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Original article

Green synthesis of copper nanoparticles from *Nigella sativa* seed extract and evaluation of their antibacterial and antiobesity activity

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Summary In this study, the copper nanoparticles were synthesized using various concentrations (5%, 6 %, 8%, and 10%) of *Nigella sativa* seed extracts, and their antibacterial and antiobesity effect was evaluated. The optimum particle size (98.23 nm) of nanoparticles was obtained at an extract concentration of 8%. FTIR results confirmed the functional groups responsible for copper ion reduction, The synthesized nanoparticles exhibited high antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli* having inhibition zone of 25 mm and 24 mm, respectively. Significantly ($P < 0.005$) percent lipase and amylase inhibition assay of copper nanoparticles confirmed their antiobesity activities.

Keywords Amylase inhibition assay, antibacterial activity, black cumin, copper nanoparticles (CuNPs), lipase inhibition assay, *Nigella sativa*.

Introduction

Nanotechnology is one of the most promising technologies used in different fields of science, which practised at the nanoscale (about 1–100 nm) (Kirtane *et al.*, 2021). Nanotechnology and nanoscience are studies including the use of small-size and large surface-to-volume ratios that result in physical and chemical differences in their properties like biological properties, mechanical properties, catalytic activity, sterical properties, materials science, melting point, electrical conductivity, thermal conductivity, optical absorption, medical imaging, filters, nanocomposites, filters, hyperthermia of tumours, and drug delivery in comparison to the bulk of the same chemical components (Ambika & Sundrarajan, 2015; Khatoon *et al.*, 2023). The increase in surface energy of these nanoparticles also boosted their biological effects (Khan *et al.*, 2019). Therefore, by regulating shape and size at a nanometre scale, it is possible to design and produce materials with novel uses. Nanoparticles display shape and size-dependent properties, which are of interest for various uses such as bio-sensing, catalysts to optics, computer transistors, antimicrobial activity, electrometers, wireless electronic logic, memory schemes, and chemical sensors (Alavi & Moradi, 2022; Dat *et al.*, 2023). Metal nanoparticles are extensively used in the areas of medicine and pharmacy.

The most widely utilised nanoparticles in biomedical applications and the rapidly developing fields of nanobiotechnology, detection chronocoulometry, resonance imaging, amplified voltammetric, and Raman

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spectroscopy are gold, nickel oxide and silver nanoparticles (Kunc *et al*., 2022). Moreover, copper can induce apoptosis in chronic lymphoid leukaemia (B cell-chronic lymphocytic leukaemia). Its wide range of uses in integrated circuits, bio-labelling, sensors, filters, cell electrodes, antimicrobial deodorant fibres, and antimicrobials have drawn the attention of researchers (Ahluwalia *et al*., 2018). Similarly, silver nanoparticles can be used in different areas of medicine, various industries, packaging, animal husbandry, cosmetics, health, accessories, and the military, due to their antimicrobial applications (Pandit, 2015). Silver nanoparticles show effective antimicrobial properties against harmful organisms such as *Bacillus subtilis, Escherichia coli, Vibrio cholera, Syphilis typhus, Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Firdhouse & Lalitha, 2016; Siddiqi *et al*., 2018). Furthermore, metal nanoparticle biosynthesis (green chemistry method) is eco-friendly without using toxic, harsh, costly chemicals (Rabiee *et al*., 2023). For instance, the chemical reduction method (e.g., sodium borohydride, hydrazine hydrate, ethylene glycol, and dimethylformamide (DMF)) used to produce silver nanoparticles may cause the harsh substance to absorb on the nanoparticles' surfaces, leading to toxicity issue. Although nanoparticles have long been produced chemically and physically, recent developments highlight the crucial role of microorganisms and biological systems in developing metal nanoparticles (Das *et al*., 2017). Due to their increasing success and convenience in producing nanoparticles, the use of organisms in this field is rapidly growing (Shevchenko *et al*., 2022). The organisms utilised in the synthesis of nanoparticles range from simple prokaryotic cells (such as bacterial cells) to complex eukaryotes. The ability of organisms to develop metal nanoparticles has shown a novel and exciting approach to producing these natural nanofactories (Ghosh *et al*., 2021).

Plants' different parts such as stem, fruit, root, flower and leaf are the good source of phytochemical (i.e., polyphenols, terpenoids, polyols) which help in detoxification of heavy metal, bioreduction of metallic ions, and environmental problems (Jadoun *et al*., 2021). It also contains the capping and reducing agents that show the higher stabilising and binding properties to prevent the steric and agglomeration hindrance (Sidhu *et al*., 2022). It also helps in inhibition of nanoparticles over growth and stabilising the nanoparticles interaction within the medium (Javed *et al*., 2020). At present, commercially the demand of the green synthesis nanoparticle is increasing in different sectors such as food, pharma, energy, chemical. Therefore, the present study was conducted to develop the green synthesis of copper nanoparticles by using *Nigella sativa* seeds to check their antibacterial and anti-obesity efficiency.

Materials and methods

Materials

Nigella sativa (black cumin) seeds were obtained from the high-altitude region of Kinnaur, Himachal Pradesh. The various chemicals used in the research work were of analytical grades such as copper sulphate ($CuSO_4.5H_2O$), potassium iodide, iodine crystal, sodium acetate ($C_2H_3NaO_2$), acetic acid (CH_3COOH), sodium hydroxide (NaOH), hydrochloric acid (HCl), nitric acid (HNO_3), lecithin ($C_{35}H_{66}NO_7P$), sodium cholate ($C_{24}H_{39}NaO_5$), glyceryl trioleate ($C_{57}H_{104}O_6$), tris base ($C_4H_{11}NO_3$) and lipase were procured from Sigma Aldrich, St. Louis, MO, USA of A.R. grade. Antibiotic (Ampicillin), nutrient agar, was used of HiMedia, Mumbai, India. Bacterial Cultures (i.e., *Staphylococcus aureus* (MTCC 3160), *Escherichia coli* (MTCC 443), *Klebsiella pneumonia* (MTCC 3384), and *Pseudomonas aeruginosa* (MTCC 424)) were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. Milli Q water, double distilled water, and various classes "A" certified glassware was used throughout the research.

