles with *Nigella sativa* seed extracts synthesis of copper oxide nanoparticles ( $\text{Cu}^{\text{O}}$ -NPs) using *Nigella sativa* seed extracts

For the synthesis of Cu nanoparticles ( $\text{Cu}$ -NPs), 20 mL aqueous extract of *Nigella sativa* seeds was added to 20 mL of 0.01M aqueous  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  solution in a screw-capped 250 ml Erlenmeyer conical flask. It was then kept in the dark for 24 hours at room temperature (Figure 1). At the end of the reaction time, the resulting dark brown or greenish brown colored solution showed the synthesis of copper nanoparticles (Nagar *et al.*, 2018; Yan *et al.*, 2020; Kumar *et al.*, 2023; Kulkarni *et al.*, 2013) [2, 3, 10, 11].

### 2. Characterization of green synthesized copper oxide nanoparticles using *Nigella sativa* L. seed extract (aqueous)

Characterization of synthesised nanoparticles for investigating the size and morphology in this research was done by recording the UV-Vis spectra using a Shimadzu UV spectrophotometer. Also, the image of morphology and the particle shape and size possessed by synthesised nanoparticles was assessed using MIRA3TESCAN-XMU FESEM to record Field Emission Scanning Electron Microscopy (FESEM) images. Further, a JASCO (FT/IR-6200) spectrophotometer was utilized for analysing the FT-IR spectra in this research. The characteristic diffraction peaks of synthesised nanoparticles were observed for all the samples. The XRD technique was used for determining phase distribution, crystallinity, and purity of the synthesized

nanoparticles. Strong diffraction peaks at  $2\theta$  values were recorded in the XRD spectra (Yan *et al.*, 2020; Ağçeli *et al.*, 2020; Thiruvengadam *et al.*, 2019) [3, 13, 14].

### 4. Results and Discussion

A visible change in color of the nanoparticle solution synthesised using aqueous extract of *Nigella sativa* seeds was observed, endorsing the synthesis of copper nanoparticles. Figure 2(b) depicts the synthesis of NS- $\text{Cu}^{\text{O}}$ -NPs by reducing the copper sulphate pentahydrate using the aqueous extract of *Nigella sativa* seeds. The change in color from light blue to dark brown or greenish brown (Figure 2 (a, b) was due to the excitation of the surface plasmon resonance (SPR) phenomenon which is the absorbance of visible electromagnetic radiation due to collective oscillation of surface electrons, which is also a result of interaction between the free electrons presents on the surface of metal NPs and light of specific wavelengths (Yan *et al.*, 2020; Prakash *et al.*, 2023; Thiruvengadam *et al.*, 2019; Wu *et al.*, 2020; Amaliyah *et al.*, 2020; Chandraker *et al.*, 2020; Mali *et al.*, 2020; Kausar *et al.*, 2022) [3, 7, 14, 15, 16, 17, 18, 19]. A previous report (Kausar *et al.*, 2022) [19] has suggested that the  $\text{Cu}$ -NPs synthesised using aqueous extract of *M. longifolia* resulted in the development of a color change from pale yellow to dark brown over the course of 24 h, marking it as the first indication of the formation of  $\text{Cu}$ NPs in the solution, which is in concordance with the present study. Similarly, a study (Valli *et al.*, 2015) [20] suggested that brown color can be established in the reaction mixture of plant extract and  $\text{CuSO}_4$ .

