Biological applications of Phytosynthesized gold nanoparticles using leaf extract of Dracocephalum kotschyi

Article *in* Journal of Biomedical Materials Research Part A · November 2018

DOI: 10.1002/jbm.a.36578

CITATIONS
5

READS
77

4 authors, including:

Azam Chahardoli
Razi University
11 PUBLICATIONS **72** CITATIONS

SEE PROFILE

Nasser Karimi
Razi University
90 PUBLICATIONS **711** CITATIONS

SEE PROFILE

Ali Fattahi
Arizona State University
61 PUBLICATIONS **438** CITATIONS

SEE PROFILE

Biological applications of Phytosynthesized gold nanoparticles using leaf extract of *Dracocephalum kotschyi*

Azam Chahardoli[a], Naser Karimi[a*], Ali Fattahi[b*] and Iraj Salimikia[c*]

[a]Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran.

[b]Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran.

[c]Department of Pharmacognosy, Faculty of Pharmacy, Lorestan University of Medical Sciences, Khorramabad, Iran.

*Corresponding authors:

Naser Karimi. Tell /Fax: +988334274545. E-mail address: nkarimi@razi.ac.ir.

Ali Fattahi. Tell/Fax: +988337243182-5. E-mail address: a.fatahi.a@gmail.com.

Iraj Salimikia. Tell/Fax: +988334274545. Eamil: salimikia.iraj@lums.ac.ir

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jbm.a.36578

Abstract

In this work, biosynthesis potentials of *Dracocephalum kotschyi* leaf extract for the production of gold nanoparticle (AuNPs) were studied, and the biological (catalytic, antibacterial, antioxidant and anticancer) activities of studied AuNPs were evaluated. Different analytical techniques including UV–vis spectroscopy, X-ray diffraction (XRD), fourier transform-Infrared spectroscopy (FT-IR), scanning electron microscope (SEM), energy dispersive X-ray analysis (EDX) and transmission electron microscopy (TEM) were used for the characterization of AuNPs. Moreover, Different testing methods were used for evaluating biological activities of biosynthesized AuNPs. The formation of AuNPs was confirmed by color change and UV–visible spectroscopic analysis. FE-SEM and TEM images were used to characterize phytosynthesized AuNPs which were predominantly spherical in shape with size in the range of 5–21 nm. These spherical NPs were found to be 39.79 ±5 nm in size as determined by DLS particle size analyzer. XRD pattern confirms the crystalline nature of the biosynthesized nanoparticles. The phytoconstituents involved in the reduction and stabilization of nanoparticles have been identified using FTIR spectra. The Phytosynthesized AuNPs showed effective antioxidant, antibacterial and catalytic reduction activities. Furthermore, they have inhibited H1229 and MCF-7 cancer cell lines proliferation in a dose-dependent manner. These results have supported that *D. kotschyi* leaf extract was very efficient for the synthesis of AuNPs, and synthesized NPs showed enhanced biological activities which make them suitable for biomedical applications.

Keywords: Biological activities**,** *Dracocephalum kotschyi*, Gold nanoparticles, Green synthesis, Leaf extract

1. Introduction

In recent years, the synthesis of nanoparticles (NPs) through various biological resources such as bacteria, fungi, yeast, and plant extracts has attracted considerable attention due to the broad range of natural resources with important bioactive compounds [1,2]. The green synthesized NPs are non-toxic, cost efficient and they have a wide application in various sectors of science [3,4]. Moreover, plant-based synthesis of nanoparticles is more advantageous over microbial synthesis because it does not require culturing and cell maintenance [5,6], and plant extracts are a good source of reducing agents.

Gold nanoparticles (AuNPs) owing to their unique and tunable surface plasmon resonance (SPR), have various applications in biological, industrial and commercial fields [7,8]. These NPs were used for cancer cell detection, drug delivery, encapsulation of drugs, antimicrobial agents, anticancer efficacy, nerve cell signaling stimulation, sensor analysis, membrane filtration and nano-barcodes [9,10]. In recent years, various plant materials such as *Euphorbia hirta* [11], *Aerva lanata* [12], *Cinnamomum zeylanicum* [13], *Dendropanax morbifera* [14] and *Albizia amara* [15] have been studied in this direction.

Nowadays, for the effective elimination of dye contaminants, the biosynthesis nanocatalysts are widely used [16]. Both the reduced metals and the plant compounds cornea act synergistically in catalytic degradation of organic dyes [17]. The catalytic

activity of green synthesized AuNPs by plant extracts has been reported recently with their capability to reduce the methylene blue dye [18,19]. The AuNPs have also been used recently as free radical scavengers and anticancer agents both in *in-vitro* and *in-vivo* models [20,21].

Dracocephalum kotschyi is a wild growing vegetative plant belongs to the family Labiatae. In Iranian traditional medicine, It is used for the therapy of congestion, headache, stomach and liver disorders [22]. Furthermore, it has antispasmodic, anti-nociceptive, antioxidant, antibacterial, antifungal and immune modulatory properties [23-25]. Anticancer effect of Xanthomicrol from leaves of *D. kotschyi* has been reported [26-28].Here we reported the rapid synthesis of AuNPs using *D. kotschyi* leaf extract and evaluated theirs *in vitro* biological applications (catalytic, antibacterial, antioxidant and cytotoxic activities).

