Green synthesis of copper nanoparticles using *Cinnamomum zelanicum* extract and its applications as a highly efficient antioxidant and anti-human lung carcinoma

Haocong Liu\textsuperscript{a,b}, Guan Wang\textsuperscript{c}, Jinpeng Liu\textsuperscript{b}, Kejun Nan\textsuperscript{b}, Jie Zhang\textsuperscript{b}, Lihong Guo\textsuperscript{b} and Yang Liu\textsuperscript{d}

\textsuperscript{a}Health Science Center, Xi’an JiaoTong University, Xi’an, China; \textsuperscript{b}Oncology Hospital, Xi’an International Medical Center Hospital, Xi’an, China; \textsuperscript{c}Department of Radiotherapy, Shaanxi Provincial Cancer Hospital, Xi’an, China; \textsuperscript{d}Department of Pediatrics, Chongqing Bishan People’s Hospital, Chongqing, China

**Abstract**

*Cinnamomum zelanicum* is one of the popular medicinal plants. The plant has many pharmaceutical uses in traditional medicine. In this study, copper nanoparticles were synthesized using the aqueous extract of *Cinnamomum zelanicum*. The CuNPs were characterized using different techniques such as EDX, FE-SEM, XRD, and FT-IR. The FE-SEM results confirm spherical morphology for the nanoparticles with size of 19.55 to 69.70 nm. For investigating the antioxidant property of CuNPs, the DPPH test was used in the presence of butylated hydroxytoluene as the positive control. To survey the cytotoxicity and anti-human lung carcinoma effects of CuNPs, MTT assay was used on the NCI-H2126, NCI-H1437, NCI-H1573, and NCI-H661 cell lines. DPPH test revealed similar antioxidant potentials for *Cinnamomum zelanicum* aqueous extract, CuNPs, and butylated hydroxytoluene. Copper nanoparticles had very low cell viability and anti-lung carcinoma properties dose-dependently against NCI-H2126, NCI-H1437, NCI-H1573, and NCI-H66 cell lines without any cytotoxicity on the normal cell line. The best result of anti-lung carcinoma properties of CuNPs against the above cell lines was seen in the case of the NCI-H2126 cell line. According to the above findings, the copper nanoparticles containing *Cinnamomum zelanicum* can be administrated in humans for the treatment of several types of lung carcinoma.

**1. Introduction**

*Cinnamomum zeylanicum* is from Plantae kingdom, Tracheobionta subkingdom, Spermatophyta superdivision, Magnoliophyta division, Magnoliopsida class, Laurales order,

**CONTACT** Yang Liu  \texttt{liuyang20210309@sina.com} Department of Pediatrics, Chongqing Bishan People’s Hospital, 9 Shuangxing Avenue, Biquan Street, Bishan District, Chongqing, 402760, China

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Lauraceae family, and Cinnamomum genus. There are 250 species in the Cinnamomum genus, and the main species of this genus are Cinnamomum cassia Presl Cassia, Cinnamomum verum Presl True, Cinnamomum burmannii Blume, Cinnamomum loureirii Nees, Cinnamomum tamala Nees and Eberm, and Cinnamomum cordatum [1]. The main antioxidant compounds of C. zelanicum include cinnamic acid, 2,4-dihydroxy cinnamic acid, 2,5-dihydroxy cinnamic acid, o-coumaric acid, 3,4,5-trihydroxy cinnamic acid, sinapic acid, ferulic acid, caffeic acid, p-coumaric acid, camphor, linalool, benzyl benzoate, (E)-cinnamyl acetate, p-cymene, α-terpineol, (E)-β-caryophyllene, (E)-cinnamaldehyde, eugenol, and cinnamaldehyde [1]. Previously, the anti-inflammatory, antioxidant, antifungal, and antibacterial, anti-diabetic, hypolipidemic, and anti-tyrosinase properties of this species have been confirmed. In traditional medicine, the people use C. zelanicum for the prevention, control, and treatment of blood disorders such as iron deficiency, fauvism, hemolysis, hemolytic anemia, and thrombocytopenia and several cancers such as breast, ovarian, prostate, bone marrow, and gastric cancers [1].