Extraction of plant extract

Nigella sativa seeds were extracted using the aqueous solvent (double distilled water) extraction method (1:10). Firstly, the grinding of *Nigella sativa* seeds was done, and a 5 g sample was dissolved in an aqueous solvent. After that, the liquid extract was placed on the hot plate at 30 °C for 40 min to give the treatment, and allowing the mixture to cool at the room temperature. Then filtered, the extract mixture by using Whatman filter paper no.1 and centrifugation was done for 10 min at 5000 rpm. The supernatant was collected and stored in glass bottles for further use (Kanth *et al*., 2019).

Synthesis of copper nanoparticles (CuNPs) using plant extract

The reduction of copper sulphate with seed extract was made by the method given by Singh *et al*. (2016). And 0.05 M solution of copper sulphate (i.e., 1.25 g) taken in the 100 mL of the conical flask was heated up to 80 °C on a hot plate (Thermo Fisher Scientific, Mumbai, India), and 25 mL of seed extract was added dropwise with micropipette with constant stirring by magnetic stirrer at 150 rpm (M3D, Eltak DIGIMAG, India). Copper sulphate reduction occurred, and the colour changed from blue to green, confirming the complete reaction. For optimization of plant-stabilised copper nanoparticles

(CuNPs), the concentration of copper sulphate was kept constant (i.e., 25 mL), and different concentrations of seed extract (5%, 6%, 8%, and 10%) were used, respectively. The different concentration of herbal extract was used to observe the reduction on the size of copper ions due to presence of flavones, phenols, polysaccharides, and terepenoids (Mittal *et al*., 2013).

Characterisation of green synthesised CuNPs

UV–Visible spectrophotometer

Chemical reduction of copper sulphate by plant extract in the reaction mixture was observed by using UV–Visible Spectrophotometer (Evolution 201, Thermo Fischer Scientific India Pvt. Ltd, Mumbai). It is also used to measure the solution's concentration and identify organic compounds by determining the maximum absorption of the solution. Measurements were then recorded in the range between 280–500 nm. The quartz cuvettes of 2.5 mL with 1 cm path length were used (Kaushik *et al*., 2018).

Fourier transform infrared spectroscopy (FTIR)

Fourier-transform infrared spectroscopy (Agilent Technology) was used to find out the functional group present in the ethanolic herb extract of ratio 1:10 (1 g extract and 10 mL ethanol) at a range 4000–6500 cm^{-1} with Attenuated Transmission (ATR) and an internal reflection accessory comprised of Composite Zinc Selenide (ZnSe) and Diamond 144 crystals (Shimadzu IR Prestige-21 equipment) (Depciuch *et al*., 2018).

Dynamic light scattering (DLS)

The dynamic light scattering technique was used to determine the particle size of copper nanoparticle samples at 25 °C. Multiple scattering is a component of the laser diffraction method. Based on the Mie scattering theory, it has been used to measure the powder's particle size distribution. The experiment was conducted in a computer-controlled particle size analyser [ZETA Sizers nano series (Malvern Instruments Nano Z.S.)] to determine the particle size distribution (Wasilewska *et al*., 2009).

Applications of green synthesised CuNPs

Agar-well diffusion method

The test organisms were freshly seeded on a plate, and the antibacterial potential of the nanoparticles was allowed to permeate into the medium and interact with them. The diameter of the generated inhibition zones was used to calculate their sizes. An overnight bacterial culture was inoculated on the Petri plates with 20 mL of nutrient agar medium, and the nutrient agar (N.A.) was allowed to solidify. Then inoculum (100 μL) was allowed to dry in laminar airflow (Sanco Vertical Laminar Air Flow, India) for 5 min. The seeded agar plate was punctured with 6 mm diameter wells using a cork borer. Using a micropipette, 100 μL of the copper nanoparticles solution was added. Antibiotic discs with ampicillin were employed as a positive control. The plates were then kept in the incubator (Adarsh Company, ISO-certified India) for 24 h at 37 °C. By measuring the diameter of the zone of inhibition that developed around the well, the antibacterial activity was evaluated. The test microorganism's growth and germination are often inhibited by antibacterial drugs that diffuse into the agar, and the diameters of these inhibition zones were determined (Nalawade *et al*., 2016).

Lipase inhibition assay

Lipase inhibitory assay of the extracts was performed using the method given by Kumar *et al*. (2020). The solution of the substrate was made in 0.1 M TES buffer (9 mL) (pH 7.0) by mixing 10 mg of lecithin, 5 mg of sodium cholate, and 80 mg of glycerol trioleate. And 10 μL of lipase solution was mixed with the 20 μL of sample and 20 μL of substrate solution in a microplate well and then incubated at 37 °C for 30 min. The optical density was measured at 550 nm using an ELISA (Biotek) reader. Lipase inhibitory (%) activity was calculated using the formula:

$$\text{Lipase inhibition (\%)} = \{1-(OD2-OD1)/(OD4-OD3) \times 100\}$$

Where, OD1 = the solution optical density of plant extract, lipase, and substrate, OD2 = the solution optical density of plant extract and substrate, OD3 = the solution optical density of lipase and substrate, OD4 = the solution optical density of substrate.