a) copper sulphate solution b) synthesised copper oxide nanoparticles

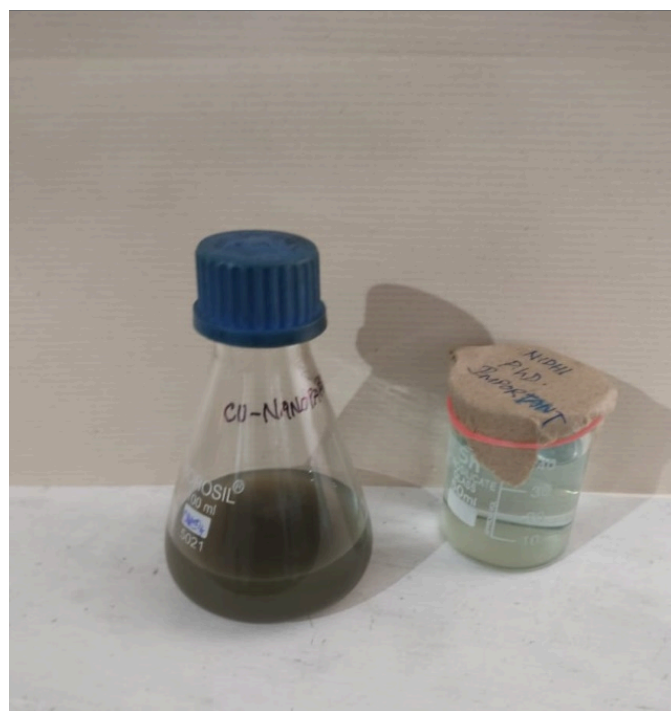
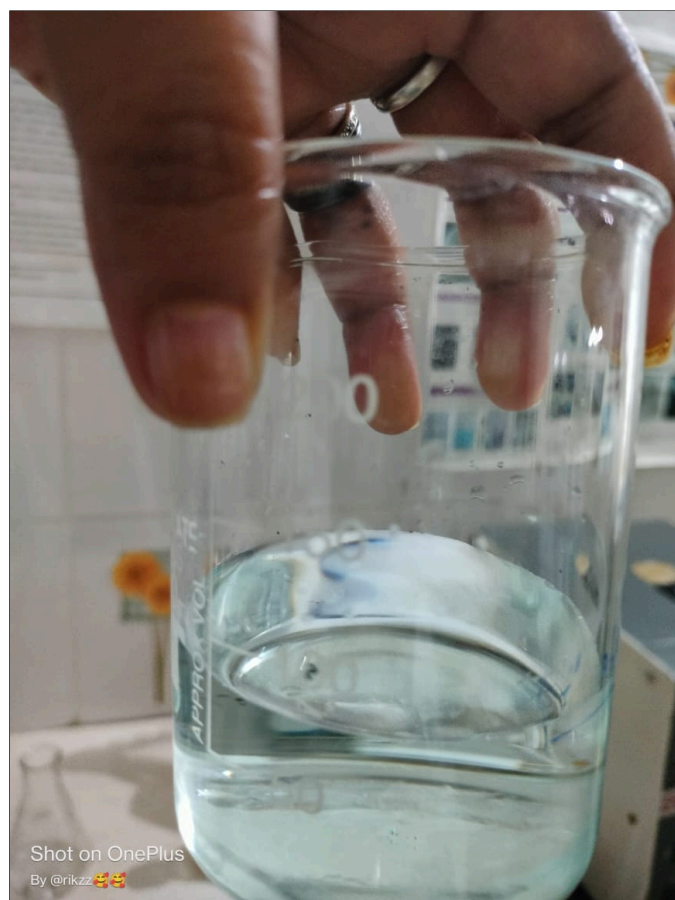


Figure 2. a) copper sulphate solution b) prepared copper nanoparticles

### UV/Vis spectroscopic analysis

In the present study, the formation of green-synthesised copper oxide nanoparticles ( $\text{Cu}^{\text{O}}$ -NPs) was initially confirmed using UV-Vis spectrophotometric analysis. The spectrum exhibited a prominent absorp-

tion peak at 375.3 nm (in a spectrum width of 200-600 nm). It is a characteristic surface plasmon resonance (SPR) band for  $\text{Cu}^{\text{O}}$ -NPs, arising from the excitation of valence electrons to the conduction band. The absence of any absorption peak in the 550-650 nm range clearly indicated that pure metallic copper was not formed; and the

phytochemicals present in the extract facilitated the capping, formation and stabilization of the oxidised form  $\text{Cu}^{\text{O}}$  and prevented further agglomeration. (Figure 3) confirmed the synthesis of copper oxide nanoparticles ( $\text{Cu}^{\text{O}}$ -NPs). In accordance with the present study, a peak at 326 nm absorption maximum of photosynthesized CuNPs using the leaf extracts of *A. houstonianum* (Chandraker *et al.*, 2020) [17]; at about 350 nm for Cu-NPs, using the leaf extract of *T. cordifolia* (Prakash *et al.*, 2023) [7] was previously reported. Green synthesis of copper nanoparticles from *Nigella sativa* seed extract by observing the UV-visible spectrum absorption peak at 500–280 nm was previously reported (Kumar *et al.*, 2023) [10], and a maximum absorption between 550–600 nm at about 560 nm was reported (Jayadev *et al.*, 2021) [22], that doesn't fall with the present work. A distinct study (Yan *et al.*, 2020) [3] showed a peak at 564 nm, confirming the formation of the copper nanoparticles. The absorption band in the present work is in discordance, as it indicates the formation of CuNPs in the colloidal solution because of the known fact that CuNPs show characteristic absorption peaks in the range of 200–800 nm (Akpanudo *et al.*, 2024) [21].