Metallic nanoparticles have received a great attention due to their applications in different branches of technology including pharmaceuticals, electronics, photonics, sensing technologies, therapeutics and antimicrobial products [2, 3]. Moreover, nanoparticles interact with plants causing different morphological and physiological modifications. Their effects are reported to be positive or negative depending on the physico-chemical, size, concentration, and composition characteristics of nanoparticles as well as plant species [2, 4]. The current control measures are focused mainly on inhibiting diffusion of disease to uninfected plants. Recent works are concentrated on the application of nanoparticles for controlling plant diseases [2–5]. Due to the recent advances in research on metallic nanoparticles, the copper nanoparticles are effective antifungal agents due to their powerful cytotoxic activity toward a broad range of microorganisms compared to other metals [4–6]. Copper nanoparticles made fastidious attention because of its wide applications in chemical, electrical, optical, bioremediation, sensor, and biological fields [5–8]. Several methods for the synthesis of copper nanoparticles are under consideration. These include chemical and physical methods. However, they are expensive, time-consuming, and environmentally toxic. A green synthesis is proposed as a simple, cost-efficient and environment friendly method. The biological synthesis of nanoparticles uses different biomaterials such as bacteria, yeast, algae, fungi, and plants [6–9]. The use of plants attracts the attention of researchers as a simple biosynthesis process compared to other conventional methods. Also, due to the high availability and low cost of the medicinal plants, the copper nanoparticles green-synthesized using natural compounds significantly have increased [6–8]. Copper nanoparticles green-synthesized using medicinal plants have been abundantly used in the biomedical sciences for the treatment of many diseases. Every year, the notable applications of copper nanoparticles green-synthesized are being gained and this trend is continuing [9, 10]. The results of many studies have indicated the significant antifungal effects of copper nanoparticles green-synthesized by plants in the cure of candida diseases and their antibacterial properties in the treatment of Streptococcus, Staphylococcus, Pseudomonas, Salmonella, and Bacillus infectious. Also, copper nanoparticles synthesized by plants have been formulated due to the antiviral, antibacterial, antioxidant, anti-parasitic, anti-inflammatory, antifungal, wound healing, and anti-cancer properties [8]. The main therapeutic properties of copper nanoparticles green synthesized by plants are anticancer effects [8–10].

In the present study, we decided to investigate the anti-lung carcinoma potentials of copper nanoparticles formulated by C. zeylanicum against the NCI-H2126, NCI-H1437, NCI-H1573, and NCI-H661 cell lines.
2. Materials and methods

2.1. Material

Dimethyl sulfoxide (DMSO), Antimycotic antibiotic solution, hydrolysate, decamplmaneh fetal bovine serum, Ehrlich solution, 4-(Dimethylamino)benzaldehyde, 2,2-diphenyl-1-picrylhydrazyl (DPPH), carbazole reagent, borax-sulphuric acid mixture, Dulbecco’s Modiﬁed Eagle Medium (DMED), and phosphate buffer solution (PBS) all were achieved from Sigma-Aldrich company of USA.

2.2. Preparation and extraction of aqueous extract

First, the dried leaves of C. zeylanicum were grounded. Then, 100 g of the sample was macerated in 1000 mL of boiling water for 6 h. After that, the extract was ﬁltrated and evaporated to concentrate. Finally, the extract was placed in a freeze drier for 72 h. The obtained extract as brown powder was kept in cold place.