2. Materials and Methods

2.1. Chemicals and Regents

Chloroauric acid ($HAuCl_4$), 1,1-Diphenyl-2- picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, DMSO, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), trypsin, aluminum chloride ($AlCl_3$), Mueller Hinton broth, methylen blue (MB), quercetin, gallic acid and methanol were purchased from Sigma–Aldrich, USA.

2.2. Preparation of leaf extract

The leaves of *D. kotschyi* were collected from research greenhouse of Razi University campus (Kermanshah, Iran) and washed properly to remove any traces of dust and impurities, and then they had been shade dried. 10 g of prepared fine powder was mixed

in 200 ml boiled water in 250 ml Erlenmeyer flask. The mixture was stirred at boiling point for 15 min, then cooled, centrifuged and filtered to get the appropriate aqueous extract.

2.3. Synthesis and characterization of AuNPs

For the green synthesis of AuNPs, 10 ml of *D. kotschyi* leaf extract was mixed with 90 ml of 1 mM aqueous solution of $HAuCl_4$ in 150 ml beaker. Then, the mixture was stirred continuously for 10 min at room temperature. The color change occurred from yellow to dark purple after 15 min, which confirming the reduction of Au^{+} to Au° and the formation of AuNPs. The biosynthesized AuNPs were characterized by scanning the aliquot sample in the wavelength range of 300–800 nm and recorded the maximum absorption in Shimadzu UV-2400 spectrophotometer at a resolution of 1 nm. The hydrodynamic diameter of phytosynthesized AuNPs was performed by Dynamic light scattering (DLS) and also, zeta-potential of NPs was carried out using ZETA Seizers Nanoseries (Malvern Instruments Nano ZS) to study the size and stability of the phytosynthesized AuNPs .

The XRD measurements were carried out on an APD 2000- Italian structures X-ray generator operated at a voltage of 40 kV and a current of 30 mA with Cu K^{-1} radiation. FTIR spectra of biosynthesized AuNPs and the plant extract are recorded using an IR prestige 21 Shimadzu spectrometer using the KBr pellet. The scanning electron microscope (SEM) analysis for determination the surface morphology of phytosynthesized AuNPs and energy dispersive X-ray analysis (EDX) for determine the elemental composition of these NPs were performed by MIRA3 FEG-SEM (Tescan,

Czech). TEM images are obtained using LEO 906, 80 kV transmission electron microscope.

2.4. Antibacterial activity

The antibacterial activity of biosynthesized AuNPs was assayed by Minimum Inhibitory Concentration (MIC) method using the standard broth dilution manner (CLSI-M07-A8). Live cells of pathogenic strains of gram-positive bacteria (*Staphylococcus epidermidis* (ATCC 12228), *Bacillus subtilis* (ATCC 6633), and *Staphylococcus aureus* (ATCC 43300)) and gram-negative bacteria (*Escherichia coli* (ATCC 25922), *Serratia marcescens* (ATCC 13880), and *Pseudomonas aeruginosa* (ATCC 27253)), at final concentrations of 5×10^5 CFU/ml were inoculated into 96 well plates. Then, 50 µl of the biosynthesized AuNPs added to each well and the test plates were incubated at 37 °C for 24 h. After this period, their concentrations were recorded. The negative (pure medium) and positive (medium containing bacteria) controls were maintained. The MIC was calculated based on the lowest concentration of biosynthesized nanoparticles that inhibit the bacterial growth.

2.5. Cytotoxicity studies

The cytotoxicity test of the biosynthesized AuNPs was performed *in vitro* against human breast cancer cell line (MCF-7) and a human non-small cell lung cancer cell line (H1299) using 3-(4,5-dimethylthiazol-2-yl-)-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, 180 µl of cell suspension at a concentration of 5×10^4 cells/ml was seeded in 96-well plates for 24 h. Following 24 h growth, 20 µl of NP suspensions at varying concentrations (10, 25, 50, 100 and 150 µg/ml for MCF-7 cell line and 10, 25, 50, 100 and 200 µg/ml for H1299 cell line), which were freshly prepared in distilled water, was

added to each well. Untreated cells were used as a control. After 48 h, cells were rinsed, and 100 µl of fresh medium containing 0.5 mg/ml of MTT was added to each well. Plates were incubated for an additional three h. Each well was then washed with 50 ml of PBS for removing medium containing unreacted MTT. Then, 150 ml of DMSO was added to each well to dissolve the formazan crystals. Finally, the absorbance was determined on an ELISA plate reader (Bio-Rad, Model 680, USA) with a test wavelength of 570 nm and a reference wavelength of 630 nm to obtain sample signal. All conditions were done in four replicate in three independent experiments for each cell line. The cell viability was expressed as follows;

Viability [%] $=A_s/A_c \times 100$

Where A_s and A_c are the mean absorbance of AuNPs treated and control cells respectively.

