Heliyon 11 (2025) e41397

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Euphorbia serpens extracts mediated synthesis of copper oxide nanoparticles and their biological applications

Sana Idrees [a,1], Banzeer Ahsan Abbasi [a,*,1], Javed Iqbal [b,**], Mohsin Kazi [c]

[a] *Department of Botany, Rawalpindi Women University, 6th Road, Satellite Town, Rawalpindi, 46300, Pakistan*
[b] *Department of Botany, Bacha Khan University, Charsadda, Khyber Pakhtunkhwa, Pakistan*
[c] *Department of Pharmaceutics, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh, 11451, Saudi Arabia*

A B S T R A C T

With the passage of time there is enormous development in the field of science and technology, however, human health remained the utmost concern. There are different strategies that helps us to treat various diseases but they have adverse reactions on our bodies. Nanobiotechnology is the advanced field consisting of new techniques and fabrication procedures for nanostructures for making drugs more effective against diseases in less time. These bio-nanoparticles easily target diseased cells, bind to them and damage the diseased tissue. Green synthesis of CuONPs is multi-purposed and can also be used for several bio-applications. The present study involves fabrication of economically viable copper oxide nanoparticles using aqueous plant extracts. The medicinal plants used in current study were *Euphorbia serpens.* The orchestrated nanoparticles were validated using several analytical techniques; Ultraviolet–Visible spectroscopy, XRD, SEM, FT-IR (Fourier-transform infrared), EDS, FT-IR and DLS (Dynamic light scattering). The average/mean size of the greenly fabricated nanoparticles was found to be 20.39 nm. Further, the biosynthesized NPs were evaluated for multiple biological activities. The bactericidal properties of green synthesized NPs were demonstrated using different bacterial strains revealing significant antibacterial potentials. The fungicidal properties were also demonstrated using different fungal strains showing significant antifungal potential. The minimum inhibitory concentration (MICs) values were calculated for different bacterial and fungal strains via disc-diffusion method. Further, our *Euphorbia serpens* orchestrated nanoparticles revealed excellent cytotoxic potentials against nascent brine shrimp larvae (IC50: 32.88 μg/mL) and have shown potential antioxidant properties. In conclusion, the asynthesized nanoparticles have shown significant *in vitro* bio-potentials. In future, different *in vitro* activities; antileishmanial, anticancer, enzyme degradation assays and different *in vivo* studies using animal models are recommended to further investigate the role of these NPs in different biomedical applications. Also, the formulations of multi-organic functional groups and biometallic NPs with regulated/balanced surface chemistry is worthy for the consideration.

1. Nanotechnology: A new frontier

Nanotechnology is one of emerging field of science, engineering and technology dealing materials at nanoscale.The term nano is derived from Greek language, mean minute/microscopic [1]. Nanoscience involves the study and applications of extremely small things and can be used in all sciences such as chemistry, biology, physics, material science and engineering [2,3]. Nanotechnology has diversified applications; imaging, modeling, designing, production and utilizing structural devices and systems by manipulating molecules in atoms at nanoscale (atomic, molecular and macromolecular level/scale). It involves the manipulation of matter with at least one dimension in size ranging from 1 to 100 nm (nm) [4].

* Corresponding author.
** Corresponding author.
E-mail addresses: sanaidrees348@gmail.com (S. Idrees), banzeer.abbasi@f.rwu.edu.pk (B.A. Abbasi), javed89qau@gmail.com (J. Iqbal), mkazi@ksu.edu.sa (M. Kazi).
[1] Equally Contributed.

https://doi.org/10.1016/j.heliyon.2024.e41397
Received 3 December 2023; Received in revised form 29 November 2024; Accepted 19 December 2024
Available online 20 December 2024

Nanotechnology is currently applied as a tool to focus the darkest avenues of medical sciences through several ways like sensing [5], targeted drug delivery [6], imaging [7], gene delivery systems [8], and artificial implants [9]. Nanotechnology have potential to make significant impact on society and has already being embraced by industrial sectors including information and communication sectors, food technology, energy technology and in medicine and play significant role in reduction of environmental pollution [10]. In nano-science and technology, the concept of safer-by- design is utilized to make sure the designs of nanostructures to avoid any toxic effect on human health. During the fabrication of nanostructures safety and lesser toxicity is ensured for formulating nanomedicines [11].

1.1. Nanoparticles

Nanoparticles hold specific properties due to their extremely small size, shape, surface charge and large surface-to -volume ratio, which determine both physical and chemical attributes (biological, mechanical, thermal and electrical and catalytic properties) compared to their bulk materials [10,12]. It has been reported that green nanomaterials are rapid, faster and ecofriendly source that can be used in different bio-applications [13]. Based upon their fabrication, different categories of nanomaterials were investigated; nanocubes, nanoflower, nanowires, nanotubes and nanohelices [12] and on basis of structures they are classified as regular, crystalline and amorphous [14].

Different researches have been conducted for production of nanostructures and are being studied for multiple biological properties like antimicrobial, anti-leishmanial, antiglucoma, anticancer and antidiabetic properties [14]. Recently, different types of nano-structures are used in the formulation of nanomedicines; neocrystalline, liposomes, micelles, polymers, carbon nanotubes, metal and metal oxides as well as nanobiomolecules [15]. Researchers are now engaged in fabricating different metal and metal oxide nano sized particles with unique structures and functional properties based on their nature, shape, size and synthesis protocol [16,17].

1.2. Different routes for the fabrication of nanoparticles

The biosynthesis of nanostructures using plants, natural polymers, algae and fungi have distinguishing physicochemical properties [18]. There are different routes to fabricate nanostructures such as physical method, (thermal decomposition, electrode position, hydrothermal etc.) and chemical (micro-emulsions, co-precipitation chemical precipitation procedures etc. [19] (Fig. 1). The physical methods generate heterogeneous nanoparticles with high energy consumption and require more than 350 °C which make its synthesis more expensive [20]. The common methods utilized in physical approach of nano sized particles fabrication include electrons spraying, evaporation-condensation, laser ablation and laser pyrolysis [21]. Different nanoparticles have been fabricated through physical method such as gold, silver, lead, copper by successfully utilizing the evaporation condensation methods [22,23].

Chemical methods are more preferable due to less energy requirements during stabilization of nanostructures with high precision in shape, size, dimension, composition and structure [20]. The chemical approaches involve metals, salts, harsh reducing agents and organic solvents comprising of multiple functional groups; thiols and amines that interact with particles surface and stabilize their growth [24,25].

Using chemical routes for the fabrication of nanostructures might lead to generate noxious toxic wastes, which are unfriendly to environment and human health [26]. Also, these methods have more chances of accumulation of nanostructures [27]. Physical

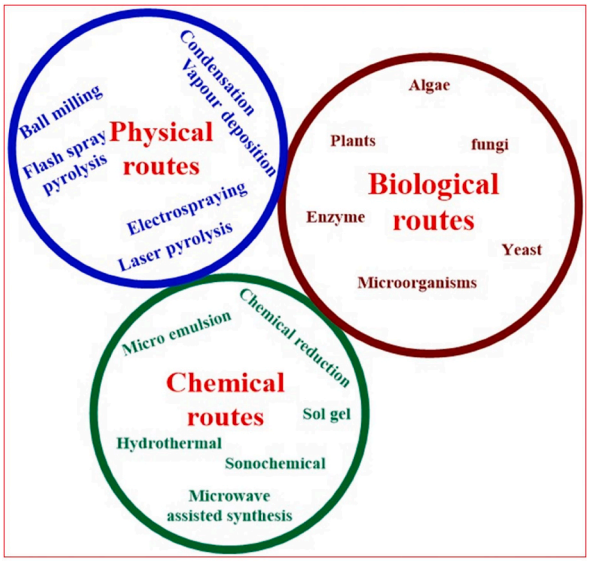

Fig. 1. Different routes for the synthesis of nanoparticles including physical, chemical and biological synthesis methods.

fabrication procedures are also considered as costly due to high energy. Due to high energy demands and noxious waste generation physical and chemical methods were diverted to cheap, ecofriendly and sustainable methods demands [28,29]. Physicochemical routes are not only expensive but also possess environmental and biological hazards. Therefore, scientists are trying to design green methods for the orchestration of nanostructures having modulated size, shape and surface chemistry [30].

Biological fabrication of nanoparticles (utilizing different medicinal plants, bacteria, algae, diatoms and fungi) is one of the advanced area and is considered as non-toxic, greener, cheap and is mostly performed at ambient temperature and pressure [31,32]. Bio-based nanoparticles use bio-safe, ecofriendly, potential for easy scalability, reproducible, in-expensive chemicals from various medicinal plants, microbes and many other natural resources, without the involvement of multiple steps for synthesis and eliminate the use of hazardous chemicals [33,34].

1.3. Medicinal plants and their green chemicals

Medicinal plants extracts are used to made large number of traditional medicines and also provide health security to large segment of world population [35]. A vital portion of the world population is totally dependent on plant based therapies based on the data collected from local community [36]. According to recent research, the natural products are widely used in the formulation of various medicines. The medicinal plants have attractive bioactive compounds and rich phytochemistry to fabricate novel nanomaterials for vast applications (Fig. 2) [37,38]. The green chemicals-based nanoparticle fabrication offers high reproducibility due to the consistent composition of functional groups/phytochemicals that acts as stabilizing and reducing agents, ensuring environment friendly nanoparticle preparation [39]. In this prospective, the green compounds have been found to be involved in biological activities such as antifungal, anticancer, anti-plasmodial and antimicrobial properties [10,40].Different phytochemicals have been collected from the medical plants and has been used for treatment of several diseases in form of drugs and then utilized in the orchestration of nanoparticles [40]. Due to increasing microbial hostility to antibiotics there is an imperious need to develop fresh and pioneering green products based antibacterial agents [10].