Amylase inhibition assay

The amylase inhibition activity was calculated using the method given by Kumar *et al*. (2020). The solution of the substrate was prepared by mixing 500 mg of soluble starch in 0.4 M NaOH (25 mL) and heating at 100 °C for 5 min. Using HCl, the solution pH was adjusted to 7.0, and the volume was made to 100 mL using distilled water. Different concentrations of the solution of plant extract were made using acetate buffer (pH 6.5). And 20 μL of the α-amylase solution was mixed with 20 μL of sample and 40 μL of substrate solution in a microplate well and then incubated at 25 °C for 15 min. To stop the reaction, 80 μL of 0.1 M HCl and 200 μL of 1 mM iodine solution were added. The optical density was recorded at 650 nm using a UV–Visible

Spectrophotometer. Amylase inhibitory (%) activity was calculated using the formula:

$$\text{Amylase inhibitory activity (\%)} = \{1 - (OD6 - OD5)/(OD8 - OD7) \times 100\}$$

Where, OD5 = the solution optical density of plant extract, amylase, and starch, OD6 = the solution optical density of plant extract and starch, OD7 = the solution optical density of amylase and starch, OD8 = the solution optical density of starch.

Dialysis bag test (glucose uptake assay)

To study the effect of glucose movement, a dialysis membrane with a pore size of 2000 KDa (6 cm × 15 mm) was used. To expand, the membrane was immersed in distilled water. And 1 mg/10 mL extract was added to the membrane sealed from one end. Then, 15 mL of 0.22 mM glucose solution was dissolved, incubated for 4 h at 37 °C, and centrifuged for 20 min at 4800 rpm. And 45 mL of 0.15 M sodium chloride (NaCl) was then added to the tube. Lastly, the movement of the glucose from the membrane to the outer solution was recorded at different time intervals such as 15, 30, 60, 120, 240, 360, and 480 s using a glucometer (Accu-Chek Active Glucose Monitor) (D'Souza *et al*., 2014).

Results and discussion

Synthesis and optimisation of plant-synthesised CuNPs

Figure 1a,b depicted the synthesis of CuNPs by reducing the copper sulphate pentahydrate using the plant extract of *Nigella sativa* seeds. The change in colour from blue to green confirms the fabrication of CuNPs. After that, *Nigella sativa* seeds extract was used to stabilise the freshly prepared CuNPs. Different concentrations (5%, 6%, 8%, and 10%) of CuNPs were used to optimise the CuNPs and UV–visible characterisation was used to select suitable CuNPs, as shown in Fig. 2a,b. The fabrication process confirmed the UV–visible spectrum at 500–280 nm.

The collective oscillation of conduction electrons at the surface of metal nanoparticles causes them to absorb visible electromagnetic radiation. Generally, CuNPs exhibit SPR at around 500–600 nm. Furthermore, UV–visible spectroscopy results revealed no shift in the strong absorption of the plant-synthesised CuNPs with increasing concentrations of plant extracts. After the plant-synthesised CuNPs were optimised, the UV–visible spectrum from the above-given concentration of nanoparticles was selected. The concentration chosen for *Nigella sativa* synthesised CuNPs was 8% due to higher reduction of copper ions into nanoparticles through presence of bioreducing agents such as enzymes, phenols, flavonoids, terpenoids in extract. Even though *Nigella sativa* extract acts as stabilising and reducing agent in presence of metal salt (Mohamad *et al*., 2014).

Characterisation of plant-synthesised CuNPs

Average particle size

Dynamic light scattering was used to determine the practical size of CuNPs at 25 °C. It was conducted to measure the powder's particle size distribution. The average particle size of plant-synthesised CuNPs is depicted in Fig. 3a. The particle size of plant-synthesised CuNPs, i.e., *Nigella sativa* seed (K.J.), is 98.23 nm. The particle size results demonstrated a considerable change

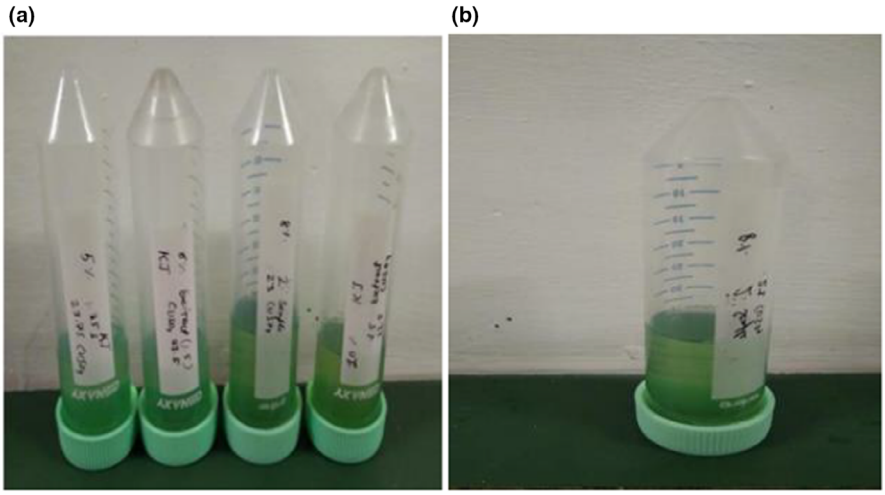

Figure 1 (a and b) Plant-synthesised copper nanoparticles (CuNPs) at different concentrations (*Nigella sativa* seed extract) i.e. (a) 5%, 6%, 8% and 10% (b) 8%.