Earlier results showed peak maxima at 207 nm in the UV spectrum of *Piper retrofractum* extract (Amaliyah *et al.*, 2020) [16]; an absorption band in the  $\text{Cu}^{\text{O}}$  NPs biosynthesized from the leaves of *Calotropis gi-*

*gantean* (Sharma *et al.*, 2015) [23] displaying the two absorption bands at 291 and 355 nm, at 290 nm using the *T. terrestris* seed extract (Meena *et al.*, 2023) [24], at 269 nm using *C. paniculatus* leaf extract (Mali *et al.*, 2020) [18], at 340 nm using *Parthenium hysterophorus* aqueous extract (Nzilu *et al.*, 2023) [25], at 256 nm from *Morinda citrifolia* leaf extract (Priya *et al.*, 2023) [26], peak at a wavelength of 414 nm using *C. spinosa* extract (Khatami *et al.*, 2020) [27] indicating the presence of Cu-NPs. A comparable report showed a noticeable peak at 325 nm due to the interband transition of the core electrons of the  $\text{Cu}^{\text{O}}$  NPs synthesised using the extract of mint leaves (Mahmoud *et al.*, 2021) [28]. Previous results showing UV-Vis spectra of  $\text{Cu}^{\text{O}}$  NPs with characteristic peak at 385 nm, exhibiting an SPR using propolis (Khalkhal) extract (Hajizadeh *et al.*, 2022) [29], absorption peaks at 337 and 266 nm using JC-CuNPs (Ghosh *et al.*, 2020) [30], an absorptivity at 378 nm using extract of *M. pinnata* flower (Thiruvengadam *et al.*, 2019) [14]; a similar absorption peak at 340 nm was obtained using *C. vitiginea* leaf extract (Wu *et al.*, 2020) [15] as a reducing agent that supports the result of the present work. Hence, the optical behaviour observed in the present investigation was found to be distinct from the literature reporting metallic copper nanoparticle formation.