2.6. DPPH free radical scavenging activity assay

DPPH free radical scavenging activity was assayed by a standard method [29]. Briefly, 1 ml of 0.1 mM DPPH (in methanol) was added to different concentrations (100, 200, 300, 400 and 500 µg/ml) of plant extract and biosynthesized AuNPs. The reaction mixture was well stirred and incubated in the dark for 30 min. After incubation, the absorbance was determined at 517 nm against a blank. In this test, DPPH with methanol was used as a control. The percentage of inhibition or scavenging of free radicals was determined by the following formula:

Percentage of inhibition (%) = [(Absorbance $_{Control}$ − Absorbance $_{sample}$)/Absorbance $_{Control}$] × 100.

2.7. Total phenol content assay

The total phenolic compounds in plant extract and biosynthesized AuNPs samples were determined using the Folin–Ciocalteu method [30]. For this, 0.5 ml of the leaf extract and biosynthesized AuNPs solutions at varying concentrations of 100, 200, 300, 400 and 500 µg/ml were added to 2.5 ml of freshly prepared Folin – Ciocalteu reagent (0.2 N) and mixed for 5 min. Then, 2 ml of the 75 g/l sodium carbonate (Na_2CO_3) added to it. After two h incubation at room temperature, the absorbance of the reaction mixture was measured at 760 nm against the methanol as a blank. Gallic acid was used as a standard for drawing the calibration curve. The total phenolic content was expressed in µg of gallic acid equivalents.

2.8. Total flavonoid content assay

The modified Quettier – Deleu (2000) method [31], was used for determination of total flavonoid content in the plant extract and biosynthesized AuNPs. Aluminum chloride solution (in 2% methanol) was mixed with different concentrations (100, 200, 300, 400 and 500 µg/ml) of the plant extract and biosynthesized AuNPs. After the 15 min incubation, the absorbance was recorded at 415 nm against the blank sample. The total flavonoid content was determined using a standard curve of quercetin and was expressed as quercetin equivalents (µg QE).

2.9. Catalytic activity

The catalytic activity of biosynthesized AuNPs was evaluated against methylene blue dye by Nakkala *et al.* (2016) method [17]. In brief, AuNPs (100 µg/ml) was added to 10 ml of methylene blue (10^{-3} M) solution. Reduction of dye was determined by measuring the absorption maxima in the range of 200–800 nm at regular time intervals using UV–visible spectrophotometer.

3. Results

3.1. Synthesis and analysis of AuNPs

The green synthesis of AuNPs through plant extracts was carried out using $HAuCl_4$. After 15 min from the addition of leaf extract of *D. kotschyi* to the $HAuCl_4$ solution at room temperature, the change in the color was observed from yellow to dark purple. The dark purple color indicates bio-reduction of Au^{3+} to Au^0 and formation of AuNPs. The UV–vis spectroscopic analysis of AuNPs formation at different time intervals is shown in Figure 1. The characteristics surface plasmon resonance (SPR) peak for biosynthesized AuNPs was observed at 537 nm which confirmed the formation of these NPs.

The XRD analysis of biosynthesized AuNPs for its crystal structures is shown in Fig. 2. The diffraction intensities of biosynthesized AuNPs were recorded in the range of 20–80° (Figure 2). Four peaks observed at 38.3 °, 44.7 °, 64.8 ° and 77.6° which indexed to (111), (200), (220) and (311) reflections planes crystalline structure, respectively.

FT-IR analysis was carried out to identify the reducing and stabilizing ability of *D. kotschyi* leaf extract (Figure 3). The infrared spectrum showed absorption bands of *D. kotschyi* leaf extract at 3390.86, 2931.80, 1720.50, 1604.77, 1419.61, and 1076.28 that shifted to 3421.72, 2924.09, 1708.93, 1620.21, 1427.32, and 1072.42 in biosynthesized AuNPs respectively. The absorption bands at 3421, 2924 and1708 correspond to hydroxyl functional group of alcohols or N–H of amines, O-H stretch or C-H stretch of alkens, and C=O stretch of carboxylic acids, respectively. The bands at 1620 and 1427 correspond to C=C stretch of alkenes or N-H bend of amines and amides and C-H in-plane bend of alkenes or O-H bend of carboxylic acids, respectively. The bands at 1620

and 1427 correspond to C=C stretch of alkenes or N-H bend of amines and amides and C-H in-plane bend of alkenes or O-H bend of carboxylic acids, respectively.

The DLS analysis was used to find out the hydrodynamic size of nanoparticles. DLS revealed the average size of the AuNPs was found to be 39.79 ± 5.3 nm at 25°C as shown in Figure 4(a) and the intensity of peaks were found to be 91%. The polydispersity index was 0.335. Stability of AuNPs was measured through zeta potential, which was -11.4 mV as shown in Figure 4(b).

The transmission electron microscopy (TEM) gives information about the size, morphology, shape and dispersion of green biosynthesized AuNPs which is shown in Figure 5. The TEM image shows that biosynthesized AuNPs have nearly spherical shape, highly dispersed and small size of 5-21 nm. Also, the surface morphology of Phytosynthesized AuNPs was analyzed by SEM and they were found to be most of the particles are small and spherical in shape as shown in Figure 6. This observation is in good agreement with the data from UV–visible and TEM analysis indicating that the Phytosynthesized AuNPs were in similar spherical shape. Furthermore, The EDX spectrum showed a strong Au signal (at two different places with 51.65 %W at range of 2.1; 8.5; 9.8 keV supporting the formation of AuNPs) along with weak signals for O, C, and N (Figure 7).