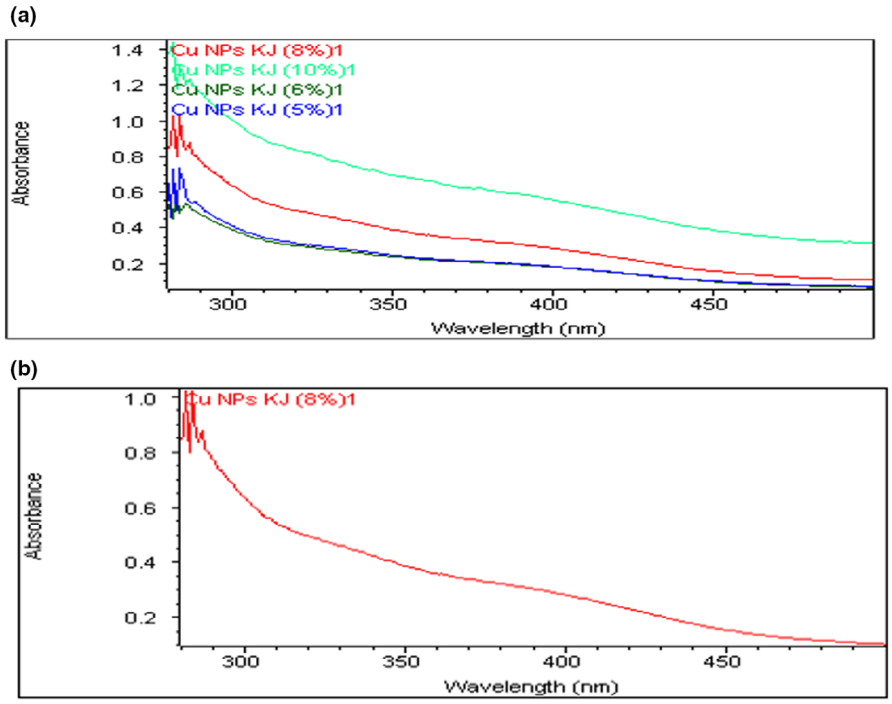

Figure 2 (a and b) UV–Visible spectrum of different concentrations of plant (*Nigella sativa* seed extract) synthesised copper nanoparticles (CuNPs) i.e. (a) Spectrum at different concentrations (b) 8%.

in average nanoparticle particle size with an increase in plant-synthesised CuNPs concentration. Compared with another study, the outcomes of particle size are directly connected to the enhanced absorbance value of the nanoparticle (Zahir *et al*., 2015). The presence of the free form of the copper molecule in the solution might significantly increase the average particle size due to the limited available binding site in the plant extract. The free copper molecule tends to interact with the individual copper molecule and coalesce copper molecule, resulting in a larger average particle size of the nanoparticles with an increase in the concentration of the plant-synthesised CuNPs (Dang *et al*., 2011; Sebeia *et al*., 2020). Therefore, plant-synthesised copper nanoparticles demonstrated a reduced absorbance value during UV–Visible characterisation and particle size in the 1–100 nm (Ahmed *et al*., 2016). As a result, the plant-synthesised CuNPs was chosen for further characterisation and application.

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy was used to categorise the potential biological ability or functional groups of plant extracts involved in stabilising CuNPs, as shown in Fig. 3b. The FTIR spectrum supports the development of plant-synthesised CuNPs (*Nigella sativa* seed) in the 4000–5000 cm^{-1} region. The results demonstrated that the vibrational bands observed in the sample between 1000–3500 cm^{-1} closely resemble the hydroxyl group (O=H) at 3263 cm^{-1} with strong and broad intensity. This revealed stretched and H-bonded vibrations and an alkyne group (CC) around 2100–2260 cm^{-1}. Furthermore, the existence of (CC) at 2243 cm^{-1} revealed alkyne stretching vibrations with variable strength. The vibrational band 1620–1680 cm^{-1} is similar to the alkene group (C=C) at 1648 cm^{-1} but with stretched vibrations of varying intensity. The different peak locations in the spectrum show that the plants extract need for CuNPs synthesis was variable because plant extract was employed to synthesise CuNPs that prevent nanoparticle aggregation and contribute to secondary structures. The functional groups are created due to the interaction between plant extracts and the CuNPs responsible for producing plant-synthesised CuNPs. Shifrina *et al*. (2019) reported the presence of hydroxyl group, alkyne and alkene groups in plants with copper nanoparticles, which validated the findings. The elemental analysis confirmed the presence of significant copper elements with the minor functional compounds reported by Rajesh *et al*. (2018).

Applications of plant-synthesised CuNPs

Antibacterial activity

The plant-synthesised CuNPs were significantly evaluated against four bacterial cultures, which were

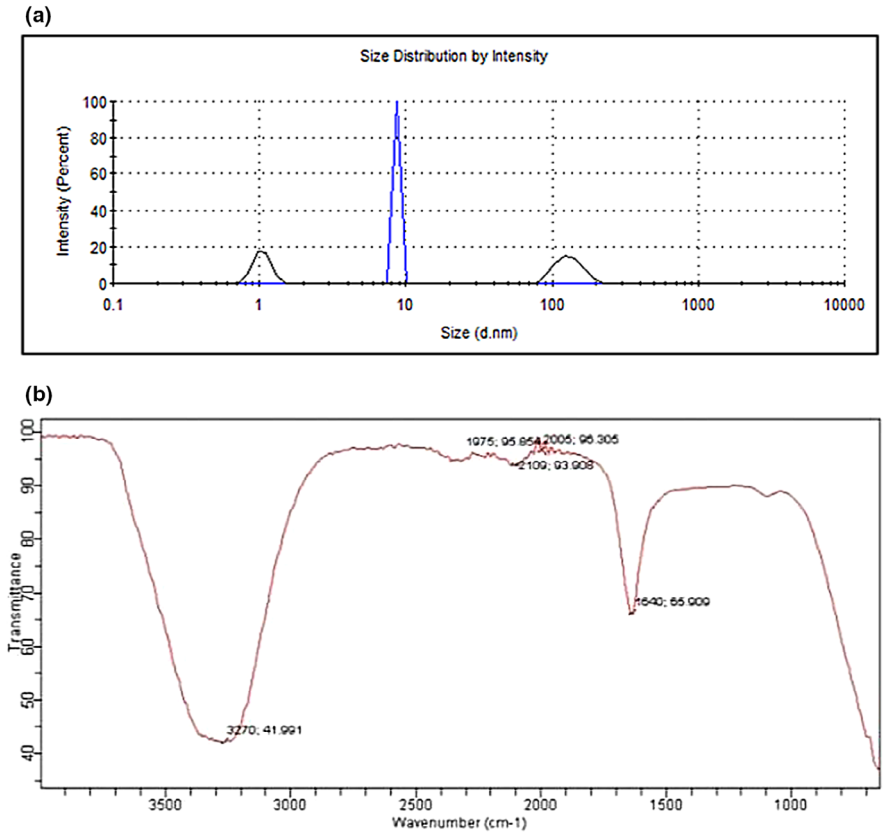

Figure 3 (a) The average particle size of plant-synthesised copper nanoparticles (CuNPs). (b) Fourier Transform Infrared Spectroscopy spectrum of plant-synthesised copper nanoparticles (CuNPs).

Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, and *Pseudomonas aeruginosa*, against the positive control, i.e., antibiotic (Ampicillin) and the distilled water as the negative control as shown in Fig. 4a–d. The result revealed that *Nigella sativa* synthesised CuNPs with *Klebsiella pneumoniae* showed a significantly ($P < 0.005$) lower zone of inhibition (22 mm) compared with *Staphylococcus aureus* (23 mm), *Escherichia coli* (24 mm), and *Pseudomonas aeruginosa* (25 mm). Whereas *Escherichia coli* (20 mm), *Klebsiella pneumoniae* (19 mm), *Staphylococcus aureus* (18 mm), and *Pseudomonas aeruginosa* (17 mm) showed a significantly ($P < 0.005$) lower zone of inhibition with the antibiotic compared with *Nigella sativa* synthesised CuNPs as shown in Table 1. These bacterial strains showed sensitivity towards the antibiotic with the clear zone around the well through the agar well diffusion method. A virtuous inhibitory zone was observed for all four bacterial strains against the plant-synthesised CuNPs. The result showed the plant-synthesised CuNPs have effective antibacterial potential. The antimicrobial results against the bacterial strains with the positive control results of the zone of inhibition were, i.e., for *Phaseolus lunatus* (15 mm, 18 mm, 19 mm, and 21 mm) and *Cephalanthus occidentalis* (16 mm, 14 mm, 18 mm and 18 mm) for plant-synthesised CuNPs against the *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli*, and *Pseudomonas aeruginosa*, respectively. The studies revealed that plant-synthesised CuNPs had shown high antibacterial activity due to the differences in the membrane structures of the bacterial pathogens. Moreover, CuNPs showed their bactericidal activity mainly due to the release of copper cations (Cu^{+}) which then get attached electrostatically to the bacteria's cell wall. Furthermore, this metal ion is not only connected to the cell walls but also penetrates inside the surface of the membrane (Kruk *et al.*, 2015; Rajoriya *et al.*, 2016; Patil *et al.*, 2017).

Lipase inhibition assay

Table 2 represents the % lipase inhibition assay results at different concentrations and IC 50 (%) of plant-synthesised CuNPs. The study revealed that with

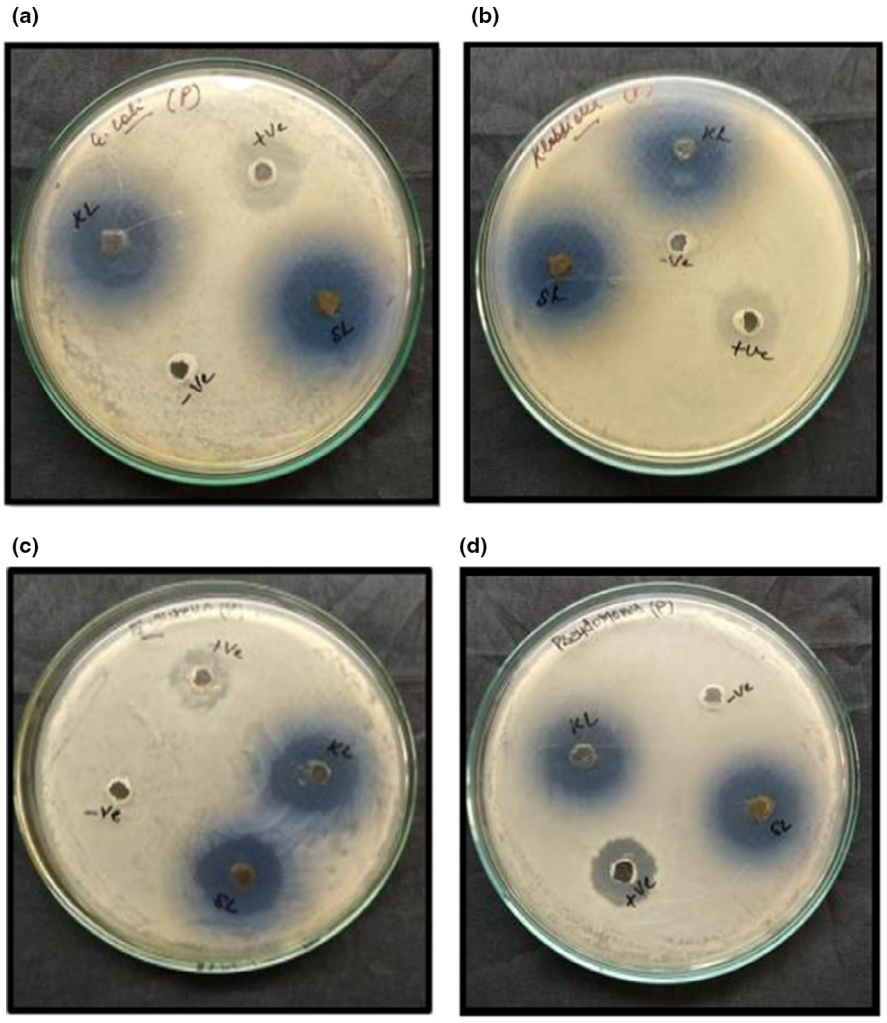

Figure 4 (a–d) Antibacterial activity of *Nigella sativa* seeds synthesised copper nanoparticles (CuNPs): (a) *Klebsiella pneumonia*, (b) *Escherichia coli*, (c) *Staphylococcus aureus* (d) *Pseudomonas aeruginosa*.

increase in concentration 20 μg mL^{-1}, 40 μg mL^{-1}, 60 μg mL^{-1}, and 80 μg mL^{-1}, the percentage lipase inhibition significantly ($P < 0.005$) increased such as 33.20%, 46.00%, 51.30%, 66.00%, whereas the IC 50 value is 57.54. Compared with other studies, in the *Achillea millefolium* rubra seeds, the percentage lipase inhibition and IC 50 were 43.21% and 24.67. Similarly, Ramson's seeds also showed 43.22% lipase inhibition activity and 15.22 IC 50. The *Nigella sativa* seeds show the higher property of reducing the fat content due to the hydrolysis process, which helps to inhibit the dietary lipids conversion into fatty acids (Nalawade *et al.*, 2016). The herb also helps to remove fat from the body by controlling the fat digestion process during the gastrointestinal process (Patil *et al.*, 2017; Maideen, 2020).