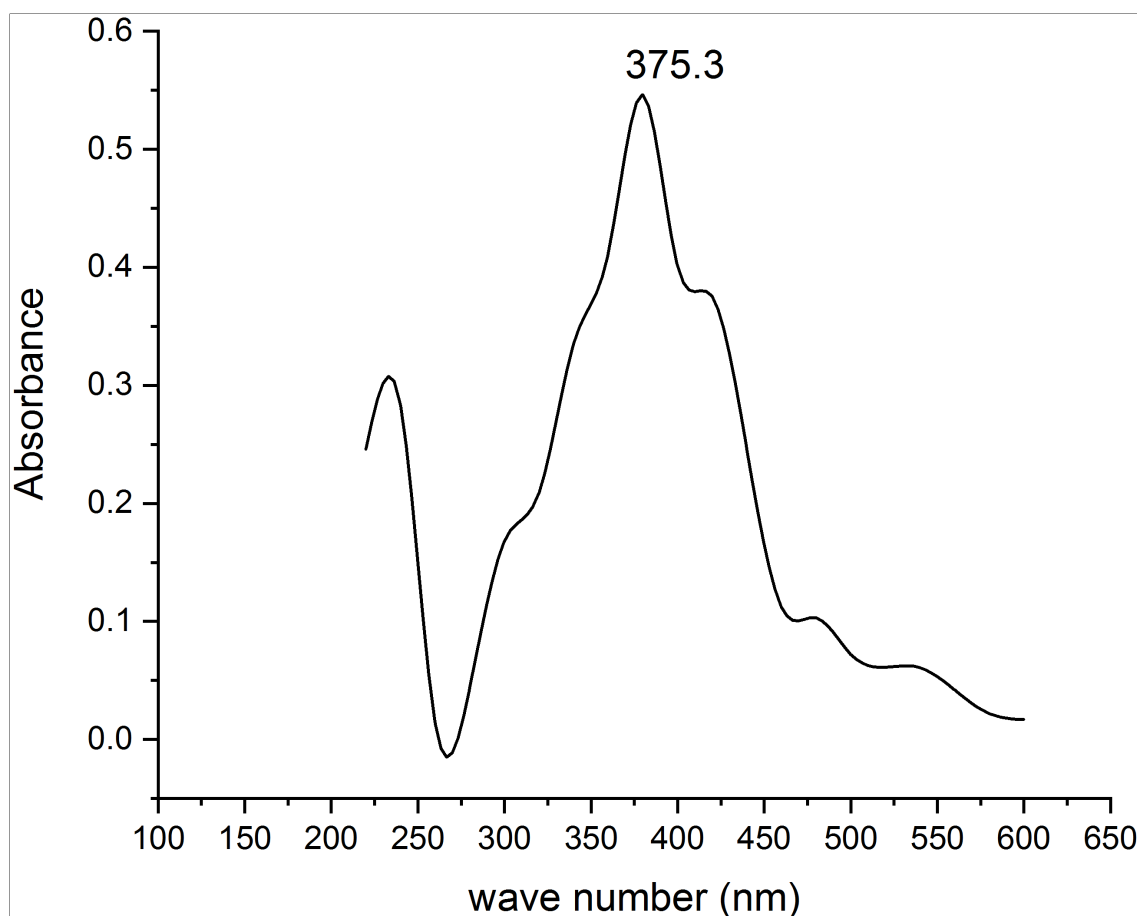


**Figure 3.** UV-Visible spectroscopy of copper nanoparticles synthesised from *Nigella sativa* seeds extract (aq.)

## 2. X-ray diffraction (XRD) and crystallinity analysis

The X-ray diffraction (XRD) data were used to analyze and identify the crystalline structure, phase orientation, purity and particle size of the Copper oxide nanoparticles ( $\text{Cu}^{\text{O}}$ -NPs). In the present study, characterization was performed using powder of NS- $\text{Cu}^{\text{O}}$ -NPs. The diffraction pattern displayed distinct and sharp peaks, reflecting the highly crystalline nature of the sample. The diffracted intensities were recorded from 30 to 80 at 2 theta angles. The synthesis method employed a green approach using *Nigella sativa* seed extract (aqueous), which stabilises and partially oxidises the copper nanoparticles due to the bioactive compounds present in the extract. The *Nigella sativa*

aqueous extract served as a reducing agent that converts  $\text{Cu}^{2+}$  ions into  $\text{Cu}^{\text{O}}$  or  $\text{Cu}_2\text{O}$  as well as a capping agent that stabilized nanoparticles and prevented aggregation via polyphenols, flavonoids, or other antioxidants.

The XRD peaks were observed for Green-synthesised  $\text{Cu}^{\text{O}}$ -NPs at  $2\theta = 32.27^\circ, 34.82^\circ, 36.56^\circ, 39.69^\circ, 48.17^\circ, 57.04^\circ, 63.23^\circ$ , and  $68.21^\circ$ . When matching these data points with standard reference files, the analysis showed a dual-phase characteristic. On the basis of the peaks observed at  $2\theta$  values, the data reported a mixture of  $\text{Cu}_2\text{O}$  and  $\text{Cu}^{\text{O}}$  with possible traces of  $\text{Cu}^{\text{O}}$  present in the sample. The intensity range (0–100 a.u.) indicated a high degree of crystallinity (Figure 4). A prominent characteristic peak was observed at a  $2\theta$  position of  $36.56^\circ$

that closely matches the (111) plane of  $\text{Cu}_2\text{O}$ , a cubic copper(I) oxide, and has the highest intensity. Peaks near  $32.27^\circ$  and  $34.82^\circ$  correspond to  $\text{Cu}^0$ 's (110) and (Nagar *et al.*, 2018) [2] planes. The underlying structural framework correlates with the face-centred cubic (FCC) crystal lattice of metallic copper, corresponding to the Joint Committee on Powder Diffraction Standards (JCPDS) card no. 04-0836. Simultaneously, the peak at  $36.56^\circ$  confirms the presence of a highly crystalline  $\text{Cu}^0$  phase. The diffraction peaks in the present report are in line with crystallographic planes of face-centred cubic (FCC) Cu crystals reported in previous reports (Ghosh *et al.*, 2020; Suresh *et al.*, 2013) [30, 31]. All peaks observed in the study match FCC symmetry and crystalline nature, which is in alignment with earlier literature (Thiruvengadam *et al.*, 2019; Kausar *et al.*, 2022; Hajizadeh *et al.*, 2022) [14, 19, 29] showing crystalline Cu-NPs with FCC symmetry using JCPDS No: 04-0836. The results of the present study, with peak values at 2 $\theta$  and the crystalline nature of NS-CuONPs, agree with previous work. The peak positions and crystallographic planes reported in the present report are in agreement with literature standards (Prakash *et al.*, 2023; Chandraker *et al.*, 2020; Akpanudo *et al.*, 2024; Jayadev *et al.*, 2021; Sharma *et al.*, 2015; Meena *et al.*, 2023; Priya *et al.*, 2023; Hajizadeh *et al.*, 2022; Ghosh *et al.*, 2020; Suresh *et al.*, 2013) [7, 17, 21, 22, 23, 24, 26, 29, 30, 31]. This suggested that the green synthesis process successfully generated a hybrid material containing both metallic FCC copper and localized oxide forms.