1.4. Green fabrication of copper-oxide nanoparticles

In these days, biological synthesis of copper oxide nanoparticles emerged as cost-effective and alluring plate form, the alliance of medicinal plants and metal oxide attracted many scientists to construct nanomaterials with diverse applications. Some recent reports documented CuONPs biological synthesis are already available [41–43]. Recent studies have indicated that copper nanoparticles have excellent anti-microbial activity against *E.coli* and *staphylococcus* species and it's antifungal properties were also reported [43,44].

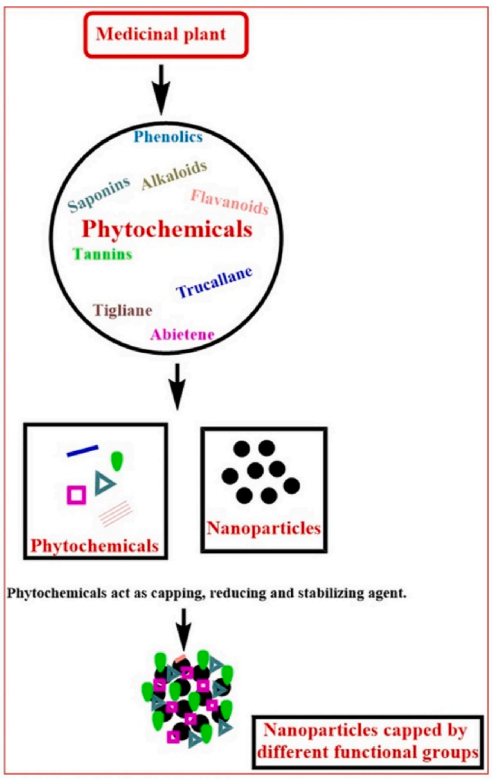

Fig. 2. Green chemicals of in the aqueous extract of the plants which serves as capping, reducing and stabilizing agents.

Previously, various greenly synthesized CuONPs has been notably used for the degradation/decolorization of organic dyes. In several studies, the CuO nanoparticles have been increasingly used in marine antifouling paints and as antimicrobial-antifouling agents in aquaculture industry [45]. Further, the unique nature of CuONPs made them imperative agent for being used in nanomedicine. Despite of significant applications of green CuONPs in biomedicine, their toxic effects elucidated by many researchers working on different invertebrates and vertebrates (especially mammalian cells) is utmost concern for their use for therapeutic and diagnostic purposes [46, 47]. Also, CuONPs are also being reported to generate reactive oxygen species (ROS) in the living cells that always leads to the damage of DNA and cellular organelles. The surface charge, nanomaterial dissolution and size are key factors contributing to toxicity caused by CuONPs [48–50]. So, non-toxicity and biocompatibility are two essential parameters for any nanomaterial that has to be employed in clinical research. The CuONPs has been widely utilized for different nanomedical purposes due of their significant bactericidal and fungicidal properties and are also employed as potential disinfectants against nosocomial infections [51].

The green fabricated copper nanostructures are efficient, ecofriendly, cheap and play a vital role in different biological properties. Copper is an essential microelement present in all living organism with distinct properties; two different redox states (oxidized (Cu^{2+}) and reduced (Cu^{+}) form). It is important for survival and provides important catalytic cofactor in redox chemistry for protein that performs basic protein functions in human body [52,53]. Also, it is a trace element that altered the function and structure of blood and immune cells.

1.5. *Euphorbia serpens (ES)*

Euphorbia serpens, also known as sand mat (as it grows prostately), included in Euphorbiaceae family [54]. The branches of this herb grows (maximum length might be 70 cm) from the base [55]. The greyish green leaves are without any marks, turns to purple color at the end of season, are up to 8 mm long, approximately 3 mm wide small, whole, orbicular, etiolate, oval with notched tip [55]. The glands of inflorescence (cyathium) have thin appendages white in color. The fruit is capsule which is approximately 2 mm in diameter. The seeds are quadrangular, even and soft (≈1–1.5 mm long). Up till now, *E. serpens* is the least explored plant. The phytochemicals discovered so far includes flavonoids, tannins, euphol, abietene, tirucallane, tigliane and saponins. The ethnobotanical surveys and deep literature review have reported the different medicinal values of *E. serpens.* The plant is used for the treatment of diseases, such as skin irritations, and body pain. The plant is used as a cure for microbial illness and snake or scorpion bites. The antifungal, insecticidal, antioxidant propensities of *E. serpens* raw extracts and ES based AgONPs has been reported so far [54,55]. Green synthesis of nanoparticles by utilizing biological extracts is recently attracting a great deal of attention in field of biomedicine due to their efficient and ecofriendly behavior [56,57]. The aim of our current study is to develop green, cost-efficient and simple approach to fabricate *Euphorbia serpens* aqua extract mediated CuO nanostructures. Further, the asynthesized CuO nanoparticles will be characterized followed by diverse biological potentials. Fig. 3 reveals the detailed study mechanism for the fabrication and characterization of *E. serpens* based CuO nanoparticles.

1.6. *Sampling of E. serpens and extraction*

The medicinal plant *Euphorbia serpens* (family: Euphorbiaceae) was selected after ethnobotanical surveys and literature review. The whole plant; root, stem, leaves, flowers of *E. serpens* was identified and was sampled from Jasmine garden, Rawalpindi in month of May and June. The collected plants were rigorously washed using tap water to remove associated debris and dust particles and dried under shade to remove any surface moisture and water content. Fine powder obtained from completely dried plant was used to attain

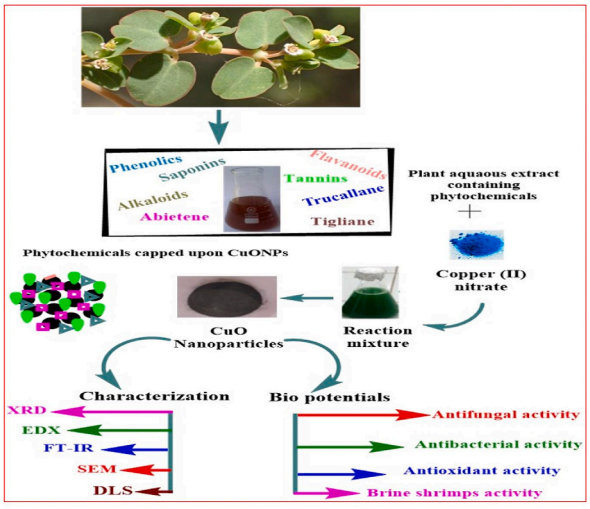

Fig. 3. Detailed step by step study mechanism for the fabrication and characterization of *E. serpens* based CuO nanoparticles.

aquaous plant extract. Approximately, 40 g light green plant powder was mixed with 100 mL ultrapure water. This mixture/suspension was continuously heated at 90 °C and stirred utilizing hotplate for 2 h for acquiring aquaous plant broth of *E. serpens*. The filtration and centrifugation of obtained suspension was carried out in order to separate the unwanted aggregates/accumulates of plant. The resultant light green solution was considered as aqueous broth of *E. serpens* which was further used for the reduction of copper acetate salt.

1.7. Green fabrication of CuO nanoparticles from E. serpens

The fabrication of *E. serpens* based CuONPs was executed following the optimized procedures of [58]. The obtained filtrate was used for the green synthesis of CuONPs. The plant broth was then utilized as a strong reducing, stabilizing and capping agent to convert copper acetate salt to their metallic form. The synthesis of CuONPs was achieved by reducing 2 gm copper (II) acetate (sigma aldrich) precursor salt using 100 mL of *Euphorbia serpens* plant extract. The reaction was performed at 60 °C for 2hr upon hotplate. The resultant reduced material was washed thrice, centrifuged and dried in an oven at 100 °C/3hr. The resultant nanostructures was then grounded and dried again for 2hr at 100 °C. The material obtained was regarded as CuONPs. After accomplishing room temperature, the obtained *E. serpens* based CuONPs was stored in 2 mL eppendorf tubes, parafilmed and were stored in dry and dark place. The *E. serpens*-CuONPs were used for detailed physical characterization. Also, synthesized *E. serpens*-CuONPs were investigated for multiple bio-applications.

1.8. Physical characterization for E. serpens-CuONPs

Different spectral and microscopic studies were utilized to examine the electro-optical, physical, thermal and chemical studies of greenly synthesized *E. serpens* orchestrated CuONPs. Following are the techniques utilized for the characterization of *E. serpens*-CuONPs.

1.8.1. Ultraviolet–visible spectroscopy

The bio-reduction of the copper (II) oxide salt into nano metal particles was observed via ultraviolet–visible spectrophotometer. The copper ions reduction was observed by a simple change in the color of the plant extract from light brown to greenish-black. To study the spectrum obtained via ultraviolet–visible spectroscopy, a stock solution of the copper oxide nanostructures (5 mg/5 mL) was prepared and properly sonicated using Sonicator machine. To achieve this purpose, the CuONPs were scanned and absorbance was measured in the range of 200 nm–600 nm using ultravoilet–Vis spectrophotometer (Shimadzu, Tokyo, Japan) using a quartz cuvette. The quartz cuvette (approximately 1 cm length) was utilized for measuring the absorbed spectra of light with the reference to ultrapure water.

1.8.2. Crystal analysis of E. serpens-CuONPs

The phase purity, structural analysis and crystallinity of *E. serpens*-CuO nanostructures were determined by the planes of X-rays differection patterns and 2 theta points. The differectiometer was well-equipped with Cu/K radiations ($\lambda = 1.5406$ °A) utilized 40 kV voltage and current of approximately 30 mA. The mean crystal sizes were calculated using XRD spectra by Scherer approximation.