2.3. Green synthesis of CuNPs

The green synthesis of copper nanoparticles was carried out according to a previous study [11]. A 40 mL of C. zeylanicum extract (1 g in 10 mL of deionized water) was added to 40 mL of 0.3 M Cu(NO₃)₂·3H₂O in a flask. Then, the mixture was heated and stirred at 65°C for 24 h. The CuNPs was formed as dark brown participates during the reaction time. The CuNPs was washed with water and ethanol for three times and then centrifuged at 15000 rpm for 10 min. Finally, the precipitate was dried in an oven at 55°C.

2.4. Determination of the antioxidant property of copper nanoparticles

At the beginning of the study, 100 mL of methanol (50%) was added to the 39.4 g of DPPH. Also, several concentrations of Cu(NO₃)₂·3H₂O, C. zelanicum aqueous extract, and CuNPs i.e. 0-1000 μg/mL were considered. The above DPPH was added to the various concentrations of Cu(NO₃)₂·3H₂O, C. zelanicum aqueous extract, and CuNPs and all samples were transfer to an incubator at the temperature of 37°C. After 30 min incubating, the absorbances were measured at 517 nm. In this study, methanol (50%) and butylated hydroxytoluene (BHT) were negative and positive controls, respectively. Acceding to the following formula, the antioxidant properties of Cu(NO₃)₂·3H₂O, C. zelanicum aqueous extract, and CuNPs were determined in detail [4]:

\[
\text{DPPH free radical scavenging (\%) = (Control - Test/Control) \times 100}
\]

2.5. Determination of anti-lung carcinoma effects of copper nanoparticles

In this research, the following cell lines have been used for investing the cytotoxicity and anti-lung carcinoma effects of the Cu(NO₃)₂·3H₂O, C. zelanicum aqueous extract, and CuNPs using the common cytotoxicity test i.e. MTT assay:

I. Lung carcinoma cell line: NCI-H2126
II. Lung carcinoma cell line: NCI-H1437.
III. Lung carcinoma cell line: NCI-H1573.
IV. Lung carcinoma cell line: NCI-H661.
V. Normal cell line: HUVEC.

For culturing the above cells, penicillin, streptomycin, and Dulbecco’s modified Eagle’s medium (DMEM) were used. The distribution of cells was 10,000 cells/well in 96-well plates. Then, all samples were transferred to a humidified incubator with 5% CO₂ at the temperature of 37°C. After 24h incubating, all cells were treated with several concentrations i.e. 0-1000 μg/mL of Cu(NO₃)₂.3H₂O, C. zelanicum aqueous extract, and CuNPs, then incubated for 24 h. Cu(NO₃)₂.3H₂O, C. zelanicum aqueous extract, and CuNPs were sterilized using the radiation of ultraviolet for 2 h. Finally, 5mg/mL of MTT was added to all wells and all samples of Cu(NO₃)₂.3H₂O, C. zelanicum aqueous extract, and CuNPs were transferred to an incubator at the temperature of 37°C for 4 h. The percentage of cell viability of samples was measured at the absorbance of 570 nm and according to the following formula [12]:

\[
\text{Percentage of cell viability (\%)} = \frac{\text{Sample absorbance}}{\text{Control absorbance}} \times 100
\]

2.6. Statistical analysis

The obtained results were fed into SPSS-22 software and analyzed by one-way ANOVA, followed by Duncan post-hoc test \((p \leq 0.01)\).

3. Results and discussion

3.1. Chemical characterization of CuNPs

XRD analysis: The XRD diffraction pattern is the method to evaluate compounds crystallinity. The XRD pattern of CuNPs is shown in Figure 1. The formation of copper nanoparticles with small size and well crystallizing is approved to this result. The obtained data for the signal at different of 2θ values were compared with the standard database of PDF card No. 04-012-7238. The signals at 35.77, 38.97, 48.88, 58.48, 61.68, 65.81, 66.41, 68.24, 73.58, and 75.41 are indexed as (-111), (111), (-202), (202), (-113), (022), (-311),

![Figure 1. XRD Pattern of CuNPs.](image-url)
(-221), (221), and (-222) planes. This data is matched to those of copper oxide in formula of CuO. The crystal size of CuNPs was calculated using Scherer’s equation. The result revealed a 17.70 nm for the crystal. In our review of the literature, a range of 12-40 nm has been reported for the crystal size of copper nanoparticles [13–16].