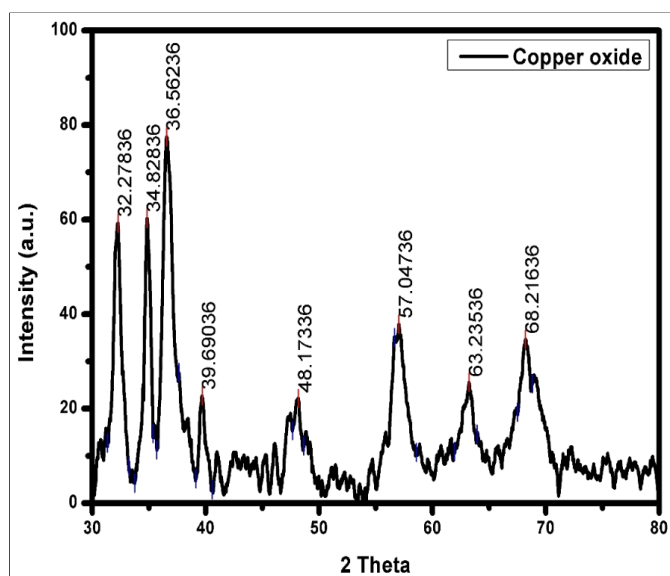


**Figure 4.** XRD Spectrum of Copper Oxide Nanoparticles

The absence of amorphous humps suggests well-crystallized phases, which is a hallmark of green synthesis methods. The particle size was estimated using the Scherrer Equation and assuming FWHM values for the peaks observed in the study. By applying Scherrer's mathematical equation to the dominant reflection peak at  $36.5^\circ$ , the average crystalline size of these nanoparticles was calculated to be approximately 22 nm. The crystallite size, estimated using the Scherrer Equation, indicated the nanoparticles were in the 20–50 nm range, which is in agreement with the report (Suresh *et al.*, 2013) [31]. To estimate the crystallite size, the Scherrer Equation was used:  $D = \frac{K\lambda}{\beta \cos \theta}$ ; where  $K$  = Shape factor (commonly 0.9);  $\lambda$  =  $1.5406 \text{ \AA}$ ;  $\beta$  = Full width at half maximum (FWHM) of each peak in radians;  $\theta$  = Diffraction angle ( $2\theta/2$ ) (Thiruvengadam *et al.*, 2019; Akpanudo *et al.*, 2024; Meena *et al.*, 2023; Ghosh *et al.*, 2020; Suresh *et al.*, 2013) [14, 21, 24, 30, 31]. Therefore, the present work observed that XRD data indicated a mixture of  $\text{Cu}_2\text{O}$  and  $\text{Cu}^0$  phases, stabilized by bioactive compounds from *Nigella sativa* (aqueous) seed extract. The particles are nano-sized, highly crystalline, and potentially functionalized for enhanced biological activity.