1.8.3. Surface morphology via SEM

Surface morphology and actual particle size of the prepared CuONPs was investigated utilizing SEM (EM-NOVA-FEI-SEM-450 equipped with EDX detector). To fix CuONPs and to prevent their spread, many drops of the solution were carefully applied to the carbon tape covered-stub. The microscopic study was accomplished via SEM for the illustration/analysis of shape, size, stability and nature (accumulation/agglomeration/dispersed) of *E. serpens*-NiONPs. The sample was placed on the SEM machine and images of 50 Kx magnifications were taken. The EDX spectral studies determined the presence of adsorbed functional groups on the surface of NPs and to confirm its synthesis.

1.8.4. Zeta potential, PDI and dynamic size (d-nm)

For understanding the interactions between nanostructures and solutions (water molecules), the evaluation of surface/interface charge and the migration of nanostructures, DLS was carried out. The zeta size and potential studies were performed by DLS utilizing ζ-sizer equipment's (Malverns UK).

1.8.5. Fourier transmission-Infra red spectroscopic studies

Fourier transmission-Infra red spectroscopic studies was carried out to identify the several bioactive functional groups up on the surface of NPs directly involved in the stabilization and reduction of *E-serpens*-CuONPs. To achieve this purpose, CuONPs was scanned among the range of 500–4500 cm^{-1} and different spectra were recorded to determine various functional groups present upon the surface of ES- CuO nanoparticles participated in the fabrication and enhancing bio-potentials of nanomaterials.

1.9. Biological potentials of E. serpens-CuONPs

1.9.1. Cytotoxic activity of E. serpens-CuONPs

Six dilutions at concentration of 1000, 500, 250, 125, 63, 32 μg per ml from stock solution were used as testing samples against

brine shrimp lethality assay. All concentrations were taken in glass vials and solvent was evaporated. Hatching of eggs (*Artemia salina)* was carried out in a bi-partitioned tray providing artificial sea water as a medium of their hatching. Sea water was prepared by adding sea salt (3.8 g) into 1 L of distilled water and dissolved it by continuous stirring. Hatching tray consists of a small and large section. A pinch of eggs was taken and added to larger compartment and covered it with aluminium foil while other section remain uncovered for illumination under light source. After incubation period of 1–2 days hatched nauplii moved toward light source. Nauplii (30) were collected via micro pipette and transferred to each vial containing 5 ml of sea water along with evaporated ES- CuONPs sample. Vial for positive control included vincristine sulphate, sea water and nauplii, while, DMSO, sea water and nauplii were maintained as negative control. After incubation of 24 h dead nauplii were counted in all vials and percent mortality was compared with positive control. The BSCA experiment was performed in triplicate and IC_{50} values were determined using graph prism pad software.

1.9.2. Antiradical potency of E. serpens-CuONPs

2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay, (TAC) total antioxidant capacity and (RPA) reducing power assay were used for evaluation of antioxidant potential of ES- CuO NPs. A procedure reported by Sarwar et al. [59] was employed for determining the potential of all samples/concentrations against DPPH reagent with minor changes. The Microplate reader (Bioteck), DPPH (2, 2-diphenyl-1-picrylhydrazyl) in solid form purchased from Sigma (USA), DMSO (VWR, BDH, chemicals), ascorbic acid Sigma (USA), ethanol and ES- CuONPs are required for performing experiment. For preparing stock solution, 1 mg from ES based CuONPs was taken and dissolved in DMSO (1 ml) via sonication. The DPPH solution (0.004 %) was prepared in methanol. A 96 wells plate was used for DPPH assay. Each concentration (10 μl) to be tested was added to 190 μl of DPPH in respective well in order to make the final volume of 200 μl. Ascorbic acid (Sigma Aldrich, USA) was taken as positive standard and DMSO was utilized as negative control. Then reaction mixture was incubated for half an hour in dark at room temperature. Those wells that changed their color and turned yellow were observed. Changing color determines ES-CuONP's oxidation potential. Absorbance of testing samples was measured using microplate reader at wavelength of 515 nm. Following formula was used for calculation of free radical scavenging activity at different doses.

$$\text{Percent scavenging of sample} = \frac{\text{Abs(C)} - \text{Abs (test sample)}}{\text{Abs(C)}} \times 100$$

A procedure explained by Dridi et al. [60] was utilized for the assessment of TAC of ES- CuONPs. The ascorbic acid (Sigma Aldrich, USA), ammonium molybdate (Sigma Aldrich, USA), sulfuric acid, sodium phosphate (Sigma Aldrich, USA) and ES- CuONPs are required for performing experiment. Further, for preparation of stock solution a quantity of 1 mg from all ES- CuO NP's was taken and dissolved in 1 ml of DMSO. The DMSO lacking ES- CuONPs was used as negative standard while ascorbic acid as positive standard. The testing samples were assessed for total antioxidant capacity by taking 100 μl stock solutions (for each sample) and mixing it with 900 μl of reagent solutions that comprised of 28 mM sodium phosphate, 0.6 M sulfuric acid and 4 mM ammonium molybdate. Resulting mixtures were then incubated for 90 min at 95 °C. After cooling, their incubation was carried out at room temperature. Absorbance of each testing dose was recorded via microplate reader at 630 nm. DMSO (100 μl) was used as blank and different concentrations of ascorbic acid were used for calibration curve. Consequently, total antioxidant capacity was expressed as mg/g dose equivalents to ascorbic acid.

Furthermore, procedure outlined by Gecer et al. [61] was used for analysis of reduction potential of ES- CuONPs samples to be tested. The requirement of TRP includes gallic acid, solution of potassium ferricyanide (1 %)(Sigma Aldrich, USA), ferric chloride solution (0.1 %), 0.2 M PB (pH 6.6) (Sigma Aldrich, USA), trichloroacetic acid (10 %) (Sigma Aldrich, USA) and ES- CuONPs. The ES-CuONPs (1 mg) was dissolved in 1 ml of DMSO for preparation of stock solutions. For positive control gallic acid at final concentration of 100 μg/ml was used for evaluation of total reduction potential of ES- CuONPs. Total reducing power was evaluated by taking stock solution (100 μl) (for each dose) and mixing it with 1 % solution of potassium ferricyanide (250 μl) and phosphate buffer (200 μl). Mixtures were then incubated at 50 °C/20 min. Then trichloroacetic acid (200 μl) was added after for acidification of reaction mixtures. After that they were centrifuged for 10 min at 3000 rpm. Supernatant was taken and 0.1 % $FeCl_2$ solution was added. Later on, 200 μl from reaction mixtures was transferred to a 96 wells plate. Absorption was measured on microplate reader at 630 nm. Gallic acid was kept as positive standard and reduction potential was expressed as gallic acid Eq.mg/g of ES- CuONPs.

1.9.3. Antimicrobial studies of E. serpens-CuONPs

Kirby-Bauyer or disc-diffusion test was used as outlined by Abbasi et al. [62] for assessment of bactericidal and fungicidal potential of ES-CuONPs nanostructures. Five cultures (*E. coli* ATCC#: 15224, *P. aeruginosa* ATCC#: 9721, *S. aureus* ATCC#: 25923, *K pneumonia* ATCC#:4617 and *B. subtilis* ATCC# 6633*)* supported on SDA medium (Sigma Aldrich, USA), ES-CuONPs, discs of Whatman's filter paper # 1 (autoclaved), petri plates, DMSO, (Merck Germany), vernier caliper and Demeclocycline are requirement of bactericidal activity. However, Four cultures (*Candida albican* FCBP#: 478, *Mucor* racemosus FCBP#: 030, *Aspergillus niger* FCBP#: 0918, (*A. flavus* FCBP#:64) and *Fusarium solani* FCBP# 291*)* supported on SDA medium, ES-CuO nanoparticles, discs of Whatman's filter paper # 1 (autoclaved), petri plates, DMSO, (Merck Germany), vernier caliper and terbinafine are required for fungicidal activity.

The ES-CuONPs were exploited for potential antifungal and antibacterial assays. In antibacterial test, agarose solid media (Sigma Aldrich, USA) was used for cultivation/spreading of different bacterial species on different plates while in fungicidal assay, SDA agar was used for this purpose. Filter discs dipped and dried with different doses of ES-CuONPs were placed at distant positions upon media. The petriplates with spreaded bacteria/fungus (depending upon assay) upon media and dose laden discs were incubated at 37 °C/24 h (bactericidal assay) and 25 °C/24 h (fungicidal assay) respectively. Amphotericin B and Oxytetracycline were exploited as controls in

fungicidal and bactericidal assay respectively. The bactericidal and fungicidal affects were recorded while assessing inhibitory zones around the discs. MIC values for both assays were calculated.

1.10. Phyto-synthesis of Euphorbia serpens-CuONPs

1.10.1. Color monitoring and ultraviolet–visible spectrophotometery

After the successful fabrication of CuONPs, the change of color of plant broth from light brown to dark green solution was observed as shown in Fig. 4. Color change is the first indication of fabricated CuONPs. Furthermore, the maximum plasmon resonance of the solution was recorded at 373 nm (Fig. 5). Our results of ultraviolet–visible spectrophotometery are congruent to standard electron-spectrometric peaks for CuONPs. The result of optical analysis is in line with the studies of Kumar et al. [63] and Renuga et al. [64] in which greenly orchestrated CuONPs (*Brassica oleracea* var. *italic* - CuONPs and *Rubus glaucus* - CuONPs respectively) were thoroughly observed via various characterization methods and different *invitro* and *invivo* bio-properties were also studied. The plant extract of *Euphorbia serpens* contain flavonoids, tannins, euphol, abietene, tirucallane, tigliane and saponins that might have active role in the successful fabrication and stability of asynthesized nanoparticles.