3.2. Antibacterial efficiency of synthesized AuNPs

In the present study, MIC values against the strains of *S. marcescens, S. epidermidis* and *E. coli* calculated as 250 µg/ml and it was 62.5 µg/ml for *B. subtilis* (Table 1). Also, in treatment with plant leaf extract, MIC for *B. subtilis* and *S. marcescens* was 250 µg/ml.

3.3. Antioxidant activities

DPPH scavenging activity of plant leaf extract and biosynthesized AuNPs at various concentrations (100, 200, 300, 400 and 500 µg/ml) suggests that plant leaf extract was more effective than biosynthesized AuNPs and the antioxidant activity increased with increasing concentrations of plant leaf extract in the medium (Figure 8). The highest DPPH free radical scavenging activity of plant leaf extract was 90.2% at highest concentration (500 µg/ml) which it was 6.7 fold of the biosynthesized AuNPs with 13.5% inhibition activity at same concentration. In the case of biosynthesized AuNPs at concentrations higher than 200 µg/ml, there was a reduction in the inhibition percentage of DPPH.

3.4. Catalytic activity

In the present study, the dye degradation potential of biosynthesized AuNPs was tested against the MB and their absorbance, with a maximum absorption at 665 nm, was measured at different intervals of time to assess degradation (Figure 9). The relative absorbance of the MB following treatment with biosynthesized AuNPs calculated up to 62% degradation after 48 h.

3.5. Cytotoxic Properties

Green synthesized AuNPs were evaluated for their potential cytotoxicity against of MCF-7 and H1299 cell lines by MTT assay. MTT reduction by H1299 cells significantly decreased after a 48 h treatment with 10 µg/ml dose of biosynthesized AuNPs and 100 µg/ml dose of plant extract (Figure 10 (a)). The IC_{50} of biosynthesized AuNPs was calculated to be around 10 µg/ml. Furthermore, cytotoxicity of biosynthesized AuNPs and plant extract against the MCF-7 cell line has been shown in Figure 10 (b). IC_{50} for

biosynthesized AuNPs and plant extract calculated less than 10 µg/ml and exceed of 150 µg/ml respectively.

3.6. Phenolic compounds content

Phenolic compounds were studied by measuring the total phenol and flavonoid content in crude extract and biosynthesized AuNPs (Figure 11). The amount of total phenol in highest concentration (500 µg/ml) of biosynthesized AuNPs was calculated 143 µg/ml as compared to those found in plant extract with the amount of 310 µg/ml, in same concentrations (Figure 11(a)). Also, the total flavonoid content of biosynthesized AuNPs and plant extract were evaluated as 597 µg/ml and 1144 µg/ml in highest concentration (Figure 11(b)).

4. Discussion

The green synthesis of AuNPs through *D. kotschyi* leaf extract was carried out using $HAuCl_4$. The most important phytochemical constituents present in *D. kotschyi* leaf extract are reported to contain flavonoids (luteolin-7-O-glucoside, apigenin-7-O-glucoside (cosmosiin), luteolin 3'-O-β- D -glucuronide, luteolin, apigenin, cirsimaritin, iso-kaempferide, penduletin, xanthomicrol, calycopterin), a phenolic compound (rosmarinic acid) [32], and protein compounds, which can be responsible for the reduction of Au^{3+} ions. Similar results were also reported by many researchers[33-36]. For instance, previous reports have obtained AuNPs by using plant extracts at a longer incubation period, higher temperature, and greater ratio of plant extract to Au solution. For example, the incubation time was 5 h for *Nerium oleander* leaf extract at 60 °C [37], 5 h for *Piper longum* fruit extract [17], 60 min for *Moringa oleifera* petals [38], and 30 min for *Hovenia*

dulcis fruit extract [39], at room temperature. It was two h for plant extract of *Commelina nudiflora* at 37 °C [9]. The fast reduction of $HAuCl_4$ to AuNPs in our work can be attributed to the most important phytochemical constituents present in *D. kotschyi* leaf extract containing the flavonoids (luteolin-7-O-glucoside, apigenin-7-O-glucoside (cosmosiin), luteolin 3'-O-β- D -glucuronide, luteolin, apigenin, cirsimaritin, iso-kaempferide, penduletin, xanthomicrol, calycopterin) and a phenolic compound (rosmarinic acid). These compounds as reducing and capping agents are responsible for the formation and stability of AuNPs.

Among the techniques used for determination shape and size of the NPs in aqueous solution, UV-visible absorption spectroscopy is the primary one [40], which is due to its high sensitivity to morphological differences, interparticle interactions and refractive index of the medium. The change of color from yellow to dark purple is a characteristic feature of AuNPs formation due to the excitation of SPR in AuNPs at 537 nm that was confirmed in this study. The SPR properties of the metallic nanoparticles occur due to the oscillations of free electrons on the surface of the NPs when they align in resonance with the wavelength of irradiated light [40-42]. The colour change did not occurred after complete reduction of Au^{3+} to Au^0 was continued until 24 h without any significant shift in the peak wavelength to control the stability of the biosynthesized AuNPs. The intensity variation of the peaks relates to the greater formation of AuNPs on increasing the quantity of the phytochemicals substances presents in *D. kotschyi* leaf extract as bioreductants. As shown in Figure 1, there is a large gap between the absorbance spectrum at interval 10 and 20 min which, indicates that reaction took place and gets

completed in first 10 min and after it, the reaction rate gets slowed down without any significant change in total absorbance of reaction mixture.