Amylase inhibition assay

The amylase (%) inhibition assay results of different concentrations and IC50 of the plant-synthesised CuNPs is shown in Table 2. It was observed that 80 μg/mL concentration showed a significantly ($P < 0.005$) higher inhibition effect of 76.00% and IC 50 46.34 compared with other concentrations 60 μg mL^{-1}, 40 μg mL^{-1}, and 20 μg mL^{-1} that showed the following results 53.90%, 43.00%, and 37.50%, respectively. Kumar *et al.* (2020) reported in a study that *Andrographis paniculata* shows a significantly ($P < 0.005$) lower (%) inhibition assay and IC 50 (3.29% and 0.68). The main mechanisms shown by the *Nigella sativa* seeds help to decrease the digestion of glucose and carbohydrate, which target to reduce postprandial hyperglycemia (Akhilnath *et al.*, 2019). It

Table 1 Zone of inhibition of *Nigella sativa* seeds CuNPs (mm) with microorganisms

Microorganism	Zone of inhibition of *Nigella sativa* seeds CuNPs (mm)
Staphylococcus aureus	
Antibiotic	18
CuNPs	23
Escherichia coli	
Antibiotic	20
CuNPs	24
Klebsiella pneumoniae	
Antibiotic	19
CuNPs	22
Pseudomonas aeruginosa	
Antibiotic	17
CuNPs	25

Table 2 % of inhibition of lipase and amylase assay for *Nigella sativa* seeds synthesised copper nanoparticles (CuNPs)

S. No.	Concentration (μg mL^{-1})	% lipase inhibition	I.C. 50 (%)	% amylase inhibition	I.C. 50 (%)
1	20	33.20 ± 1.15^{a}	57.54	37.50 ± 0.27^{a}	46.34
2	40	46.00 ± 1.00^{b}		43.00 ± 1.00^{b}	
3	60	51.30 ± 0.90^{c}		53.90 ± 0.30^{c}	
4	80	66.00 ± 1.00^{d}		76.00 ± 1.00^{d}	

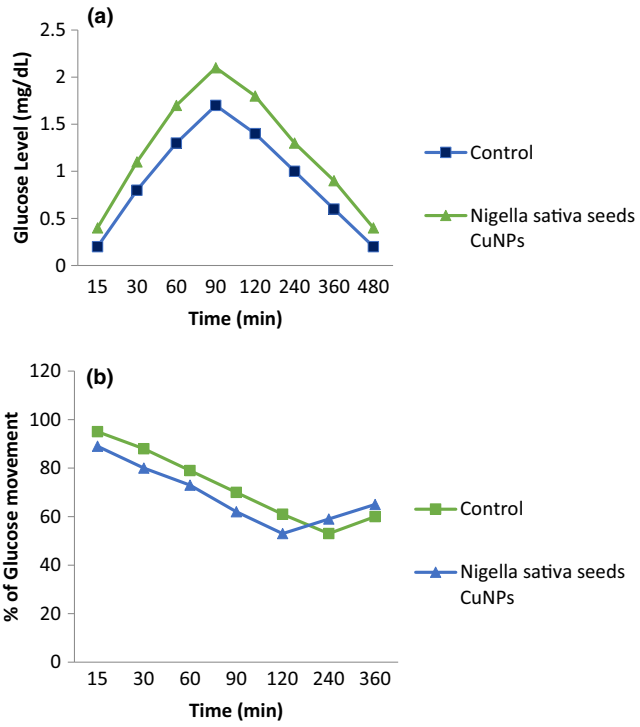

Figure 5 (a) Glucose level (mg dL^{-1}) of *Nigella sativa* seeds synthesised CuNPs. (b) Percentage of glucose movement of *Nigella sativa* seeds synthesised copper nanoparticles (CuNPs).

also helps to reduce the hydrolysis process in the enzymatic reaction of carbohydrate complexes (Kumar *et al.*, 2020).

Glucose assay

The ameliorating high glucose level was analysed by the *Nigella sativa* seeds synthesised by CuNPs to check the property against the diabetic condition. The glucose and glucose movement percentage levels are shown in Fig. 5a,b. The level of glucose and percentage of glucose movement was observed at different time intervals such as 15 min, 30 min, 60 min, 90 min, 120 min, 240 min, 360 min, and 480 min. In this study, the higher retention time for the glucose in dialysis membrane was observed at 90 min (2.1 mg dL^{-1}) compared with 15 min (0.4 mg dL^{-1}), 30 min (1.1 mg dL^{-1}), and 60 min (1.7 mg dL^{-1}). Similarly, *Nigella sativa* seeds synthesised CuNPs (2.1 mg dL^{-1}) showed significantly ($P < 0.005$) higher glucose retention time compared with control 1.7 mg dL^{-1} at 90 min. Interestingly, the study observed that glucose retention time is reduced at 120 min in both *Nigella sativa* seeds synthesised CuNPs and control (1.8 mg dL^{-1} and 1.4 mg dL^{-1}, respectively). The percentage of glucose movement is significantly ($P < 0.005$) lower (53%) in *Nigella sativa* seeds synthesised CuNPs compared with control (61%) at 120 min. The main mechanism behind these *Nigella sativa* seeds synthesised CuNPs show the quenching property with the glucose molecules, which helps to improve glucose uptake due to glucose level reduction (Kumar *et al.*, 2020, 2022).