1.10.2. Surface morphology of Euphorbia serpens - CuONPs

To investigate the interface morphology SEM was used. The SEM of *Euphorbia serpens* CuONPs with the 80,000x magnification and is presented in Fig. 6 (A). The results in fig. indicate accumulated/agglomerated nano sized particles with huge surface area and mono to poly-dispersed nature of *Euphorbia serpens* orchestrated CuONPs particles.

1.10.3. Elemental analysis for Euphorbia serpens orchestrated CuONPs

The energy dispersive (EDX) x.ray spectrum was analyzed to study the availability/presence of different elements in our *Euphorbia serpens* based CuONPs. The intense signals was perceived at 0.2 KeV, 0.4 KeV, 0.9 KeV, 8.1 KeV and 8.9 KeV corresponds to the presence of C, O and Cu elements. The carbon signal (0.2 KeV) is attributed to the supporting grid (made of C), however other signals are attributed to the presence of O and Cu. The EDX spectral pattern for *Euphorbia serpens* mediated CuONPs is shown in Fig. 6 (B).

1.10.4. Crystallinity of greenly orchestrated Euphorbia serpens – CuONPs

The differection signals/patterns of the *Euphorbia serpens* - CuONPs were explored via X.ray differectiometer and the resultant pure crystalline pattern is illustrated in Fig. 7 (A). The sharp peaks of *Euphorbia serpens* - CuONPs are observed at 2 theta values (with referred planes) of 32.41 (110), 36.63 (002, 111), 38.22 (111, 200), 47.72 (202), 54.72 (020), 57.49 (202), 60.39(113), 68.05 (311), 69.63 (220), 74.13 (311) and 76.63 (004). The sharp peaks patterns are in match with standard PDF code of ICSD 45–0937. The average/mean size of *Euphorbia serpens* - CuONPs was recorded as 20.39 nm**.** The miller indexation calculated via Scherrer-equation ($L = K\lambda/\beta.Cos\theta$) is also depicted in Fig. 7 (B). Our diffectiometric patterns results were compared with mentioned ICSD database for CuONPs. No additional characteristic diffectiometric peak for any additional product was observed which signifies the purity of greenly fabricated CuONPs. The results of XRD differectiometric pattern are in agreement to the reports of [65,66]. Further, the XRD pattern also indicates that the asynthesized green procedure used for fabrication of *Euphorbia serpens* - CuONPs is controllable, repetitive, pure and has the capability of reducing and capping defects.

1.10.5. Fourier transmission-Infra red spectroscopic analysis

The fourier transmission-Infra red spectroscopic analysis was performed to study the chemical composition of interface/surface of *Euphorbia serpens* orchestrated CuONPs (Fig. 8). The highest/intense transmittance signals of fourier transmission-Infra red spectra was observed at 545.17, 657.43, 1025.26, 1729.59, 3005.93, 3288.45 and 3579.43 cm^{-1}. The intense peaks at 545.17 and 657.43 are assigned to the presence of Cu–O bond. The transmission peak at 1025.26, 1729.59, 3005.93, 3288.45 and 3579.43 cm^{-1} are

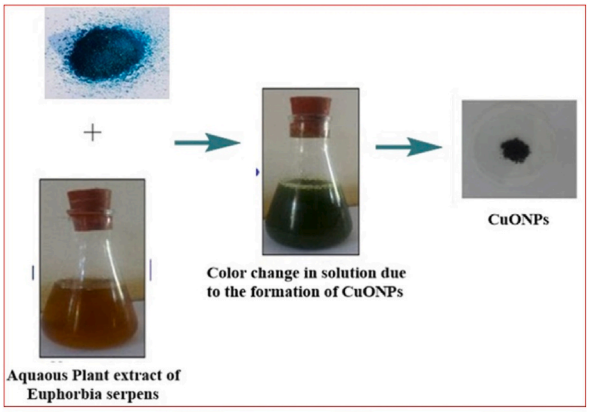

Fig. 4. Color change of aqueous plant extract after the addition of copper acetate salt.

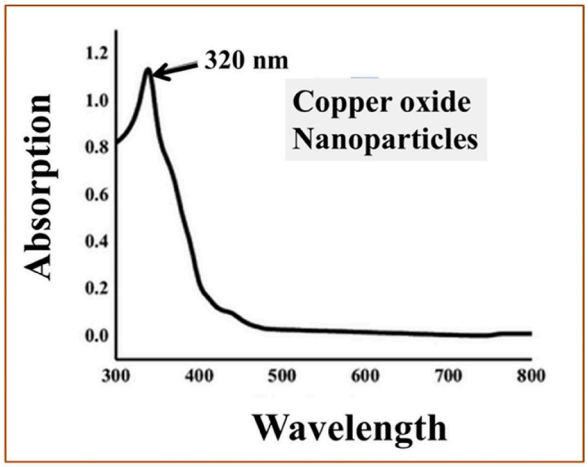

Fig. 5. UV–visible spectroscopy for *Euphorbia serpens* mediated copper oxide nanoparticles.

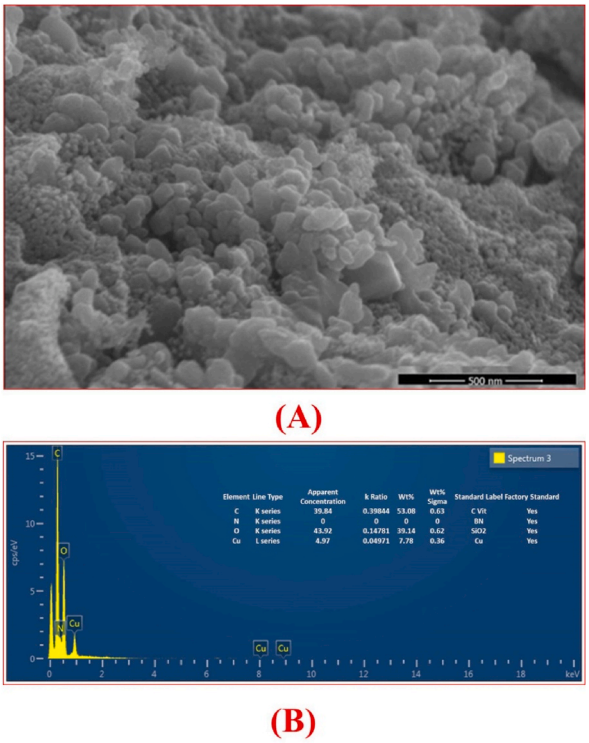

Fig. 6. (A) Scanning electron micrograph for greenly synthesized CuONPs and (B) EDX spectral analysis for ES-CuONPs illustrating pure CuONPs.

attributed to alkyl amine, carboxyl acid, alkenes, –OH group for alcohols and amine primary respectively. The adsorption/attachment of functional groups presents upon the surface of ES- CuO nanoparticles have participated in the orchestration of nanostructures and also they might have potential role in increasing the bio-properties of greenly fabricated nanomaterials.

1.10.6. Zeta potential, PDI and size

For recording the hydro-dynamic size, index of poly-dispersity and ζ-potentials, DLS was performed. The ζ-size of approximately 123.4 d nm has been recorded. This size is truly elucidating the poly-dispersed nature with the aggregation between orchestrated nanoparticles. The ζ-potential of *Euphorbia serpens* orchestrated CuONPs was noted to be positive (i.e. +32.1) which depicts true physical stability of our *Euphorbia serpens* orchestrated CuONPs. The PDI of asynthesized CuONPs was recorded as 1 which clearly indicates the dispersed/scattered distributions of the pore size. The spectroscopic results of z-potential, PDI and z-size are illustrated in Fig. 9 (A&B).

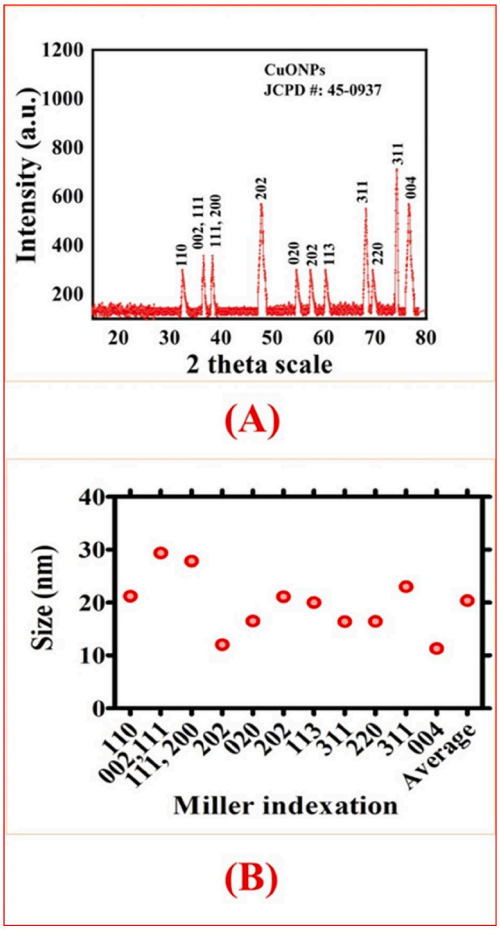

Fig. 7. (A) XRD pattern for ES-CuONPS and (B) Miller indexation via Debye-scherrer's equation.

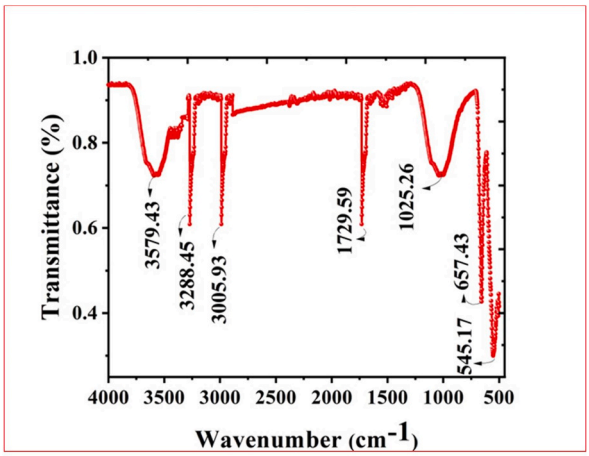

Fig. 8. FT-IR pattern for ES-CuONPs.