**EDX analysis:** To characterize metallic nanoparticles, EDX is a qualitative technique to detect the elemental compositions of the NPs. Figure 2 shows the EDX diagram of CuNPs. The results approve the successful synthesis of copper nanoparticles by the presences of the signals below of 1Kev (for CuLα), around 8 Kev (for Cu Kα), and below 9 Kev (for Cu Kβ) [17]. The around 0.5 keV for O Lα confirm the presences of oxygen in the CuNPs, that shows the nanoparticles may be formed as copper oxide, previous studies on green synthesis of CuNPs using plants extracts have reported the formation of copper oxide [18]. The other signal around 0.20 keV for CLα exhibits the presence of carbon in nanoparticles. This signal for carbon and also oxygen can be contributed to carbon and oxygen of secondary metabolite of C. zelianicum extract that are linked to the surface of nanoparticles.

**SEM analysis:** FE-SEM technique is sufficient method to investigate morphology of nanoparticles. The FE-SEM images of CuNPs are shown if Figure 3. The nanoparticles are formed in a spherical morphology in the range size of 19.55 to 69.70 nm. The green synthesized CuNPs, exhibit a tendency to aggregate that is known as a general property for green synthetic metallic nanoparticles using plants extracts [13, 19–22]. In our review of literature, different sizes from 5 to 100 nm have been reported for the green-synthesized copper nanoparticles using plants extracts [23–26].

**FT-IR analysis:** FT-IR technique is another qualitative method in characterization of nanoparticles. The presences of the bands in the specific wavenumber regions reveals sufficient information about the metallic nanoparticles. For example, if the nanoparticles are formed as metal oxide, the peaks at 400 to 700 cm⁻¹ belong to metal oxygen bond. The presences of the peaks at the other region are attributed to the different bonds for organic compounds in the plant extract that bind to the nanoparticles. The FT-IR spectra of CuNPs is shown in Figure 4. The peaks at wavenumbers of 437, and 602 cm⁻¹ (belong to Cu-O bond) confirm the formation of CuO that is in accordance to the XRD and EDX results. A previous study has reported the peaks for green synthetic CuNPs with a little difference in wavenumber [27], Furthermore, the peaks at 3418 and 2928 cm⁻¹ (O-H and aliphatic C-H stretching), 1419 to 1650 cm⁻¹ (C=C and C=O stretching), (1042 cm⁻¹ -C-O stretching) belong to the various bonds of organic compounds in C. zelianicum extract that exist as the plant secondary metabolites. These compounds can be comprised...
different class of compounds such as phenolic, flavonoid, triterpenes, which were reported previously.

3.2. Antioxidant properties of copper nanoparticles synthesized using C. zelanicum leaf aqueous extract

In recent years, researchers evaluated plants and bio mediated synthesized nanoparticles for antioxidant activity. The green-synthesized copper nanoparticles exhibit higher antioxidant activity for the formation of free radicals into the living system [28, 29]. The
copper nanoparticles have redox properties and play a significant role in deactivating free radicals in the living system [29].

For determining of antioxidant properties of several materials such as medicinal plants and metallic nanoparticles green-synthesized by medicinal plants the free radicals are used that the most main of them is DPPH. In the high antioxidant capacities of several materials, the color of the DPPH molecules changes from violet to the pale yellow or colorless [100]. In our study, the antioxidant effects of the copper nanoparticles synthesized using C. zeylanicum leaf aqueous extract were evaluated by DPPH assay revealed concentration-dependent effects i.e. an increase in the concentration of the copper nanoparticles leads to an increase in antioxidant activities. In the concentrations of studied, the best result was seen in the high concentration or 1000 μg/mL (Figure 5).