Conclusion

To summarise for the first time, green synthesis of copper nanoparticles (CuNPs) using *Nigella sativa* seeds' extract of different concentrations (5%, 6%, 8%, and 10%) were successfully prepared through green methods which showed several benefits, such as easy availability, eco-friendliness, non-toxicity, and economic feasibility. The proposed approach for green synthesis method showed a high potential for the different industrial applications. Furthermore, 8% prepared green synthesised CuNPs by *Nigella sativa* seeds' extract was finalised based on particle size, functional group, and antibacterial activity. The CuNPs showed a significantly ($P < 0.005$) higher lipase, amylase, and glucose assay activity. Hence, we conclude synthesised CuNPs by *Nigella sativa* seeds' extract can be utilised for the future promising application due to its less toxicity properties in therapeutic applications.

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Author contributions

Mukul Kumar: Conceptualization (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); project administration (equal); resources (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal). **Deepika Kaushik:** Investigation (equal); methodology (equal); software (equal). **Ashwani Kumar:** Methodology (equal). **Prerna Gupta:** Writing – review and editing (equal). **Charalampos Proestos:** Supervision (equal); writing – review and editing (equal). **Emel Oz:** Writing – review and editing (equal). **Elif Orhan:** Writing – review and editing (equal). **Jasjit Kaur:** Validation (equal). **Mohammad Rizwan Khan:** Writing – review and editing (equal). **Tahra Elobeid:** Writing – review and editing (equal). **Matteo Bordiga:** Writing – review and editing (equal). **Fatih Oz:** Supervision (equal); writing – review and editing (equal).

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Conflicts of interest

Prerna Gupta: Visualization and software (equal). The authors declare no conflict of interest.

Ethical approval

Ethics approval was not required for this research.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.16359.

Data availability statement

The data that support the findings of this study are available from the authors upon reasonable request.

References

Ahluwalia, V., Elumalai, S., Kumar, V., Kumar, S. & Sangwan, R.S. (2018). Nano silver particle synthesis using Swertia paniculata herbal extract and its antimicrobial activity. *Microbial Pathogenesis*, **114**, 402–408.

This article is important for our manuscript. Because the paper reports the detailed description of the methodology used for synthesis of nanoparticles by using plant extract.

Ahmed, S., Ahmad, M., Swami, B.L. & Ikram, S. (2016). A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. *Journal of Advanced Research*, **7**, 17–28.

Akhilnath, V.S., Satish, S. & Shabaraya, A.R. (2019). Effect of Betula utilis bark extract on cafeteria diet-induced obesity in rats. *European Journal of Biomedical*, **6**, 441–445.

Alavi, M. & Moradi, M. (2022). Different antibacterial and photocatalyst functions for herbal and bacterial synthesized silver and copper/copper oxide nanoparticles/nanocomposites: a review. *Inorganic Chemistry Communications*, **142**, 109590.

This article is important for our manuscript. Because this paper reports the detailed review on nanoparticles and its antibacterial and photocatalyst functions.

Ambika, S. & Sundrarajan, M. (2015). Green biosynthesis of ZnO nanoparticles using Vitex negundo L. extract: spectroscopic investigation of interaction between ZnO nanoparticles and human serum albumin. *Journal of Photochemistry and Photobiology B: Biology*, **149**, 143–148.

Dang, T.M.D., Le, T.T.T., Fribourg-Blanc, E. & Dang, M.C. (2011). Synthesis and optical properties of copper nanoparticles prepared by a chemical reduction method. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, **2**, 15009.

Das, R.K., Pachapur, V.L., Lonappan, L. *et al.* (2017). Biological synthesis of metallic nanoparticles: plants, animals and microbial aspects. *Nanotechnology for Environmental Engineering*, **2**, 1–21.

Dat, N.M., Nam, N.T.H., An, H. *et al.* (2023). Green synthesis of copper oxide nanoparticles for photodegradation of malachite green and antibacterial properties under visible light. *Optical Materials*, **136**, 113489.

Depciuch, J., Kasprzyk, I., Drzymała, E. & Parlinska-Wojtan, M. (2018). Identification of birch pollen species using FTIR spectroscopy. *Aerobiologia*, **34**, 525–538.

D'Souza, S., Murata, H., Jose, M.V. *et al.* (2014). Engineering of cell membranes with a bisphosphonate-containing polymer using ATRP synthesis for bone targeting. *Biomaterials*, **35**, 9447–9458.

Firdhouse, M.J. & Lalitha, P. (2016). Biogenic silver nanoparticles–synthesis, characterization and its potential against cancer inducing bacteria. *Journal of Molecular Liquids*, **222**, 1041–1050.

Ghosh, S., Ahmad, R., Zeyaullah, M. & Khare, S.K. (2021). Microbial nano-factories: synthesis and biomedical applications. *Frontiers in Chemistry*, **9**, 626834.

Jadoun, S., Arif, R., Jangid, N.K. & Meena, R.K. (2021). Green synthesis of nanoparticles using plant extracts: a review. *Environmental Chemistry Letters*, **19**, 355–374.

Javed, R., Zia, M., Naz, S., Aisida, S.O., Ain, N.U. & Ao, Q. (2020). Role of capping agents in the application of nanoparticles in biomedicine and environmental remediation: recent trends and future prospects. *Journal of Nanobiotechnology*, **18**, 1–15.

Kanth, M., Hussain, A., Shrivastva, P.K., Sharma, M., Tripath, J. & Khan, M.A. (2019). Extraction, qualitative and quantitative determination of secondary metabolites of bergenia ciliata (how) sternb rhizome. *Journal of Drug Delivery and Therapeutics*, **9**, 407–411.

Kaushik, R., Chawla, P., Kumar, N., Janghu, S. & Lohan, A. (2018). Effect of premilling treatments on wheat gluten extraction and noodle quality. *Food Science and Technology International*, **24**, 627–636.