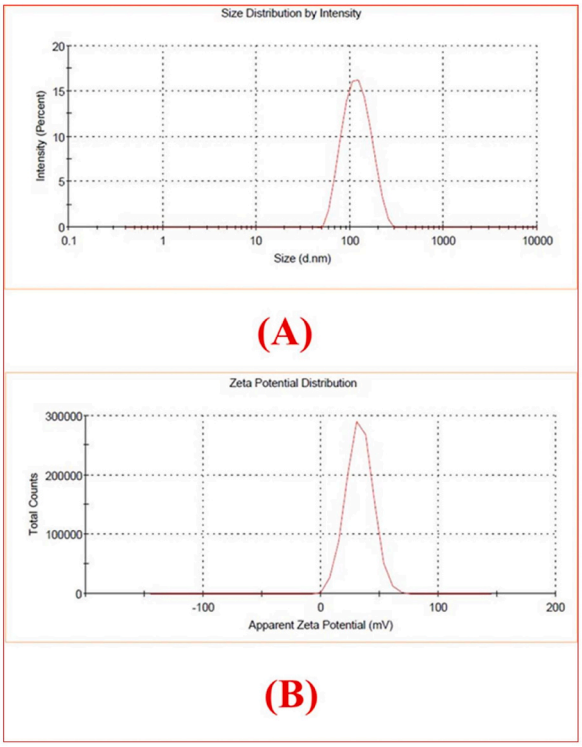

Fig. 9. DLS studies for ES-CuONPs and (A) Zeta size; Positive zeta Potential for ES-CuONPs.

1.11. Evaluations of bio-activities of Euphorbia serpens mediated CuONPs

1.11.1. Antioxidant potentials

1.11.1.1. Reducing power assay. Fig. 10 illustrates TRP property of different doses of *Euphorbia serpens*- CuONPs in terms of Gallic-acid Eq.mg/g of nanoparticles calculated at 200-1 μg/ml. In our study, highest efficiency (88.12 %) in terms of reducing power was recorded at the highest concentration (200 μg/ml) and lowest (4.43 %) at 1 μg/ml. All the tested doses have manifested good TRP results which clearly signify that our *Euphorbia serpens*- CuONPs have excellent TRP potential. Our results of TRP studies are congruent to previously published reports of Vinothkanna et al. [67] and Araya-Castro et al. [68].

1.11.1.2. Total antioxidant capacity (TAC). The TAC content for different doses of *Euphorbia serpens* - CuONPs was investigated by phosphomolybdate procedures and was presented/expressed as ascorbic acid equivalent mg per g of nano sized particles suspension as illustrated in Fig. 10. The highest TAC (86.62 %) was observed at highest dose (200 μg/mL). All the tested concentrations have exhibited potential TAC property which have revealed our asynthesized CuONPs have excellent reducing potential. The *Euphorbia serpens* - CuONPs showed concentration dependent response. The TAC analysis of the current study is congurent with the previously

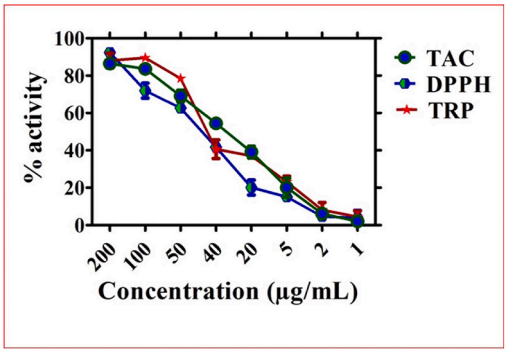

Fig. 10. Antioxidant properties for greenly synthesized CuONPs including DPPH activity, TRP assay and TAC activity.

published data on *Lactuca sativa, Tamarindus indica, Hibiscus rosa-sinensis*, *Azadirachta indica*, *Moringa oleifera* and *Murraya koenigii* based CuONPs [69,70].

1.11.1.3. DPPH assay. Free radical scavenging capabilities of *Euphorbia serpens* - CuONPs are shown in Fig. 10. The maximum antiradical property of 92.22 % was recorded at higher concentration of 200 μg/mL. Ascorbic acid (Sigma Aldrich, USA) being a powerful radical scavenger was used for comparison in this assay. All concentrations of *Euphorbia serpens* - CuONPs exhibited excellent scavenging property. The results signify that our *Euphorbia serpens* - CuONPs are potential scavenger and are able to play their significant role as antioxidant agents. The previous reports of Santhosh Kumar & Shanmugam et al. [71] and Sepasgozar et al. [72] also revealed that greenly fabricated CuONPs using *Magnolia champaca* and *Achillea nobilis* have shown excellent scavenging potentials. The results of this study are in agreement with aforementioned studies.

1.12. Bactericidal activity

The pathogenic bacterial strains are considered to be causative agent of many common and deadly diseases. Conventionally, many green broth based drugs were used against these agents for curing various diseases. However, the frequent/continuous uses of natural/synthesized drugs lead to increasing resistances in pathogenic bacterial species. For this reason, new formulations are under exploration by the scientific and research communities. In this aspect, bactericidal property of *Euphorbia serpens*- CuONPs was evaluated by performing antibacterial activity as shown in Fig. 11 (A). The dose range used was from 1500 to 46.875 μg/mL. Susceptibility of each concentration/dose of *Euphorbia serpens*- CuONPs (dose Range: 1500–46.874 μg/mL) was studied using selected five bacterial strains by disc diffusion method. The *Euphorbia serpens*- CuONPs showed dose dependent responses. According to our results, the highest concentration exhibited higher activity against all bacterial strains with clear zone of inhibition (ranging from 15 to 26 ± 1.5–2.01 mm). The maximum resistance was observed in case of *K.pneumonia and P. areuginosa* (MIC: 187.5 μg/mL), however, *B. subtilis* (MIC: 46.875 μg/mL) was found to be sensitive among all bacterial strains. The MIC values in case of *E. Col and S. aureus* was found to be 93.75 μg/mL. Previously, Kumar et al. [73] reported the bactericidal analysis with similar strains using *Aloe vera* orchestrated CuONPs. In their studies, they have reported *K. pneumonia and P. areuginosa* as resistant and *B. subtilis* as sensitive strain. The difference between the inhibitory zones of Kumar et al. [73] and current study might be because of the difference of green chemicals of two different plants. Furthermore, the results of the present studies revealed strong correlations with the findings of Altikatoglu et al. [74] and Tavakoli et al. [75] where they have used the *Ocimum bacilicum* and *Aloe vera* orchestrated CuONPs against similar bacterial strains and have found almost same results. Over all, all tested doses have shown significant antibacterial property with exception of 93.75 and

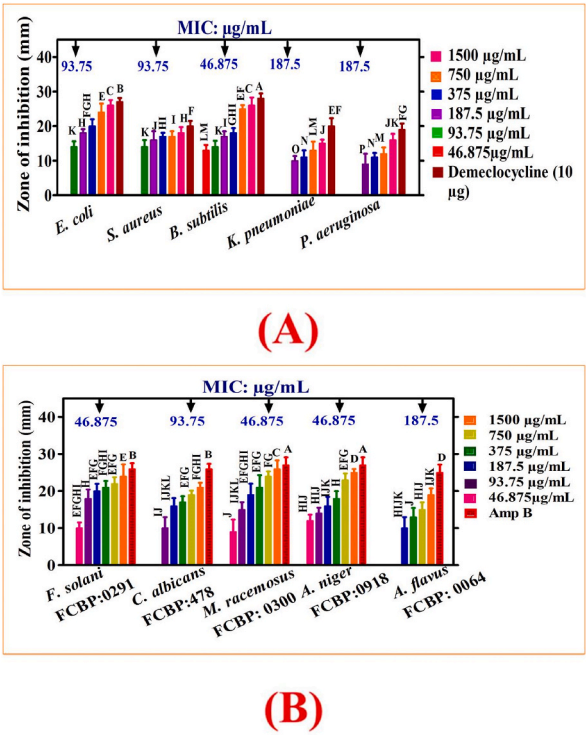

Fig. 11. Various antimicrobial activities of ES-CuONPs. Data represents the mean of three replicates and each alphabet indicates significance at $p < 0.05$ (A) MICs values of different doses of ES-CuONPs against various bacterial strains. (B) MICs values of different doses of ES-CuONPs against different fungal strains.

46.875 μg/mL in cases of *P. aeruginosa* and *K. pneumonia* and 46.5 μg/mL in case of *S.aureus* contradictory to positive standard (Demeclocycline) that has shown potential antibacterial activity against all selected bacterial strains. Over all, the results of the current study depicted excellent antibacterial properties of *Euphorbia serpens* - CuONPs. The interaction of NPs with the cell membrane receptors is highly size dependent. The mechanism of action of *Euphorbia serpens* – CuONPs is given in Fig. 12.