The crystalline structure of biosynthesized AuNPs was confirmed by XRD analysis. A similar pattern of XRD for AuNPs has been reported by Shankar *et al*.,2004; Li *et al*., 2014; and Dorosti and Jamshidi, 2016 [36,43,44]. The particle size of the biosynthesized AuNPs has been calculated using the Debye–Scherrer equation, $D = k \lambda / \beta \cos \theta$ [45] by determining the width of the (111) peak. There was no additional peak in XRD pattern, which indicates the high purity of biosynthesized AuNPs. The average crystallite size of 8.2 nm obtained from XRD is also in agreement with the size calculated from TEM images.

FT-IR analysis showed absorption bands of biosynthesized AuNPs at 3421.72, 2924.09, 1708.93, 1620.21, 1427.32, and 1072.42, which correspond to functional groups of O–H, N–H, C-H , C=O, C=C, C-O and C-O-C respectively (Figure 3). It can be clearly seen that the O–H and N–H functional group as reducing agents for the formation of nanoparticles are the main constitutional components present in the flavonoids, terpenoids, and phenols of plant extract [42]. C=O stretching was observed in the FTIR peaks of biosynthesized AuNPs, which suggests the role of it coordination in the formation and stabilization of NPs [46,47]. The bonds relate to N-H and aliphatic C-H, propose the presence of proteins on the surface of biosynthesized AuNPs. These protein molecules act as a surfactant to attach on the surface of the nanoparticles which keep away from the internal agglomeration of the AuNPs and increase their stabilization during the synthesis reaction [48,49]. The bands or functional groups such as –C-O–C-, -C–O- and –C=C- derived from heterocyclic compounds, e.g. alkaloid or flavones, and the

amide-I bond derived from the proteins which are present in the leaf extract and the capping ligands of the nanoparticles [43,50,51].

As expected, the size of particle obtained from DLS (39.79 ± 5.3 nm) and TEM (5-21 nm) is different owing to the different principles used for measurement. The AuNPs with a negative zeta-potential value in the range of -10 to -30 mV have been shown to be stable in suspension, as the surface charge prevents aggregation of the particles [52], which it was confirmed in our study by value of -11.4 mV.

As shown in Figure 5 and 6, the morphology of biosynthesized AuNPs was nearly spherical in shape with small size, which was obtained by TEM and SEM analysis. A similar observation was found that spherical size is smaller than other morphologies [36,37,53,54]. Some large images of biosynthesized AuNPs with triangle, pentagon and hexagon shapes were also observed. The formation of these large triangular shaped nanoparticles may be due to the presence of relatively weak reducing agents in the leaf extracts or consuming of reducing agents during the time and lack of enough reducing agents, which help in the slow growth process [55].

EDAX analysis was performed to control the elemental composition of phytosynthesized AuNPs. The EDX spectrum showed a strong Au signal along with weak signals for O, C, and N (Figure 7). These weak signals which, naturally occurring in *D. kotschyi* leaf extract, may indicate the binding of these phytochemicals on the surface of the biosynthesized AuNPs and act as stabilizing agents.

In this study, also, the biological effects of biosynthesized AuNPs such as antibacterial, antioxidant and cytotoxicity properties were investigated. The most antibacterial effect of

these NPs was against *B. subtilis* with MIC values of 62.5 µg/ml whereas MIC values of biosynthesized NPs against other strains were 250 µg/ml (Table 1). In a similar study, by synthesized AuNPs from *Carica papaya* [56], MIC values for *E. coli, B. subtilis* and *S. aureus* were 62.5 µg/ml, 250 µg/ml, and 125 µg/ml, respectively that was in contrast to our study. In our work, MIC for *B. subtilis* and *E. coli* were calculated as 62.5 µg/ml and 250 µg/ml while no effect was observed on *S. aureus* and *P. aeruginosa*. This different activity on different bacteria may be related to physicochemical properties of nanoparticles and difference in plant metabolites absorbed by nanoparticles' cornea that play significant roles in tolerance or susceptibility of bacteria [57]. These differences in antibacterial activities can also be linked to differences in bacterial cell wall composition [56]. Kim *et al* [58] showed that the AuNPs make bind with active sites of the bacterial cell membrane and hence, inhibit the cell cycle function. Further studies revealed that the possible mechanisms of AuNPs interaction with the external surface of the bacterial membrane include its deposit in the cell wall, penetrate through porin channels in the membrane and interrupt the membrane integrity. After inclusion to a bacterial cell, it interacts with DNA structure and inhibits DNA opening and transcription, lead to growth limitation and bacteria cellular damage [59-61].

