Biosynthesis, Characterization and Cytotoxicity of Zinc Nanoparticles Using *Panax ginseng* Roots, Araliaceae

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ABSTRACT

This study aims to biosynthesize zinc nanoparticles from the aqueous extract of *Panax ginseng* (Red Ginseng) roots. The characteristics of ZnNPs were checked using change in color, UV-Vis, SEM, SPM, AFM, FT-IR, and EDS analyses, and assessed their cytotoxicity against L20B tumor cell line using MTT assay. The change in the solution color after 3 hr on 70 °C is from yellow to the brownish color with whitish sediments. The adsorption peak of UV-vis is 340 nm as evidence of formation the Zn nanoparticles. AFM, SEM and EDS observe shapes of zinc nanoparticles which are spherical to irregular particles with rate of size 59.76 nm. The concentration 100% Zinc nanoparticles significantly (*p*<0.01) recorded best inhibition percentage 41.70% against murine fibroblast cells (L20B) which have receptors of human polioviruses. Thus, this work is considered as an auspicious first test to reduce the growth of cancers using green ZnNPs of Ginseng (*Panax ginseng*) *in vitro*.

Keywords: Anticancer activity, EDS, Red Ginseng, SPM, ZnNPs.

INTRODUCTION

Ginseng is one of Korean herbs which was used in ancient world 1. The scientific name of Ginseng is *Panax ginseng* and it is a perennial plant belongs to the Araliaceae family 2. The Greek word of Panax is meaning drug for all diseases, and its origin from two words the first *pan* and the second *αξος* meaning “all” and “medicine” respectively 3. Korean Red Ginseng has been using as a health food and food additives 4 because of its potential role in the immune improvement 5, anti-fatigue, anti-stress, anti-aging effects 6, antifungal 7, anti-diabetic 8, anticancer activity 9, and improvement of blood circulation, and serum cholesterol 10.
The chemical composition of Panax ginseng mainly consists from saponin (ginsenoside) ⁴, water-soluble sugar, acidic polysaccharides, and phenolic compounds ¹¹, thus P. ginseng had numerous pharmacological and physiological roles and opens the door toward using its extracts as a green agent to synthesize metallic nanoparticles. Few studies have enabled to Ginseng-mediated synthesize silver nanoparticles and gold nanoparticles ¹²⁻¹⁴.

Many studies were investigated the role of silver nanoparticles of Ginseng toward inhibiting human cancers which decreased the levels of mRNA and phosphorylation of receptors of epidermal growth factor in cancer cells ¹² while ¹⁴ referred to antioxidant effects by gold nanoparticles biosynthesized from Ginseng. Silver and gold nanoparticles from leaves of this plant showed antimicrobial and potent anticoagulant agents ¹³. In the last years, AuNPs were used as cosmetic products because of their role against inflammation, and to disinfect skin wounds ¹⁴.

The use of bacteria, fungi, parts of plant and their enzymes and extracts for synthesis of zinc nanoparticles have much benefits for pharmaceutical and biomedical applications ¹⁵. Also, ZnNPs are used as preservative for different products like foods, pigments, plastics, ceramics, glass, etc. ¹⁶. Most of the studies of Red Ginseng roots have referred to testing the bioactivity of its nanoparticles such as AgNPs and AuNPs. However, this recent study firstly indicates that Red Ginseng reduced zinc sulfate heptahydrate (ZnSO₄·7H₂O) and produce ZnNPs which inhibited mouse fibroblast cells (L20B cell line) in vitro. As you know that murine fibroblast cells have receptors of human polioviruses thus this study is important in the medical field.

METHODOLOGY

Red ginseng

Dried Red Ginseng roots, Panax ginseng (Figure 1), were purchased from the local market in Ramadi, Iraq which was obtained in October 2017. The pieces of roots of Red Ginseng were grinded using the Stand Blender (SAMiX, model LB6105D, China) to get its powder which will use in the extraction.