Comparative analysis of the individual antioxidant assays showed significant variations in the exertion of radical scavenging effects. Among all materials tested (Cu(NO₃)₂·3H₂O, C. zelanicum aqueous extract, and CuNPs), the copper nanoparticles indicated more excellent inhibition effects against DPPH. Standard (butylated hydroxytoluene) demonstrated similar antioxidant effects compared to the copper nanoparticles. The exact IC₅₀ of C. zeylanicum leaf aqueous extract, butylated hydroxytoluene, and copper nanoparticles were 429, 227, and 209 μg/mL, respectively (Table 1).

The reason behind the antioxidant activity of green or biosynthesized nanoparticles could be due to the presence of metabolites compounds [28–32]. Also, many researchers reported phenolic and flavonoids attached to the nanoparticles exhibited the antioxidant activity. Previously it has been indicated that C. zeylanicum leaf is rich in antioxidant compounds such as cinnamic acid, 2,4-dihydroxy cinnamic acid, 2,5-dihydroxy cinnamic acid, o-coumaric acid, 3,4,5-trihydroxy cinnamic acid, sinapic acid, ferulic acid, caffeic acid, p-coumaric acid, camphor, linalool, benzyl benzoate, (E)-cinnamyl acetate, p-cymene,

![Figure 5. The antioxidant properties of Cu(NO₃)₂·3H₂O, C. zelanicum aqueous extract, CuNPs, and BHT against DPPH.](image-url)
α-terpineol, (E)-β-caryophyllene, (E)-cinnamaldehyde, eugenol, and cinnamaldehyde [1]. Several studies were carried out in the nanotechnology field using various medicinal plants, but still, no report is available on copper nanoparticles synthesized using *C. zeylanicum* leaf aqueous extract.

3.3. Cytotoxicity and anti-human lung cancer potentials of copper nanoparticles synthesized using *C. zelanicum* leaf aqueous extract

The anticancer effects of copper nanoparticles green-synthesized by medicinal plants have been confirmed in the previous studies [33–35]. In the study of Suman et al. (2013) was clarified the anti-cervix cancer effects of metallic nanoparticles containing natural compound (*Morinda citrifolia*) against HeLa cell line. In the previous study, the metallic nanoparticles killed all HeLa cells in high doses [33]. In another study, the anti-liver cancer properties of metallic nanoparticles containing *Piper longum* leaf against Hep-2 cell lines were proved [34]. In the previous study has been indicated that metallic nanoparticles green-synthesized by *Annona quamosal* leaf have excellent anti-breast cancer potentials against MCF-7 cell line [35].

The morphological parameters of copper nanoparticles which affect anticancer properties of these nanoparticles against several cancer cell lines are size, form, and surface coating. Among the above parameters, the role of size of copper nanoparticles is the most [32]. Previously, it was showed whatever the size of copper nanoparticles reduced, the ability of these nanoparticles for transferring to the cancer cell lines and killing them increased [36].

As can be observed in Figure 3 of our study, the sizes of copper nanoparticles synthesized by *C. zelanicum* leaf aqueous extract are at the ranges of 19.55 to 69.70 nm. In the similar study, it has been indicated that copper nanoparticles at the size of 100 nm and lower have significant roles in the removing tumor cell lines [28–34].

In the recent research, the treated cells with several concentrations of the present Cu(NO$_3$)$_2$.3H$_2$O, *C. zelanicum* leaf aqueous extract, and copper nanoparticles were examined by MTT test for 48 h regarding the cytotoxicity properties on normal (HUVEC) and common lung cancer (NCI-H2126, NCI-H1437, NCI-H1573, and NCI-H661) cell lines (Figures 6–10). The absorbance rate was determined at 570 nm, which indicated extraordinary viability on normal cell line (HUVEC) even up to 1000 µg/mL for Cu(NO$_3$)$_2$.3H$_2$O, *C. zelanicum* leaf aqueous extract, and copper nanoparticles.