DPPH assay is a quick and simple method to evaluate the free radical scavenging ability of compounds. The nitrogen atom of DPPH contains odd electron, which is reduced by accepting the electron or hydrogen ion to form a corresponding yellow colored hydrazine molecule [17]. DPPH scavenging activity of plant leaf extract was more effective (6.7 fold) than biosynthesized AuNPs in dose dependent manner (Figure 8). In the case of biosynthesized AuNPs at concentrations higher than 200 µg/ml, there was a reduction in

the inhibition percentage of DPPH which can be due to aggregation and lack of full dispersion of NPs in these concentrations. Our result was accordance with the study of Nakkala *et al.* which showed that antioxidant activity of plants extract of *Piper longum* and *Costus pictus* was more than synthesized AuNPs [17,33]. Furthermore, the similar result obtained by Kuppusamy *et al.* [9] using synthesized AuNPs from *Commelina nudiflora*. The reason for the higher DPPH scavenging of plant extract as compared to biosynthesized AuNPs may be due to the phytocompounds present in plant extract which are more than ones attached to NPs [47].

In addition, the antioxidant activity of biosynthesized AuNPs were evaluated by measuring the Phenolic compounds. Our result revealed that total phenol and total flavonoid content of the extract was greater than biosynthesized AuNPs (Figure 11). which accordance to an earlier study of Phull *et al*.(2016) on synthesized AgNPs using plant extract of *Bergenia ciliata* [62]. In overall, plant biomolecules such as phenols and flavonoids are nucleophilic in nature due to the presence of aromatic rings and responsible for the chelating potential [63]. Binding of these biomolecules with metal is confirmed by measuring their content in nanoparticles. Our present observation suggested that highly potential reducing agents such as flavonoids and phenolic compounds are responsible for reducing $HAuCl_4$ to AuNPs. The greater content of flavonoids than phenols compounds in *D. kotschyi* suggested its important role as a reducing agent in AuNPs formation.

The results of our cytotoxicity assays apparently recommended that biosynthesized AuNPs inhibited potential growth of cancer cells (MCF-7 and H1299) in a dose-dependent manner. In both studied cell lines, the percentage of cell viability which

treated with plant extract was significantly greater than biosynthesized AuNPs treated cells at similar dosages (Figure 10 (a & b)). The IC_{50} of biosynthesized AuNPs was calculated to be around 10 µg/ml for H1299 and MCF-7 cell lines.

Ramalingam *et al*. (2016) showed the anticancer efficiency of AuNPs against A549 lung cancer cell line, which, 81% and 54% of cell death was observed at 20 µg/ml and14 µg/ml treatments, respectively [64]. Their results showed that IC_{50} dose efficiently induces apoptosis and necrosis of A549 cells and creation of oxidative stress and reactive oxygen species by AuNPs which might stimulate the sensitization of mitochondrial membrane that leads to actuate the apoptosis pathway [64]. Chahardoli *et al*. (2017) showed that the percentage cell death of MCF-7 and H1299 cells slowly increases with the addition of AuNPs concentration and significantly inhibited the cancer cells with the lowest amount of AuNPs [65]. However, earlier studies have been reported that interactions between different types of nanoparticles and cells are depended on shape, size and surface chemistry of the nanoparticles and the nature of cells [2,66,67].

Methylene blue (MB) is a thiazine dye that used for analysis of amounts of sulfide ions in aquatic samples. The cationic form of MB is used as an anti-malarial and chemotherapeutic agent in the aquaculture industry [68,69]. Even though MB is not considered as a very toxic dye, it can be harmful to human health with creating difficulties in breathing, vomiting, diarrhea and nausea [70]. Recently, catalytic applications of AuNPs in different reactions have attracted considerable attention. In fact, gold in bulk state is chemically inert because of the positive redox potential of this noble metal, while, at a nano-scale level due to the reduction of its redox potential to a negative value, it is becoming catalytically active for various chemicals [71,72]. Therefore, the redox potential of

AuNPs requirements to be present among the redox potential of the donor and the acceptor system until that act as an effective catalyst [73].

According to our results, the degradation of MB after 48 h treatment with biosynthesized AuNPs was calculated up to 62%. Similar catalytic activity has been reported by Chahardoli *et al.* (2017) for green synthesized AuNPs from *Nigella arvensis* which caused 44% degradation of MB dye [65] . They stated that AuNPs as electron transfer mediators acted as redox catalysts between the plant biomolecules and the dyes that are often called as electron relay effect [65]. Furthermore, synthesized AuNPs using *Piper longum* showed catalytic activity against MB after 28 h up to 64% degradation [17].These obtained results accordance to our study. In this study, the possible reasons for the low catalytic activity of biosynthesized AuNPs can be attributed to the applied form of NPs (powder form) without $NaBH_4$ and sunlight as reducing and inducing agent in the reduction of MB. Therefore, the photo-reduction, which reported in others studies, was not occurred [19,74-76]. Furthermore, in the present study, used concentration of dye was higher than other reported studies.

5. Conclusion

Using the plant extracts for biosynthesis of nanoparticles is a green method for preparation of AuNPs with plant bioactive molecules without the utilization of any toxic reducing and capping agents. We investigated the biosynthesizing AuNPs via reduction of aqueous $AuCl_4^-$ ions using *D. kotschyi* leaf extracts. The biosynthesized AuNPs were characterized by UV–vis, DLS, XRD, FT-IR, Fe-SEM, EDX and TEM to identify their nature, compositions, sizes and morphologies of particles. This nanoparticles exhibited promising antioxidant and antimicrobial activities, cytotoxicity potential and also well

catalytic activity against methylene blue. These biosynthesized AuNPs can be suitable for medicinal applications.