Extraction of Panax ginseng powder

About 20 g of the powder of P. ginseng was extracted in 200 mL Distilled Water (DW) in a 500mL-flask using magnetic stirrer hot plate for 20 minutes at 100 °C then cooled to 25 °C. The aqueous extract was filtered using filter paper Whatman No. 1 and then centrifuged for 10 minutes at 4000 cycle/min. The obtained aqueous extract was kept in the icebox as a stock solution until its use. The residue was emitted. FT-IR (Fourier Transform Infrared Spectroscopy) spectrum of this aqueous extract was performed for characterizing and com-
pared with FT-IR spectrum of the biosynthesized ZnNPs from it later.

**Biosynthesis of zinc nanoparticles**

About 0.86 g of zinc sulfate heptahydrate (ZnSO$_4$.7H$_2$O) was dissolved in 1 L of DW on the magnetic stirrer until the completion of dissolving was observed. The final concentration of ZnSO$_4$.7H$_2$O solution is $3 \times 10^{-3}$ M. Only, 100 mL of zinc sulfate solution $3 \times 10^{-3}$ M was mixed with 30 mL of the crude aqueous Ginseng extract and heated on the magnetic stirrer hotplate at 70 °C for 3 hr to synthesize zinc nanoparticles. The same test was achieved at 25 °C for 24 hr. The change in the mixture color was individually recorded at 25 °C and 70 °C.

**Characterization of ZnNPs**

Ginseng-mediated biosynthesis of zinc nanoparticles (ZnNPs) was characterized using changing of the color of the mixture solution, UV-Visible spectrum (by EMC-LAB V-1100 digital spectroscopy, Germany), FT-IR spectroscopy, AFM, SPM, SEM, and EDS analyses.

**In vitro Cytotoxicity**

The cytotoxicity of ZnNPs toward L20B tumor cell line was investigated by MTT assay as mentioned by 17,18. Firstly, 100 µl/well of $10^6$ cell/mL L20B cells was cultured in 96-well tissue culture plate. Three concentrations of 50%, 75%, and 100% of colloid ZnNPs and extract of Ginseng roots were seperately applied in this test. About one hundred microliters of each concentration was added within each well then incubated at 37 °C for 48 hr. After that, 10 µl of 5 mg/mL MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was added to each well and re-incubated at 37 °C for 4 hr. Finally, 50 µl dimethyl sulfoxide (DMSO) was added to each well and incubated for 10 minutes. L20B cells were cultured in complete medium without ZnNPs or the extract of Ginseng solution as a control. ELISA reader was used to measure the absorbance of each well at 620 nm. The growth inhibition percentage was calculated using the equation below to give the cytotoxicity:

$$\text{Growth inhibition percentage} = \frac{(\text{Optical Density of control well} - \text{Optical Density of treatment wells})}{\text{Optical Density of control well}} \times 100$$

**Statistical analysis**

The data, in triplicates, has been subjected by its mean to one way analysis of variance (ANOVA) using SAS program, version 9. The significance of differences has been determined by using Duncan’s Multiple Range Test and the probability least than 0.01 was considered to be statistically significant.
RESULTS AND DISCUSSION

UV-Visible spectrum of the biosynthesized ZnNPs from *P. ginseng* and their color were checked in this test to confirm the formation of nanoparticles. The color change of the mixture solution from yellow to the pale yellow close of the brownish color with whitish sediments was apparently recorded in tube B at peak 340 nm (Figure 1). The color arises due to excitement of surface Plasmon vibration in ZnNPs. These results agree with 19 who produced polydispersed brownish ZnNPs with absorbance peak at 310 nm using Actinomyces. This case was achieved by the heating at 70 °C for 3 hr with absorption of 3.150 cm⁻¹. The absorption of tube A (performed at the room temperature) is 2.620 cm⁻¹ that less than B tube. The heating to 70 °C is more suitable than 25 °C for forming ZnNPs from Red Ginseng roots extract due to increase of activation energy to reduce this organic molecules 20.