### 3. Fourier transform infrared (FTIR) spectroscopic analysis

Fourier transform infrared spectroscopy (FTIR) was used to categorise the potential biological ability or functional groups of plant extracts involved in reduction, capping, and stabilising Cu-NPs, as shown in Figure 5. The infrared spectrum displays a variety of absorption bands across different vibrational frequencies, representing the presence of organic biomolecules attached to the nanomaterial's surface. The results of the present study demonstrated that the vibrational bands observed in the sample ranged between  $600\text{--}3600 \text{ cm}^{-1}$ , which closely resembled the O-H stretch at  $3356.14 \text{ cm}^{-1}$ , showing a similarity with a previous finding (Kumar *et al.*, 2023) [10], in which the hydroxyl group (O=H) was observed at  $3263 \text{ cm}^{-1}$  with strong and broad intensity. The C=O stretch was observed at  $1635.64 \text{ cm}^{-1}$ , which suggested the presence of carbonyl groups from ketones, aldehydes, or amides that are potential reducing agents. The observed hydroxyl group (O=H) and C=O stretch indicated that phenolic compounds, flavonoids, or other antioxidants present in *Nigella sativa* have reduced  $\text{Cu}^{2+}$  ions to metallic copper or  $\text{Cu}_2\text{O}$ . The stabilization evidence was observed when peaks at  $1242.16 \text{ cm}^{-1}$  and below  $700 \text{ cm}^{-1}$  indicated interactions between copper and functional groups present in *Nigella sativa*. The reason for this was that these biomolecules likely capped the nanoparticles, preventing aggregation. The results have shown the  $\text{Cu}^0$ -NPs synthesis, as the peaks below  $700 \text{ cm}^{-1}$  (e.g.,  $678.94 \text{ cm}^{-1}$ ,  $601.79 \text{ cm}^{-1}$ , and others) confirmed the presence of Cu-O bonds, thus indicating partial oxidation ( $\text{Cu}^0$  or  $\text{Cu}_2\text{O}$  phases) alongside metallic copper nanoparticles. The presence of functional groups was due to the interaction between the extract and the  $\text{Cu}^0$ -NPs that was responsible for producing plant-synthesised  $\text{Cu}^0$ -NPs, and the functional groups present might be responsible for the reduction of  $\text{Cu}^+$  to  $\text{Cu}^0$  and further stabilization of  $\text{Cu}^0$ -NPs synthesised using aqueous extract of *Nigella sativa* seeds.

A study (Kumar *et al.*, 2023) [10] reported the presence of hydroxyl group, alkyne and alkene groups in plants with copper nanoparticles, which validated the findings of the present work. Previous reports showed the role of *Piper retrofractum* extracts (Amaliyah *et al.*, 2020) [16] and the corresponding functional groups towards synthesis and stabilization of CuNPs that showed similar bands at  $506$  and  $603 \text{ cm}^{-1}$  corresponding stretching vibration of Cu–O bond in monoclinic  $\text{Cu}^0$ , and using *A. houstonianum* leaf extract (Chandraker *et al.*, 2020) [17] showed a similar O–H stretch at wavenumber  $3236.34$ . The peak in the range of  $600\text{--}1000 \text{ cm}^{-1}$  (at  $850$  and  $875 \text{ cm}^{-1}$ ) attributed to stretching of  $\text{Cu}^0$  and the existence of prominent IR bands near  $400\text{--}600 \text{ cm}^{-1}$  confirmed the formation of  $\text{Cu}^0$  from a study on green synthesis of leaves extract of *Calotropis gigantea* plant (Sharma *et al.*, 2015) [23]. In a similar study on aqueous extract solution of *N. sativa* seed (Yan *et al.*, 2020) [3], the presence of many antioxidant compounds with related bonds caused excellent conditions for reducing copper in the copper nanoparticles. Similar IR spectra of the copper nanoparticles revealed peaks at  $613$ ,  $1071$ ,  $1485$ ,  $1618$ , and  $3416 \text{ cm}^{-1}$  related to the Cu–O, C=O, C–O, C–OH, and OH, respectively. Earlier findings have documented that the majority of the absorption peaks found in the plant-based extract can also be found in the FT-IR spectrum of  $\text{Cu}^0$ -NPs, often at the same locations or with just a little variation in the peak's location and intensity, and the presence of a functional group might be responsible for the bioreduction of  $\text{Cu}^+$  to CuNPs [7,10,14,15,18,19,22,24,25,26,27,29,30].

### 4. FE-SEM and morphological analysis

The surface topography, shape, and microstructural arrangement of the green-synthesised  $\text{Cu}^0$ -NPs were examined using Field Emission Scanning Electron Microscopy (FESEM). Figure 5 represents the FE-SEM images of the NS- $\text{Cu}^0$ -NPs. The FE-SEM image suggested that the particle size or the size of individual nanoparticles ranges within  $50\text{--}200 \text{ nm}$  range using a  $500 \text{ nm}$  scale bar. Due to the presence of aggregation in synthesised  $\text{Cu}^0$ -NPs, it leads to the formation of larger clusters. The microscopic images reveal that the nanomaterial predominantly exhibits a spherical to near-spherical morphology. ImageJ software analysis was utilised to measure the physical dimensions of