Khan, I., Saeed, K. & Khan, I. (2019). Nanoparticles: properties, applications and toxicities. *Arabian Journal of Chemistry*, **12**, 908–931.

Khatoon, U.T., Velidandi, A. & Rao, G.N. (2023). Copper oxide nanoparticles: synthesis via chemical reduction, characterization, antibacterial activity, and possible mechanism involved. *Inorganic Chemistry Communications*, **149**, 110372.

Kirtane, A.R., Verma, M., Karandikar, P., Furin, J., Langer, R. & Traverso, G. (2021). Nanotechnology approaches for global infectious diseases. *Nature Nanotechnology*, **16**, 369–384.

Kruk, T., Szczepanowicz, K., Stefańska, J., Socha, R.P. & Warszyński, P. (2015). Synthesis and antimicrobial activity of monodisperse copper nanoparticles. *Colloids and Surfaces B: Biointerfaces*, **128**, 17–22.

Kumar, M., Guleria, S., Chawla, P. *et al.* (2020). Anti-obesity efficacy of the selected high altitude Himalayan herbs: in vitro studies. *Journal of Food Science and Technology*, **57**, 3081–3090.

This article is important for our manuscript. Because this paper highlights the impact of herbs on obesity activity and its methodology.

Kumar, M., Kaushik, D., Kaur, J. *et al.* (2022). Assessment of anti-obesity potential and techno-functional properties of Bougainvillea spectabilis Willd. Bracts. *Separations*, **9**, 399.

This article is important for our manuscript. Because this paper shows the assessment of anti-obesity of herb.

Kunc, F., Bushell, M., Du, X., Zborowski, A., Johnston, L.J. & Kennedy, D.C. (2022). Physical characterization and cellular toxicity studies of commercial NiO nanoparticles. *Nanomaterials*, **12**, 1822.

Maideen, N.M.P. (2020). Prophetic medicine-nigella sativa (black cumin seeds)–potential herb for COVID-19? *Journal of Pharmacopuncture*, **23**, 62.

Mittal, A.K., Chisti, Y. & Banerjee, U.C. (2013). Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances*, **31**, 346–356.

Mohamad, N.A.N., Arham, N.A., Jai, J. & Hadi, A. (2014). Plant extract as reducing agent in synthesis of metallic nanoparticles: a review. *Advanced Materials Research*, **832**, 350–355.

Nalawade, T.M., Bhat, K.G. & Sogi, S. (2016). Antimicrobial activity of endodontic medicaments and vehicles using agar well diffusion method on facultative and obligate anaerobes. *International Journal of Clinical Pediatric Dentistry*, **9**, 335.

Pandit, R. (2015). Green synthesis of silver nanoparticles from seed extract of Brassica nigra and its antibacterial activity. Nusantara. *Bioscience*, **7**, 15–19. https://doi.org/10.13057/nusbiosci/n070103

Patil, M.M., Anand, T., Ilaiyaraja, N. & Khanum, F. (2017). In-vitro antioxidant and anti-obesity properties of Bauhinia variegata. *Defence Life Science Journal*, **2**, 128–132.

Rabiee, N., Ahmadi, S., Iravani, S. & Varma, R.S. (2023). Natural resources for sustainable synthesis of nanomaterials with anticancer applications: a move toward green nanomedicine. *Environmental Research*, **216**, 114803.

Rajesh, K.M., Ajitha, B., Reddy, Y.A.K., Suneetha, Y. & Reddy, P.S. (2018). Assisted green synthesis of copper nanoparticles using Syzygium aromaticum bud extract: physical, optical and antimicrobial properties. *Optik*, **154**, 593–600.

Rajoriya, P., Misra, P., Shukla, P.K. & Ramteke, P.W. (2016). Light-regulatory effect on the phytosynthesis of silver nanoparticles using aqueous extract of garlic (Allium sativum) and onion (Allium cepa) bulb. *Current Science*, **111**, 1364–1368.

Sebeia, N., Jabli, M., Ghith, A. & Saleh, T.A. (2020). Eco-friendly synthesis of Cynomorium coccineum extract for controlled production of copper nanoparticles for sorption of methylene blue dye. *Arabian Journal of Chemistry*, **13**, 4263–4274.

Shevchenko, K.G., Garkushina, I.S., Canfarotta, F., Piletsky, S.A. & Barlev, N.A. (2022). Nano-molecularly imprinted polymers (nanoMIPs) as a novel approach to targeted drug delivery in nanomedicine. *RSC Advances*, **12**, 3957–3968.

Shifrina, Z.B., Matveeva, V.G. & Bronstein, L.M. (2019). Role of polymer structures in catalysis by transition metal and metal oxide nanoparticle composites. *Chemical Reviews*, **120**, 1350–1396.

Siddiqi, K.S., Husen, A. & Rao, R.A. (2018). A review on biosynthesis of silver nanoparticles and their biocidal properties. *Journal of Nanobiotechnology*, **16**, 1–28.

Sidhu, A.K., Verma, N. & Kaushal, P. (2022). Role of biogenic capping agents in the synthesis of metallic nanoparticles and evaluation of their therapeutic potential. *Frontiers in Nanotechnology*, **3**, 105.

Singh, P., Kim, Y.J., Zhang, D. & Yang, D.C. (2016). Biological synthesis of nanoparticles from plants and microorganisms. *Trends in Biotechnology*, **34**, 588–599.

Wasilewska, M., Adamczyk, Z. & Jachimska, B. (2009). Structure of fibrinogen in electrolyte solutions derived from dynamic light scattering (DLS) and viscosity measurements. *Langmuir*, **25**, 3698–3704.

Zahir, A.A., Chauhan, I.S., Bagavan, A. *et al.* (2015). Green synthesis of silver and titanium dioxide nanoparticles using Euphorbia prostrata extract shows shift from apoptosis to G0/G1 arrest followed by necrotic cell death in leishmania donovani. *Antimicrobial Agents and Chemotherapy*, **59**, 4782–4799.