![Figure 1. UV-Visible spectrum of the biosynthesized ZnNPs from *P. ginseng* extract using at 25 °C (A) and 70 °C (B).](image)

SEM image (Figure 2) observes shapes of zinc nanoparticles which are spherical to irregular particles with a clear accumulation. Figure 3 shows histogram of the particle size distribution of the biosynthesized zinc nanoparticles which reach to average 59.76 nm. Volumes of zinc nanoparticles of 45.00 nm, 60.00 nm, and 70.00 nm are ≤10%, ≤50%, and ≤90% respectively. The lower particles diameter is 45 nm while the higher diameter is 85 nm with volumes 8.16% and 1.53% respectively. The higher amount is 17.86% for the ZnNPs with the
diameter of 65 nm. Granularity Cumulation distribution of ZnNPs also has different accumulation according to their sizes as shown in Figure 3. The zinc nanoparticles of 45 nm have the lowest accumulation of 8.16%, followed 18.88% and 36.73% for ZnNPs with diameters 50.00 and 55.00 nm respectively. The higher accumulation percentage is 100% for ZnNPs of 85.00 nm followed 98.47% and 92.35% for the sizes 80.00 nm and 75.00 nm respectively.

Figure 2. Scanning Electron Microscopy (SEM) of the biosynthesized zinc nanoparticles.

Figure 3. Histogram of particle size distribution of the biosynthesized zinc nanoparticles.
AFM shows the lateral and three-dimensional images to screen surface roughness of the Ginseng-mediated zinc nanoparticles (ZnNPs) at size image 2042.09 nm×2052.20 nm as shown in Figure 4. Surface roughness analysis exhibits some functional parameters such as roughness average of 4.26 nm, reduced summit height of 0.65 nm, core roughness depth of 15.4 nm, and reduced valley depth of 4.81 nm. Hybrid parameters are measured like surface area ratio which reaches 7.75 and mean summit curvature reaches 0.43 nm⁻¹. This is an indicator for the formation Zn nanoparticles in small sizes less than 60 nm.

Figure 5 presented the Energy Dispersive X-ray (EDS) measurement which reconfirmed that the biosynthesized nanoparticles are actuality metal ZnNPs. The existence of carbon, phosphor, sulfur and oxygen peaks observed the presence of covering organic fractions of Ginseng on the Zn nanoparticles. Weight of Zinc 3.84% compared with 0.34% and 0.73% for S and P respectively. The presence of the elemental Zn can be seen in the EDS graph that indicates the reduction of Zn ions to elemental zinc. The appearance of other elements, like K after adsorption is from the organic moieties in the watery crude extract as mentioned by 21 who confirmed that potassium is adsorbed on the surface of ZnNPs.

![Figure 4. AFM of the biosynthesized ZnNPs lateral (A), three-dimensional (B).](image)
The FT-IR spectroscopy was used to determine the chemical composition and active groups found in the studied samples (Ginseng extract and the biosynthesized ZnNPs from this extract) (Figure 6). The FT-IR spectrum of the Ginseng extract presented in Figure 6A showed finding two peaks the first 765 cm\(^{-1}\) due to the covalent bond between carbon and silicon (Si-C) and another peak 1107 cm\(^{-1}\), which is evidence of the oxygen bond with silicon in (Si-O). As well as absorption peaks at 1359 cm\(^{-1}\) and 1419 cm\(^{-1}\) belong to the homogeneous and heterogeneous bending vibration of the methylene group (-CH\(_2\)) or the methyl (-CH\(_3\)) and the absorption bands at 2889 cm\(^{-1}\) and 2923 cm\(^{-1}\) belongs to the homogeneous and heterogeneous extension vibration of the methylene group (-CH\(_2\)) or the methyl (-CH\(_3\)). The mentioned four peaks belong to the methylene group (-CH\(_2\)) and the methyl group (-CH\(_3\)) in the synthesis of amino acids, peptides or proteins and the sign of that is finding the absorption band returns to the single bond (C-C).