1.13. Antifungal properties

Harmful fungi are considered to be root cause of several common diseases. Traditionally many green chemical/broths/raw medicinal plants base drugs are employed against fungal infections. The scientific community is continuously working on new formulations due to increasing resistance against formulated drugs. In this regard, fungicidal potential of *Euphorbia serpens* orchestrated CuONPs was evaluated by performing antifungal activity. Susceptibility of each concentration/dose of *Euphorbia serpens* - CuONPs (Conc. Range: 1500–46.874 μg/mL) against five fungal strains was assessed by disc diffusion method. The *Euphorbia serpens* – CuONPs have shown concentration dependent respond. From Fig. 11 (B) it can be clearly seen that highest dose revealed highest activity against all selected fungus strains with clear inhibitory zone of 19–26 ±1–3.42 mm. The maximum resistance against all doses was observed in case of *A. flavus* (MIC: 187.5 μg/mL), however, *M. racemosus* (MIC: 46.875 μg/mL) was observed to be sensitive among all fungal strains. Previously, Vanathi et al. [76] reported the antifungal analysis with similar strains (*M. racemosus* excluded) using *Eichhornia crassipes* mediated CuONPs. In their studies, they have reported *A. flavus* as resistant strain and *F. solani* as sensitive one. The difference between the inhibitory zones of Vanathi et al. [76], and current study might be due to the use of different plant based nano sized particles. Also, our results exhibits strong correlations with the findings of Huang et al. [77] and Shammout & Awwad [78] where they have used the *Ginkgo biloba* and Bougainvillea orchestrated CuONPs against similar fungal strains and have found almost same results. Over all, all tested doses have shown significant antifungal property with exception of 93.75 and 46.875 μg/mL in case of *A. flavus* and 46.5 μg/mL in case of *C. albicans* contradictory to the selected positive standard (AmpB) that has shown strong antifungal activity against all selected fungus strains. Our results exhibited strong fungicidal potential of *Euphorbia serpens* orchestrated CuONPs. Fig. 2 describes the antibacterial mechanism of CuO NPs. A brief insight on the antibacterial and antifungal applications of CuO NPs is given in the following subsections.

1.14. Brine shrimps cytotoxicity assay (BSCA)

For the investigation of cytotoxicity and to confirm their safety level, the nascent larvae of brine shrimps were exploited. The different doses of *Euphorbia serpens* based CuONPs was studied for this test. Our greenly fabricated nanostructures fall under the category of good cytotoxic. The IC_{50} values of 32.88 μg/ml have confirmed the highest percent mortality for *Euphorbia serpens* based CuONPs as shown in Fig. 13 and 14. The *Euphorbia serpens* based CuONPs nanoparticles revealed dose dependent responses. The minimum inhibition was observed to be 17 % at the concentration of 3.91 μg/ml. The maximum recorded inhibition was 82 % at the highest dose of 500 μg/ml. The results of brine shrimp lethality/mortality assay are in agreement to previous studies [79,80]. On the whole, smaller nanostructures contain relatively larger surface area as compared to larger ones. For this reason, interactions of nanoparticles increases with biological elements/molecules, consequently triggering more toxicity (see Fig. 14).

2. Conclusion

In present study copper oxide nanoparticles are fabricated following green route while exploiting the green broth of *Euphorbia serpens.* Different bio-activities; antioxidant, antifungal, cytotoxicity and antibacterial assays of *Euphorbia serpens* - CuONPs were carried out. *Euphorbia serpens* - CuONPs have revealed strong anti-radicle activities. Furthermore, the greenly fabricated nano sized particles manifested excellent fungicidal and bactericidal properties. Also, *Euphorbia serpens* - CuONPs have shown excellent IC_{50} values against the larvae's of brine shrimps. On the whole, it can be concluded that *in vitro* antioxidant, antifungal, cytotoxic and antibacterial properties investigated in the present study presented the scientific basis and elaborated the various potential aspects of the particles under study that possibly be effective against several pathogens and can play vital role in defense mechanism. Our study is pioneer and the work should not be limited only to the targeted nanostructures, targeted plants/plant parts. The extended studies on the toxicology and biocompatibility of MNPs are needed. Our research has successfully documented the biological functions of greenly orchestrated NPs *in vitro*. Future, studies should focus on various *in vivo* experiments employing different animal models. Also, the formulations of multi-organic functional groups and biometallic NPs with regulated/balanced surface chemistry is worthy for the consideration.

CRediT authorship contribution statement

Sana Idrees: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Banzeer Ahsan Abbasi:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Formal analysis, Data curation, Conceptualization. **Javed Iqbal:** Validation, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis. **Mohsin Kazi:** Writing – review & editing, Project administration, Investigation, Funding acquisition, Data curation.

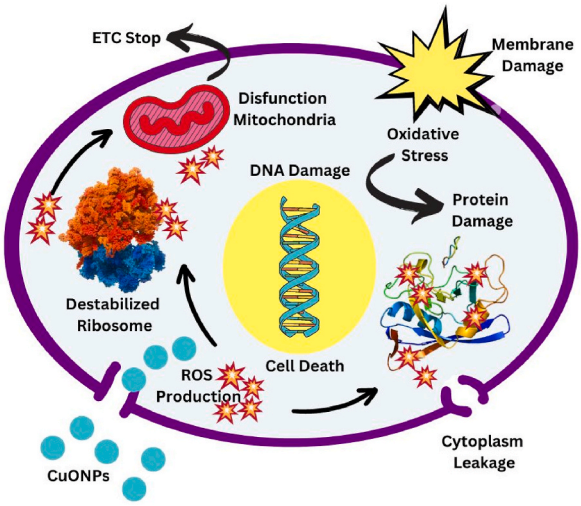

Fig. 12. Diagrammatic representation of the bactericidal mechanism of CuONPs as reported in literature.

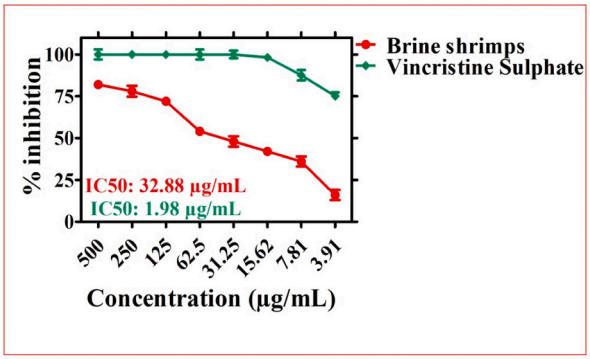

Fig. 13. Brine shrimps cytotoxicity assay for *Euphorbia serpens*-CuONPs.

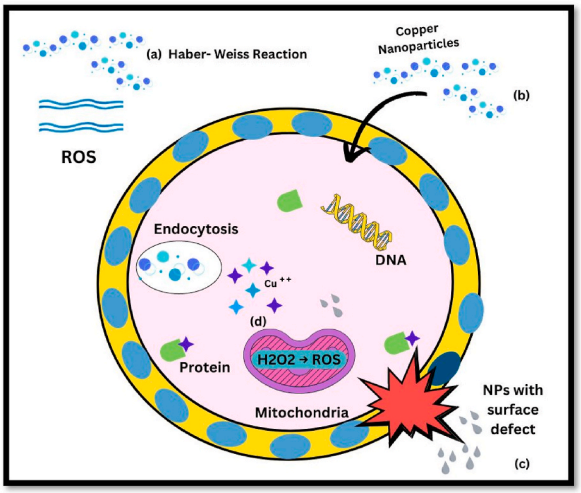

Fig. 14. Diagrammatic illustration of cytotoxicity of CuONPs as reported in literature. (a) The ROS generated outside the cells can easily enter into the cell followed by their interference with DNA that results in the genotoxicity. (b) CuONPs penetrate into the cell by receptor mediated endocytosis, followed by their entry into lysosomes, where Cu++ dissolution occur. (c) Surface defects in CuONPs can result in breaking the cellular membrane. (d) The generated ions interfere with proteins and mitochondria where they disrupt its function by synthesizing further ROS.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP2023R301), King Saud University, Riyadh, Saudi Arabia.