Acknowledgments and Funding

The authors gratefully acknowledge the Graduate School of Razi University, Pharmacy Faculty of Lorestan University of Medical Sciences and Research Councils of Kermanshah University of Medical Sciences for their financial support.

References

1. Fayaz AM, Balaji K, Girilal M, Yadav R, Kalaichelvan PT, Venketesan R. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gram-negative bacteria. Nanomed Nanotechnol Biol Med 2010;6(1):103-109.
2. Gurunathan S, Han J, Park JH, Kim J-H. A green chemistry approach for synthesizing biocompatible gold nanoparticles. Nanoscale Res Lett 2014;9(1):1.
3. Nabikhan A, Kandasamy K, Raj A, Alikunhi NM. Synthesis of antimicrobial silver nanoparticles by callus and leaf extracts from saltmarsh plant, *Sesuvium portulacastrum* L. Colloid Surf B Biointerface 2010;79(2):488-93.
4. Swamy MK, Akhtar MS, Mohanty SK, Sinniah UR. Synthesis and characterization of silver nanoparticles using fruit extract of *Momordica cymbalaria* and assessment of their in vitro antimicrobial, antioxidant and cytotoxicity activities. Spectrochim Acta Mol Biomol Spectrosc 2015;151:939-944.
5. Akhtar MS, Panwar J, Yun Y-S. Biogenic Synthesis of Metallic Nanoparticles by Plant Extracts. ACS Sustainable Chem Eng 2013;1(6):591-602.

6. Prabhu S, Poulose EK. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett 2012;2(1):1-10.
7. Rajan A, Vilas V, Philip D. Studies on catalytic, antioxidant, antibacterial and anticancer activities of biogenic gold nanoparticles. J Mol Liq 2015;212:331-339.
8. Patra JK, Kwon Y, Baek K-H. Green biosynthesis of gold nanoparticles by onion peel extract: Synthesis, characterization and biological activities. Adv Powder Technol 2016.
9. Kuppusamy P, Yusoff MM, Ichwan SJ, Parine NR, Maniam GP, Govindan N. Commelina nudiflora L. edible weed as a novel source for gold nanoparticles synthesis and studies on different physical–chemical and biological properties. J Indust Eng Chem 2015;27:59-67.
10. Ghosh S, Patil S, Ahire M, Kitture R, Gurav DD, Jabgunde AM, Kale S, Pardesi K, Shinde V, Bellare J. *Gnidia glauca* flower extract mediated synthesis of gold nanoparticles and evaluation of its chemocatalytic potential. J Nanobiotechnol 2012;10(1):1.
11. Annamalai A, Christina V, Sudha D, Kalpana M, Lakshmi P. Green synthesis, characterization and antimicrobial activity of Au NPs using *Euphorbia hirta* L. leaf extract. Colloid Surf B Biointerface 2013;108:60-65.
12. Joseph S, Mathew B. Microwave assisted facile green synthesis of silver and gold nanocatalysts using the leaf extract of *Aerva lanata*. Spectrochim Acta Mol Biomol Spectrosc 2015;136:1371-1379.

13. Smitha S, Philip D, Gopchandran K. Green synthesis of gold nanoparticles using *Cinnamomum zeylanicum* leaf broth. Spectrochim Acta Mol Biomol Spectrosc 2009;74(3):735-739.
14. Wang C, Mathiyalagan R, Kim YJ, Castro-Aceituno V, Singh P, Ahn S, Wang D, Yang DC. Rapid green synthesis of silver and gold nanoparticles using Dendropanax morbifera leaf extract and their anticancer activities. Int J Nanomed 2016;11:3691.
15. Balasubramani G, Ramkumar R, Raja RK, Aiswarya D, Rajthilak C, Perumal P. *Albizia amara* Roxb. mediated gold nanoparticles and evaluation of their antioxidant, antibacterial and cytotoxic properties. J Cluster Sci 2016:1-17.
16. Bhakya S, Muthukrishnan S, Sukumaran M, Muthukumar M. Biogenic synthesis of silver nanoparticles and their antioxidant and antibacterial activity. Appl Nanosci 2015;6(5):755-766.
17. Nakkala JR, Mata R, Sadras SR. The antioxidant and catalytic activities of green synthesized gold nanoparticles from *Piper longum* fruit extract. Process Saf Environ 2016;100:288-294.
18. Das J, Velusamy P. Catalytic reduction of methylene blue using biogenic gold nanoparticles from *Sesbania grandiflora* L. J Taiwan Inst Chem Eng 2014;45(5):2280-2285.
19. Suvith V, Philip D. Catalytic degradation of methylene blue using biosynthesized gold and silver nanoparticles. Spectrochim Acta Mol Biomol Spectrosc 2014;118:526-532.