The spectrum also showed an absorption peak at 1620 cm\(^{-1}\) due to the extension vibration of the group (C=C) in the alkene compounds and to the successive double bonds in the benzene ring in the aromatic structures. The presence of compensated aromatic rings is found in the composition of amino acids, peptides, and proteins \(^{22}\). The peak of 2290 cm\(^{-1}\) is a clear indication of the existence of stylenyl derivatives contains trinal bonds (C≡C). From the other hand, the existence of other peaks mentioned supporting the presence of double bond (C=C) on carbon atoms at 1419 cm\(^{-1}\) and 3178 cm\(^{-1}\) related to the extension vibration of groups C-O and =C-H respectively, which is due to alk-
ene compounds. Also, when reviewing the previous studies which studied the
chemical composition of some Ginseng-derived compounds, it is certain that
the extract in this study contains polyacetylenes may be in their three struc-
tures 1, (see Figure 7).

It is confirmed that the Ginseng extract contains polyacetylene compounds,
amino acids, peptides, proteins, polyphenols, and polysaccharides. There is
an extension vibration of the absorption bands at 3451 cm⁻¹, and two bending
vibration bands at 1261 cm⁻¹ and 1359 cm⁻¹ belong to the hydroxyl group (-OH)
and the peak of 1419 cm⁻¹ due to an extension vibration for the group C-O.

The absorption peak at 2705 cm⁻¹ indicates the presence of hydrogen bound
to the carbonyl group in the aldehyde group (O=C-H). That is an evidence of
the presence of non-cyclic monosugars. The two bands 603 cm⁻¹ and 638 cm⁻¹
showed the presence of the amide group (O=C-N-H) which binds successive
two amino acids in the composition of proteins or peptides. Furthermore, the
presence of the absorption peak at 3404 cm⁻¹ also belongs to the amine group
(-NH) in the synthesis of the amino acid (Arginine), or belongs to the amide
group in the peptide or protein synthesis 22,23. The presence of the carboxylic
group (-COOH) in amino acids is evidenced by the appearance of the broad
absorption range from 2410 cm⁻¹ to 3620 cm⁻¹. Moreover, a bending vibration
band at 925 cm⁻¹ is belonging to the hydroxyl group in the carboxyl group 24–27.

There is a band located at 1745 cm⁻¹ belongs to hexagonal-cyclic ketones, thus
it belongs to the group of Carbonyl (C=O). This is evidence that the extract
contains flavonoids, which belong to the category of phenols and its function to
protect the plant from the harmful effects of ultraviolet radiation. The presence
of flavonoids in the Ginseng extract was studied by others as shown in Figure
8. That also confirms that the Ginseng extract under study contains these com-
pounds. It is believed that because of the presence of hydroxyl (-OH), carbon-
oxgen (C-O), methylene (-CH₂) and methyl (-CH₃) groups, the presence of
ginsenoside compounds in the Ginseng extract is similar in structure to the
primary structure of cholesterol but is more complex in terms of the chemical
composition 28.

The FT-IR spectrum of the biosynthesized zinc nanoparticles (Figure 6B)
shows the sharp absorption peak located at 1141 cm⁻¹, the two clear peaks at
1371 cm⁻¹ and 1423 cm⁻¹ and the clear wideband at 1629 cm⁻¹. That is a clear
indicator to presence of nano-zinc atoms in the composition of the biosynthe-
sized ZnNPs from Ginseng extract, because in a study on mushrooms found
that the FTIR spectrum of silver nanostructures when it binds with the hydro-
carbons, it shows four bands located nearby and within the mentioned ranges
It was noted that this spectrum is very similar to the spectrum of FT-IR of the Ginseng extract (Figure 6A) in terms of the extension of the bands thus the chemical composition is similar, but the insulation, clarity, and the band width are better in the second spectrum (Figure 6B). The reason for this may be due to the presence of zinc atoms with good dispersion with a high surface area that enables the active groups in the electron-rich in the Ginseng extract to share their electron pairs with the empty orbitals in the outer shell of zinc.