the particles from the micrograph, yielding an average particle diameter of approximately 51 nm. The shape of the green-synthesised  $\text{Cu}^{\text{O}}$ -NPs in the present study ranges from approximately spherical, which is similar to studies (Prakash *et al.*, 2023; Mali *et al.*, 2020; Nzilu *et al.*, 2023; Priya *et al.*, 2023) [7, 18, 25, 26], to elongated or rod-like structures, typical of green-synthesized nanoparticles, where biomolecules from the extract act as stabilizers and reducing agents. The particles observed in the present study were irregularly shaped, corroborating a study (Thiruvengadam *et al.*, 2019) [14] with some tendency toward agglomeration (clustering) similar to that reported (Mali *et al.*, 2020) [18]. It may be due to the presence of hydroxyl groups in *N. sativa* seed (aqueous) extract that could be responsible for agglomeration. This result was supported by previous reports (Yan

*et al.*, 2020; Wu *et al.*, 2020; Mali *et al.*, 2020; Kausar *et al.*, 2022; Meena *et al.*, 2023; Nzilu *et al.*, 2023; Mahmoud *et al.*, 2021; Khani *et al.*, 2018) [3, 15, 18, 19, 24, 25, 28, 32]. The aqueous extract contributed to reducing Cu ions into Cu nanoparticles (CuNPs) while simultaneously capping the particles to prevent uncontrolled aggregation. However, the extent of capping or stabilization may vary depending on the synthesis conditions (e.g., concentration, reaction time, pH). The spherical-shaped  $\text{Cu}^{\text{O}}$ -NPs with an average size of ~51 nm coincide with a previous report (Meena *et al.*, 2023) [24]. A study (Wu *et al.*, 2020) [15] documented spherical nano-copper with aggregates formed and undefined-shaped NPs, while it distinctly showed an average particle size of 20 nm, which supports the present result.