References

[1] A. Abbasi, K. Ghorban, F. Nojoomi, F. Dadmanesh, Smaller copper oxide nanoparticles have more biological effects versus breast cancer and nosocomial infections bacteria, Asian Pac J. Cancer. Prev 22 (2021) 893–911.
[2] S. Khan, S. Mansoor, Z. Rafi, B. Kumari, A. Shoaib, M. Saeed, F. Shakeel, Review on nanotechnology: properties, applications, and mechanistic insights of cellular uptake mechanisms, J. Mole. Liq. 348 (2022) 118008.
[3] A. Dehnoee, R. Javad Kalbasi, S. Zangeneh, M.R. Delnavazi, A. Zangeneh, One-step synthesis of silver nanostructures using *Heracleum persicum* fruit extract, their cytotoxic activity, anti-cancer and anti-oxidant activities. Micro, Nano Lett. 18 (2023) e12153.
[4] A. Dehnoee, R. Javad Kalbasi, M.M. Zangeneh, M.R. Delnavazi, A. Zangeneh, Characterization, anti-lung cancer activity, and cytotoxicity of bio-synthesized copper nanoparticles by *Thymus fedtschenkoi* leaf extract, J. Cluster Sci. 35 (2024) 863–874.
[5] H. Altug, S.H. Oh, S.A. Maier, J. Homola, Advances and applications of nanophotonic biosensors, Nature Nanotechnol 17 (2022) 5–16.
[6] Y. Li, N. Li, W. Jiang, G. Ma, M.M. Zangeneh, In situ decorated Au NPs on pectin-modified Fe3O4 NPs as a novel magnetic nanocomposite (Fe3O4/Pectin/Au) for catalytic reduction of nitroarenes and investigation of its anti-human lung cancer activities, Int. J. Biol. Macromol. 163 (2020) 2162–2171.
[7] L. Ma, A. Ahmeda, K. Wang, A.R. Jalalvand, K. Sadrjavadi, M. Nowrozi, X. Wang, Introducing a novel chemotherapeutic drug formulated by iron nanoparticles for the clinical trial studies. App, Organomet. Chem. 36 (2022) 4996–5041.
[8] G. Wang, A. Ahmeda, Z. Malek, S. Mansooridara, A. Zangeneh, M.M. Zangeneh, Chemical characterization and therapeutic properties of *Achillea biebersteinii* leaf aqueous extract synthesized copper nanoparticles against methamphetamine-induced cell death in PC12: a study in the nanotechnology and neurology fields, Appl. Organometal. Chem. 5 (2020) 971–1012.
[9] J. Park, L. Chi, H.Y. Kwon, J. Lee, S. Kim, S. Hong, S. Decaffeinated, Green tea extract as a nature-derived antibiotic alternative: an application in antibacterial nano-thin coating on medical implants, Food Chem. 383 (2022) 132399.
[10] Q. Liu, Guan, R. Song, X. Zhang, S. Mao, Physicochemical properties of nanoparticles affecting their fate and the physiological function of pulmonary surfactants, Acta Biomater. 140 (2022) 76–87.
[11] A. Ahmeda, M.M. Zangeneh, A. Zangeneh, Green Synthesis and Chemical Characterization of Gold Nanoparticle Synthesized Using *Camellia Sinensis* Leaf Aqueous Extract for the Treatment of Acute Myeloid Leukemia in Comparison to Daunorubicin in a Leukemic Mouse Model, vol. 34, 2020 e5290.
[12] Y. Wang, S. Dong, X. Li, X. Zhang, Synthesis, properties, and multifarious applications of SiC nanoparticles: a review. Cer, Int. 48 (2022) 8882–8913.
[13] A. Saxena, R.M. Tripathi, F. Zafar, P. Singh, Green synthesis of silver nanoparticles using aqueous solution of *Ficus benghalensis* leaf extract and characterization of their antibacterial activity, Mat. Lett. 67 (2012) 91–94.
[14] B. Javed, N.I. Raja, A. Nadhman, Z.U.R. Mashwani, Understanding the potential of bio-fabricated non-oxidative silver nanoparticles to eradicate Leishmania and plant bacterial pathogens, Appl. Nanosci. 10 (2020) 2057–2067.
[15] A. Curcio, A. Van de Walle, C. Péchoux, A. Abou-Hassan, C. Wilhelm, In vivo assimilation of CuS, iron oxide and iron oxide@ CuS nanoparticles in mice: a 6-month follow-up study, Pharmaceutics 14 (2022) 179.
[16] C. Yuan, H.B. Wu, Y. Xie, X.W. Lou, Mixed transition-metal oxides: design, synthesis, and energy-related applications, Angewandte. Chemie. Int. Edition. 53 (2014) 1488–1504.
[17] A.M.E. Shafey, Green synthesis of metal and metal oxide nanoparticles from plant leaf extracts and their applications: a review, Green Process. Syn. 9 (2020) 304–339.
[18] S. Bhatia, Natural Polymer Drug Delivery Systems: Nanoparticles, Plants, and Algae, Springer, 2016, pp. 81–88.
[19] M.C. Uribe-López, M.C. Hidalgo-López, R. López-González, D.M. Frías-Márquez, G. Núñez-Nogueira, D. Hernández-Castillo, M.A. Alvarez-Lemus, Photocatalytic activity of ZnO nanoparticles and the role of the synthesis method on their physical and chemical properties, J. Photochem. Photobiol., A: Chem 404 (2021) 112866.
[20] R. Vishwanath, R, B. Negi, Conventional and green methods of synthesis of silver nanoparticles and their antimicrobial properties, Curr. Res. Green. Sustain. Chem 4 (2021) 100205, 2021.
[21] S.A. Mazari, E. Ali, R. Abro, F.S.A. Khan, I. Ahmed, M. Ahmed, A. Shah, Nanomaterials: applications, waste-handling, environmental toxicities, and future challenges–A review, J. Environ. Chem. Eng. 9 (2021) (2021) 105028.
[22] M.I. Din, R. Rehan, Synthesis, characterization, and applications of copper nanoparticles, Anal. Lett. 50 (2017) 50–62.
[23] A. Hyder, J.A. Buledi, M. Nawaz, D.B. Rajpar, Y. Orooji, M.L. Yola, A.R. Solangi, Identification of heavy metal ions from aqueous environment through gold, Silver and Copper Nanoparticles: an excellent colorimetric approach, Env.Res. 205 (2022) 112475.
[24] C.B. Ong, L.Y. Ng, A.W. Mohammad, A review of ZnO nanoparticles as solar photocatalysts: synthesis, mechanisms and applications, Renew. Sustain. Energy Rev. 81 (2018) 536–551.
[25] G.M. Nair, T. Sajini, B. Mathew, Advanced green approaches for metal and metal oxide nanoparticles synthesis and their environmental applications, Talanta. Open 5 (2022) 100080.
[26] N.A.A. Razak, N.H. Othman, M.S.M. Shayuti, A. Jumahat, N. Sapiai, W.J. Lau, Agricultural and industrial waste-derived mesoporous silica nanoparticles: a review on chemical synthesis route, J. Env. Chem.Eng. 8 (2022) 107322.
[27] H.N. Cuong, S. Pansambal, S. Ghotekar, R. Oza, N.T.T. Hai, N.M. Viet, V.H. Nguyen, New frontiers in the plant extract mediated biosynthesis of copper oxide (CuO) nanoparticles and their potential applications: a review, Env. Res. 203 (2022) 111858.
[28] G. Pal, P. Rai, A. Pandey, Green synthesis of nanoparticles: a greener approach for a cleaner future, in: Green Synthesis, Characterization and Applications of Nanoparticles, Elsevier, 2019, pp. 1–26.
[29] I. Ijaz, E. Gilani, A. Nazir, A. Bukhari, A. Detail review on chemical, physical and green synthesis, classification, characterizations and applications of nanoparticles, Green Chem. Lett. Rev. 13 (2020) 223–245.
[30] S. Chakraborty, A. Singh, A. Roychoudhury, Biogenic nanoparticles and generation of abiotic stress-resilient plants: a new approach for sustainable agriculture, Plant Stress 209 (2022) 100117.
[31] M. Patel, Green synthesis of nanoparticles: a solution to environmental pollution, in: Handbook of Solid Waste Management: Sustainability through Circular Economy, Springer Nature Singapore, Singapore, 2022, pp. 1965–1993.
[32] S. Sudheer, R.G. Bai, K. Muthoosamy, R. Tuvikene, V.K. Gupta, S. Manickam, Biosustainable production of nanoparticles via mycogenesis for biotechnological applications: a critical review, Env. Res. 204 (2022) 111963.
[33] M.Q. Nasar, A.T. Khalil, M. Ali, M. Shah, M. Ayaz, Z.K. Shinwari, Phytochemical analysis, *Ephedra Procera* CA Mey. Mediated green synthesis of silver nanoparticles, their cytotoxic and antimicrobial potentials, Medicina 55 (2019) 369.