It is known that zinc atoms have 30 electrons thus its fourth shell has one electronic pairs in the level (4s), thus the rest secondary levels 4p, 4d, and 4f which contain three, five, and seven orbitals respectively are empty and can assimilate coming electronic pairs from good atoms with negative charges are O, N, and S which find in monosaccharides, polysaccharides amino acids, peptides, proteins, polyphenols, flavonoids, polyacetylenes, and ginsenosides. Thus, the previous FT-IR spectrum (Figure 6B) exhibited that the structures of acidic polysaccharides, amino acids, ginsenosides, polyacetylenes, and polyphenols in the Panax ginseng extract are not affected because of interaction their active groups with zinc ions or zinc nanoparticles as reducing and capping agents to the synthesized ZnNPs.
Figure 6. FT-IR spectrum of extract of *P. ginseng* (A) extract and ZnNPs of *P. ginseng* (B)

Figure 7. Structures of polyacetylene compounds expected in the extract of Ginseng

Figure 8. Two structures of flavonoids compounds expected in the extract of Ginseng
Cytotoxicity of the biosynthesized ZnNPs and extracts of *Panax ginseng* Ginseng were applied against L20B tumor cell line using the colorimetric cell viability MTT assay. Three concentrations (50%, 75%, and 100%) of ZnNPs solution and aqueous extracts of Ginseng were individually achieved compared with the control. All concentrations of aqueous crude extracts did not exhibit any growth inhibition percentage as shown in Figure 9. Zinc nanoparticles of Ginseng showed growth inhibition percentages approx. 35.03%. The concentration of 100% significantly (*p*<0.01) recorded inhibition percentage of 41.70%, followed 33.30% and 30.10% by the concentration 75% and 50% respectively. The reason of that belongs to induce cytotoxicity and ROS generation in L20B tumor cell line which cause apoptosis leading to cell death and preventing their replication. Extract of *P. ginseng* composes from some pharmacological compounds like polysaccharides, flavonoids, triterpenoids, and ginsenosides which caped ZnNPs, have been included anticancer activity. The ability of the biosynthesized ZnNPs to inhibit L20B tumor cell line is considered as a potential indicator for biological activity of these green nanoparticles against the cancers *in vitro*. Saponin of Red Ginseng roots (Ginsenoside) is active compound against cancers because of its role as antioxidant agent covering the ZnNPs. The zinc in many medical and nutritional products play a promising role in host defense to prevent the initiation, promotion and development of carcinoma. A zinc nanoparticle model is useful as an approach to increase activity of zinc in treatment of cancers due to the high surface area of ZnNPs. Many studies were investigating the inhibitory role of AgNPs toward human cancers but not ZnNPs. These results agree with results of who referred to that silver nanoparticles of Ginseng decreased the levels of mRNA and phosphorylation of receptors of epidermal growth factor in cancer cells. While prepared gold nanoparticles from this plant and have antioxidant effects.
This study aims to biosynthesize zinc nanoparticles from the aqueous extract of *Panax ginseng* (Red Ginseng) roots and characterize their properties. SEM image observes shapes of zinc nanoparticles which are spherical to irregular particles with rate of size 59.76 nm. The FT-IR spectrum of ZnNPs exhibited that the numerous structures of acidic polysaccharides, amino acids, ginsenosides, polyacetylenes, and polyphenols in the Ginseng extract are not affected because of interaction their active groups to reduce and cap zinc ions to Zn⁰ and synthesizing zinc nanoparticles (ZnNPs). EDS reconfirmed that the nanoparticles formed are indeed metal ZnNPs. The concentration 100% Zinc nanoparticles significantly ($p<0.01$) recorded best inhibition percentage 41.70% against murine fibroblast cells (L20B) which have receptors of human polioviruses, thus, this work is considered as an auspicious first test to reduce the growth of cancers using green ZnNPs of Ginseng (*Panax ginseng*) *in vitro*. A zinc nanoparticle model is useful as an approach to increase activity of zinc in treatment of cancers due to the high surface area of ZnNPs.

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