In the case of lung cancer cell lines, the viability of them reduced dose-dependently in the presence of Cu(NO$_3$)$_2$.3H$_2$O, *C. zelanicum* leaf aqueous extract, and copper nanoparticles. The IC50 of *C. zelanicum* leaf aqueous extract and copper nanoparticles against NCI-H2126 cell line were 474 and 250 µg/mL, respectively; against NCI-H1437 cell line were 505 and 348 µg/mL, respectively; against NCI-H1573 cell line were 502 and 301 µg/mL, respectively; against NCI-H661 cell line were 516 and 261 µg/mL, respectively.

The best results of cytotoxicity and anti-lung cancer potentials of copper nanoparticles against the above cell lines were seen in the case of the NCI-H2126 cell line (Table 2). Likely the significant anti-lung cancer potentials of copper nanoparticles synthesized by *C. zelanicum* leaf aqueous extract against common lung cancer cell lines are linked to

| Cu(NO$_3$)$_2$ (µg/mL) | *C. zelanicum* (µg/mL) | CuNPs (µg/mL) | BHT (µg/mL) |
|----------------------|-----------------------|---------------|-------------|
| IC50 against DPPH    |                       |               |             |
| 429                  | 227                   | 209           |             |

Table 1. The IC50 of Cu(NO$_3$)$_2$.3H$_2$O, *C. zelanicum* aqueous extract, CuNPs, and BHT in antioxidant test.
their antioxidant activities. The similar researches have revealed the antioxidant materials such as metallic nanoparticles especially copper nanoparticles and ethno medicinal plants reduce the volume of tumors by removing free radicals [37].
The high presence of free radicals in the normal cells makes many mutations in their DNA and RNA, destroy their gene expression and then accelerate the proliferation and growth of abnormal cells or cancerous cells \[38, 39\].

**Figure 8.** The anti-human lung carcinoma properties of Cu(NO\(_3\))\(_2\)\(\cdot\)3H\(_2\)O, C. zelanicum aqueous extract, and CuNPs against NCI-H1437 cell line.

**Figure 9.** The anti-human lung carcinoma properties of Cu(NO\(_3\))\(_2\)\(\cdot\)3H\(_2\)O, C. zelanicum aqueous extract, and CuNPs against NCI-H1573 cell line.

The high presence of free radicals in the normal cells makes many mutations in their DNA and RNA, destroy their gene expression and then accelerate the proliferation and growth of abnormal cells or cancerous cells \[38, 39\]. The free radicals high presences in
all cancers such as breast, gallbladder, stomach, rectal, liver, gastrointestinal stromal, esophageal, bile duct, small intestine, pancreatic, colon, parathyroid, thyroid, bladder, prostate, testicular, fallopian tube, vaginal, ovarian, hypopharyngeal, throat, lung, and skin cancers indicate significant role of these molecules in making angiogenesis and tumorigenesis [39, 40]. Many researchers reported that copper nanoparticles synthesized by ethno medicinal plants have remarkable role in the removing free radicals and growth inhibition of all cancerous cells [40, 41].

4. Conclusion

In summarize, the copper nanoparticles were green-synthesized using an aqueous extract of *Cinnamomum zelanicum*. The CuNPs was characterized using common chemical techniques such as UV-Visible, FT-IR, XRD, FE-SEM, and EDS. The results approved the synthesis of nanoparticles in form of copper oxide (CuO) nanoparticles with a spherical morphology. The size of CuNPs was in the range of 19.55 to 69.70 nm which is well known as an efficient size for the synthetic nanoparticles. Copper nanoparticles indicated remarkable antioxidant and anti-lung carcinoma properties against NCI-H2126, NCI-H1437, NCI-H1573, and NCI-H661 cell lines. It appears these nanoparticles may be administrated as a chemotherapeutic drug.
Disclosure statement

No potential conflict of interest was reported by the authors.

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