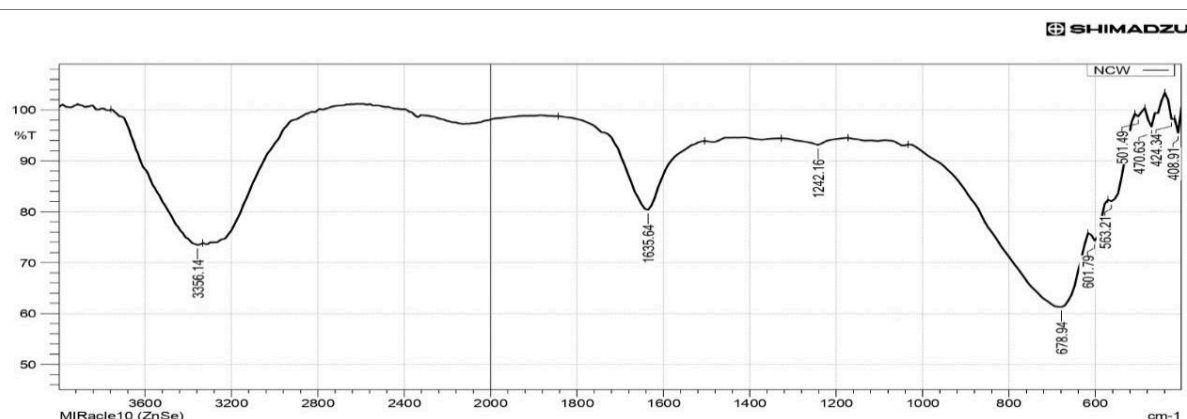


**Figure 3.3.** Fourier Transform Infrared (FTIR) analysis of copper nanoparticles

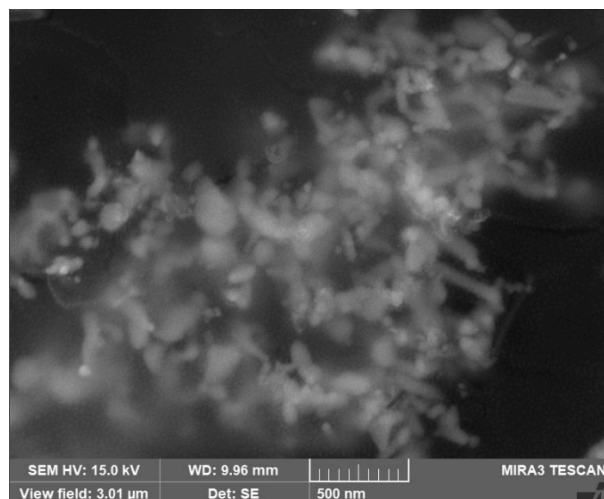


**Figure 5.** FE-SEM Analysis of copper Nanoparticles

A different result showed irregular shapes along with spherical particles in clusters in a previous work (Thiruvengadam *et al.*, 2019) [14]. A similar average size, ranging from 17–41 nm, representing the spherical morphology of green synthesised Cu-NPs, was reported (Khatami *et al.*, 2020) [27], spherical  $\text{Cu}^{\text{O}}$ -NPs with an average size of 29.2 nm (Priya *et al.*, 2023) [26]. A similar SEM study (Prakash *et al.*, 2023) [7] revealed the spherical-shaped Cu-NPs varying in size from 15–40 nm. In contrast to the agglomerated nanoparticles synthesised in the present work, a study (Nzilu *et al.*, 2023) [25] showed less evidence of agglomeration, while the average particle size determined using ImageJ software showed a similar average particle size to the previous report, at 59.99 nm. The agglomeration might have appeared due to the high surface energy of the nano-scale units causing multi-

ple small crystalline grains to clump together, forming slightly larger particles as observed under the microscope. In contrast, a large particle size of synthesized  $\text{Cu}^{\text{O}}$ -NPs in the nanometer range of ~150 nm was noted in an earlier report (Mahmoud *et al.*, 2021) [28], disagreeing with the present result. In a comparable result reported (Yan *et al.*, 2020) [3], an average size of 19.5 nm green synthesised Cu-NPs using *N. sativa* and spherical in shape was indicated using FESEM analysis. Therefore, the morphological clarity confirms the nanoscale characteristics of the synthesised material, showing a well-distributed morphology that complements the structural findings.

## 5. Conclusion

Green synthesis is a feasible alternative to conventional copper nanoparticle synthesis methods in which adverse effects are a consideration. The present study elucidated an eco-friendly, easy, less toxic, cost-effective, easy preparation, and sustainable method for the synthesis of  $\text{Cu}^{\text{O}}$ -NPs through green route using aqueous extract of *Nigella sativa* extract as reducing and capping agents. Green method for nanoparticle synthesis is safe as compared to chemical methods, as no chemical reagents or surfactants or toxic agents were used during the synthesis, it makes the process applicable to be used in different biomedical applications. The analytical characterization confirmed the dual-phase integrity of the material, revealing a crystalline network where a face-centred cubic (FCC) metallic copper arrangement co-exists with a well-defined copper oxide phase. The Green synthesis of  $\text{Cu}^{\text{O}}$ -NPs was noted by the visible color change observed, that owed to the surface plasmon resonance (SPR) during the reaction due to the presence of compounds in the extract. The nanofabrication, the size, shape, elemental composition, and structure of  $\text{Cu}^{\text{O}}$ -NPs was confirmed and characterized by UV–Vis spectroscopy, XRD, FTIR and SEM analysis. The color change was also confirmed by noting the absorption peaks in the UV-visible spectroscopy. The

above-mentioned characterization steps indicated that these NPs were synthesised in the best possible form. In conclusion, the overall study revealed that the greener method was successfully used to fabricate  $\text{Cu}^{\text{O}}$ -NPs using aqueous extract of *Nigella sativa* seeds. The overall findings confirmed that the *N. sativa* (aqueous) extract acts as both a reducing and stabilising agent, offering a simple and non-toxic synthesis route for fabrication of  $\text{Cu}^{\text{O}}$ -NPs. It can be further suggested for extensive research and scaling up the process in synthesising  $\text{Cu}^{\text{O}}$ -NPs using other plant parts of *Nigella sativa* as well as using other solvent extracts. In the future years, the utilization of other plants and plant parts for synthesizing metal nanoparticles for a variety of applications is anticipated to increase substantially. Moreover, the synthesised  $\text{Cu}^{\text{O}}$ -NPs hold promise for evaluating specific applications of these nanoparticles, including antimicrobial, catalytic, and biomedical uses. Future studies could focus on optimizing synthesis conditions and evaluating the nanoparticles' specific functional properties. Additionally, the optimization of the reaction parameters could be investigated to precisely tune the balance between the metallic and oxide phases. The dual-phase stability and controlled nanoscale dimensions observed in the study hold strong potential for environmental applications, particularly as high-surface area catalysts for wastewater purification and organic dye degradation.

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