[34] C. Wang, W. Tian, Y. Ding, Y.Q. Ma, Z.L. Wang, N.M. Markovic, S. Sun, S. Rational synthesis of heterostructured nanoparticles with morphology control, J. American. Chem. Soc. 132 (2010) 6524–6529.
[35] J.J.M. Miranda, Medicinal plants and their traditional uses in different locations, in: Phytomedicine, Academic Press, 2021, pp. 207–223.
[36] C.X. Liu, Overview on development of ASEAN traditional and herbal medicines, Chinese. Herbal. Med. 13 (2021) 441–450.
[37] Y.S. Birhan, Medicinal plants utilized in the management of epilepsy in Ethiopia: ethnobotany, pharmacology and phytochemistry. Chinese, Med 17 (2021) 1–37.
[38] F.H. Shah, S.J. Kim, Therapeutic role of medicinal plant extracts and bioactive compounds in osteoarthritis, Adv. Traditional. Med. 22 (2022) 837–844.
[39] M.H. Sharaf, A.M. Abdelaziz, M.H. Kalaba, A.A. Radwan, A.H. Hashem, Antimicrobial, antioxidant, cytotoxic activities and phytochemical analysis of fungal endophytes isolated from Ocimum basilicum, Appl.Biochem.Biotechnol. 102 (2022), 1-19.
[40] T. Kebede, E. Gadisa, A. Tufa, A. Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: a possible alternative in the treatment of multidrug-resistant microbes, PLoS One 16 (2021) e0249253.
[41] A. Atri, M. Echabaane, A. Bouzidi, I. Harabi, B.M. Soucase, R.B. Chaâbane, Green synthesis of copper oxide nanoparticles using *Ephedra Alata* plant extract and a study of their antifungal, antibacterial activity and photocatalytic performance under sunlight. Heliyon. e13484. 9 (23) 1-29.
[42] N. Bhatia, A. Kumari, N. Chauhan, N. Thakur, R. Sharma, Duchsnea indica plant extract mediated synthesis of copper oxide nanomaterials for antimicrobial activity and free-radical scavenging assay, Biocatalysis. Agri. Biotechnol. 47 (2023) 102574.
[43] A.A. Menazea, M.K. Ahmed, Synthesis and antibacterial activity of graphene oxide decorated by silver and copper oxide nanoparticles, J. Mole. Str. 1218 (2020) 128536.
[44] J. Sackey, A. Nwanya, A.K.H. Bashir, N. Matinise, J.B. Ngilirabanga, M. Maaza, Electrochemical properties of *Euphorbia pulcherrima* mediated copper oxide nanoparticles. Mat, Chem. Phy. 244 (2020) 122714.
[45] M.E. Grigore, E.R. Biscu, A.M. Holban, M.C. Gestal, A.M. Grumezescu, Methods of synthesis, properties and biomedical applications of CuO nanoparticles, Pharmaceuticals 9 (2016) 75–91.
[46] A. Katsumiti, A.J. Thorley, I. Arostegui, P. Reip, E. Valsami-Jones, T.D. Tetley, M.P. Cajaraville, M.P. Cytotoxicity, And cellular mechanisms of toxicity of CuO NPs in mussel cells in vitro and comparative sensitivity with human cells, Toxicol.in Vitro. 48 (2018) 146–158.
[47] A. Abbasi, K. Ghorban, F. Nojoomi, M. Dadmanesh, Smaller copper oxide nanoparticles have more biological effects versus breast cancer and nosocomial infections bacteria. Asian. Pacific, J. Cancer Prevention 22 (2021) 893–910.
[48] A. Thangadurai, V.S. Sreenivasan, Eco-friendly cost-effective formation of copper oxide nanostructures and its prodigious potential for environmental remediation applications, G. nest. 26 (2024) 5557–55537.
[49] C. Wang, H. Zhang, P. Liu, D.Q. Zheng, X. Hu, Comprehensive evaluation of the impact of CuO nanoparticles on nitrogen transformation in the coastal aquaculture area, J. Env. Chem. Eng. 10 (2022) 107428–107480.
[50] S. Naz, A. Gul, M. Zia, R. Javed, Synthesis, biomedical applications, and toxicity of CuO nanoparticles, Appl. Microbiol. Biotechnol. 107 (2023) 1039–1061.
[51] M. Abudayyak, E. Guzel, G. Özhan, G. Cupric, Oxide nanoparticles induce cellular toxicity in liver and intestine cell lines. Adv. Pharmaceutical, Bulletin 10 (2020) 213–235.
[52] K. Michalczyk, A. Cymbaluk-Płoska, The role of zinc and copper in gynecological malignancies, Nutrients 12 (2020) 3732.
[53] L. Shkodenko, I. Kassirov, E. Koshel, E. Metal oxide nanoparticles against bacterial biofilms: perspectives and limitations, Microorganisms 8 (2020) 1545.
[54] E.J. Mavundza, R. Street, H. Baijnath, H. A, Review of the ethnomedicinal, pharmacology, cytotoxicity and phytochemistry of the genus Euphorbia in southern Africa, South. African. J. Bot. 144 (2022) 403–418.
[55] I. Ahmad, Phytochemical and Biological Studies of *Euphorbia Serpens, Euphorbia Granulata (Euphorbiaceae) and Vernonia Cinerascens (Compositae)* (Doctoral Dissertation, Bahauddin Zakariya University Multan, Pakistan), 2009, pp. 20–46.
[56] M. Dehghani, H. Beyzaei, Z. Ebrahimnezhad, Determination of phenolic and flavonoid contents of roots and shoots of *Euphorbia serpens* kunth using different solvents, Curr. Appl. Sci. 22 (2022) 59–66.
[57] M. Pradeep, D. Kruszka, P. Kachlicki, D. Mondal, G. Franklin, G. Uncovering the phytochemical basis and the mechanism of plant extract-mediated eco-friendly synthesis of silver nanoparticles using ultra-performance liquid chromatography coupled with a photodiode array and high-resolution mass spectrometry, ACS Sustain. Chem. Eng. 10 (2021) 562–571.
[58] B.A. Abbasi, J. Iqbal, T. Mahmood, R. Ahmad, S. Kanwal, S. S. Afridi, Plant-mediated synthesis of nickel oxide nanoparticles (NiO) via *Geranium wallichianum*: characterization and different biological applications, Mat. Res.Express 6 (2019) 0850a7.
[59] M.N. Sarwar, H.G. Ali, S. Ullah, K. Yamashita, A. Shahbaz, U. Nisar, I.S. Kim, Electrospun PVA/CuONPs/bitter gourd nanofibers with improved Cytocompatibility and antibacterial properties: application as antibacterial wound dressing, Polymers 14 (2022) 1361.
[60] R. Dridi, B. Essghaier, H. Hannachi, G.B. Khedher, C. Chaffei, M.F. Zid, Biosynthesized silver nanoparticles using *Anagallis monelli*: evaluation of antioxidant activity, antibacterial and antifungal effects, J.Mole. Str. 1251 (2022) 132076.
[61] E.N. Gecer, R. Erenler, C. Temiz, I. Yildiz, Green synthesis of silver nanoparticles from *Echinacea purpurea* (L.) Moench with antioxidant profile. Particulate, Sci. Technol. 40 (2022) 50–57.
[62] B.A. Abbasi, J. Iqbal, J.A. Nasir, S.A. Zahra, A. Shahbaz, S. Uddin, T. Mahmood, Environmentally friendly green approach for the fabrication of silver oxide nanoparticles: characterization and diverse biomedical applications, Microsco. Res. Tech. 83 (2020) 1308–1320.
[63] B. Kumar, K. Smita, L. Cumbal, A. Debut, Y. Angulo, Biofabrication of copper oxide nanoparticles using Andean blackberry (Rubus glaucus Benth.) fruit and leaf, J. Saudi Chem. Soc. 21 (2017) S475–S480.
[64] D. Renuga, J. Jeyasundari, A.S. Athithan, Y.B.A. Jacob, Synthesis and characterization of copper oxide nanoparticles using *Brassica oleracea* var. *italic* extract for its antifungal application, Mat. Res. Express. 7 (2020) 045007.
[65] Y. Li, J. Liang, Z. Tao, J. Chen, CuO particles and plates: synthesis and gas-sensor application, Mat. Res. Bulletin 43 (2008) 2380–2385.
[66] M. Ahamed, H.A. Alhadlaq, M.M. Khan, P. Karuppiah, N.A. Al-Dhabi, Synthesis, characterization, and antimicrobial activity of copper oxide nanoparticles, J. Nanomat. 2014 (2014), 17-17.
[67] A. Vinothkanna, K. Mathivanan, S. Ananth, Y. Ma, S. Sekar, Biosynthesis of copper oxide nanoparticles using Rubia cordifolia bark extract: characterization, antibacterial, antioxidant, larvicidal and photocatalytic activities, Env. Sci. Poll. Res. 30 (2023) 42563–42574.
[68] K. Araya-Castro, T.C. Chao, B. Durán-Vinet, C. Cisternas, G. Ciudad, O. Rubilar, Green synthesis of copper oxide nanoparticles using protein fractions from an aqueous extract of Brown Algae Macrocystis. pyrifera 9 (Pro) (2020) 78.
[69] D. Rehana, D. Mahendiran, R.S. Kumar, A.K. Rahiman, Evaluation of antioxidant and anticancer activity of copper oxide nanoparticles synthesized using medicinally important plant extracts, Biomed. Pharmacother. 89 (2017) 1067–1077.
[70] M.Y. Pelegrino, M.Y. Kohatsu, A.B. Seabra, L.R. Monteiro, D.G. Gomes, H.C. Oliveira, C.N. Lange, Effects of copper oxide nanoparticles on growth of lettuce *(Lactuca sativa* L.) seedlings and possible implications of nitric oxide in their antioxidative defense, Env. Monitor. Assess. 192 (2020) 1–14.
[71] J. Santhosh Kumar, V. Shanmugam, Green synthesis of copper oxide nanoparticles from *Magnolia champaca* floral extract and its antioxidant & toxicity assay using Danio Rerio, Int. J. Recent Technol. Eng. 8 (2020) 5444–5449.
[72] S.M.E. Sepasgozar, S. Mohseni, B. Feizyzadeh, A. Morsali, A. Green synthesis of zinc oxide and copper oxide nanoparticles using *Achillea Nobilis* extract and evaluating their antioxidant and antibacterial properties, Bulletin. Mat. Sci. 44 (2021) 1–13.
[73] P.V. Kumar, U. Shameem, P. Kollu, R.L. Kalyani, S.V.N. Pammi, Green synthesis of copper oxide nanoparticles using *Aloe vera* leaf extract and its antibacterial activity against fish bacterial pathogens, BioNanoSci 5 (2015) 135–139.
[74] M. Altikatoglu, A. Attar, F. Erci, C.M. Cristache, I. Isildak, Green synthesis of copper oxide nanoparticles using *Ocimum basilicum* extract and their antibacterial activity, Fresenius Environ. Bull. 25 (2017) 7832–7837.
[75] S. Tavakoli, M. Kharaziha, S. Ahmadi, Green synthesis and morphology dependent antibacterial activity of copper oxide nanoparticles, J. Nanostr. 9 (2019) 163–171.

[76] P. Vanathi, P. Rajiv, R. Sivaraj, Synthesis and characterization of Eichhornia-mediated copper oxide nanoparticles and assessing their antifungal activity against plant pathogens, Bulletin. Mat. Sci. 39 (2016) 1165–1170.
[77] W. Huang, H. Fang, S. Zhang, H. Yu, Optimised green synthesis of copper oxide nanoparticles and their antifungal activity, Micro & Nano Lett. 16 (2021) 374–380.
[78] M.W. Shammout, A.M. Awwad, A novel route for the synthesis of copper oxide nanoparticles using Bougainvillea plant flowers extract and antifungal activity evaluation, Chem. Int. 7 (2021) 71–78.
[79] D.G. Arumugam, S. Sivaji, K.V. Dhandapani, S. Nookala, B. Ranganathan, Panchagavya mediated copper nanoparticles synthesis, characterization and evaluating cytotoxicity in brine shrimp, Biocatalysis. Agri. Biotechnol. 19 (2019) 101132.
[80] N. Ahmad, M. Jabeen, Z.U. Haq, I. Ahmad, A. Wahab, Z.U. Islam, M.Y. Khan, Green fabrication of silver nanoparticles using *Euphorbia serpens* Kunth aqueous extract, their characterization, and investigation of its in vitro antioxidative, antimicrobial, insecticidal, and cytotoxic activities, BioMed Res. Int. 2022 (2022) 1–30.