20. Dipankar C, Murugan S. The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from *Iresine herbstii* leaf aqueous extracts. Colloid Surf B: Biointerface 2012;98:112-119.
21. Muniyappan N, Nagarajan N. Green synthesis of gold nanoparticles using *Curcuma pseudomontana* essential oil, its biological activity and cytotoxicity against human ductal breast carcinoma cells T47D. J Environ Chem Eng 2014;2(4):2037-2044.
22. Rechinger K. Flora iranica: no. 150. Labiatae. Graz. Akademische Druck 1982;2.
23. Sonboli A, Esmaeili MA, Gholipour A, Kanani MR. Composition, cytotoxicity and antioxidant activity of the essential oil of *Dracocephalum surmandinum* from Iran. Nat Prod Commun 2010;5(2):341-344.
24. Sonboli A, Gholipour A, Yousefzadi M. Antibacterial activity of the essential oil and main components of two Dracocephalum species from Iran. Nat Prod Res 2012;26(22):2121-2125.
25. Sadraei H, Asghari G, Kasiri F. Comparison of antispasmodic effects of *Dracocephalum kotschyi* essential oil, limonene and α-terpineol. Res Pharm Sci 2015;10(2):109.
26. Saeidnia S, Gohari AR, Uchiyama N, Ito M, Honda G, Kiuchi F. Two new monoterpene glycosides and trypanocidal terpenoids from *Dracocephalum kotschyi*. Chem Pharm Bullet 2004;52(10):1249-1250.
27. Saeidnia S, Gohari AR, Ito M, Kiuchi F, Honda G. Bioactive Constituents from *Dracocephalum subcapitatum* (O. Kuntze) Lipsky. Z Naturforsch C 2005;60(1-2):22-24.

28. Jahaniani F, Ebrahimi SA, Rahbar-Roshandel N, Mahmoudian M. Xanthomicrol is the main cytotoxic component of *Dracocephalum kotschyii* and a potential anti-cancer agent. Phytochemistry 2005;66(13):1581-1592.
29. Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, Park SH, Kim SK. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci 2002;163(6):1161-1168.
30. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in *Burkina Fasan* honey, as well as their radical scavenging activity. Food Chem 2005;91(3):571-577.
31. Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M, Cazin M, Cazin J-C, Bailleul F, Trotin F. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J Ethnopharmcol 2000;72(1):35-42.
32. Fattahi M, Nazeri V, Torras-Claveria L, Sefidkon F, Cusido RM, Zamani Z, Palazon J. Identification and quantification of leaf surface flavonoids in wild-growing populations of Dracocephalum kotschyi by LC–DAD–ESI-MS. Food Chem 2013;141(1):139-146.
33. Nakkala JR, Bhagat E, Suchiang K, Sadras SR. Comparative study of antioxidant and catalytic activity of silver and gold nanoparticles synthesized from *Costus pictus* leaf extract. J Mater Sci Technol 2015;31(10):986-994.
34. Geetha R, Ashokkumar T, Tamilselvan S, Govindaraju K, Sadiq M, Singaravelu G. Green synthesis of gold nanoparticles and their anticancer activity. Cancer Nanotechnol 2013;4(4-5):91-98.

35. Muthuvel A, Adavallan K, Balamurugan K, Krishnakumar N. Biosynthesis of gold nanoparticles using *Solanum nigrum* leaf extract and screening their free radical scavenging and antibacterial properties. Biomed Prevent Nutrition 2014;4(2):325-332.
36. Dorosti N, Jamshidi F. Plant-mediated gold nanoparticles by *Dracocephalum kotschyi* as anticholinesterase agent: Synthesis, characterization, and evaluation of anticancer and antibacterial activity. J Appl Biomed 2016;14(3):235-245.
37. Tahir K, Nazir S, Li B, Khan AU, Khan ZUH, Gong PY, Khan SU, Ahmad A. *Nerium oleander* leaves extract mediated synthesis of gold nanoparticles and its antioxidant activity. Mater Lett 2015;156:198-201.
38. Anand K, Gengan RM, Phulukdaree A, Chuturgoon A. Agroforestry waste Moringa oleifera petals mediated green synthesis of gold nanoparticles and their anti-cancer and catalytic activity. J Ind Eng Chem 2015;21:1105-1111.
39. Basavegowda N, Idhayadhulla A, Lee YR. Phyto-synthesis of gold nanoparticles using fruit extract of *Hovenia dulcis* and their biological activities. Ind Crop Prod 2014;52:745-751.
40. Wiley BJ, Im SH, Li Z-Y, McLellan J, Siekkinen A, Xia Y. Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis. The J PhysChem B 2006;110(32):15666-15675.
41. Mulvaney P. Surface plasmon spectroscopy of nanosized metal particles. Langmuir 1996;12(3):788-800.
42. Nayak D, Ashe S, Rauta PR, Kumari M, Nayak B. Bark extract mediated green synthesis of silver nanoparticles: Evaluation of antimicrobial activity and

antiproliferative response against osteosarcoma. Mater Sci Eng C Mater Biol Appl 2016;58:44-52.

43. Shankar SS, Rai A, Ahmad A, Sastry M. Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. J colloid Interface Sci 2004;275(2):496-502.
44. Li X, Robinson SM, Gupta A, Saha K, Jiang Z, Moyano DF, Sahar A, Riley MA, Rotello VM. Functional gold nanoparticles as potent antimicrobial agents against multi-drug-resistant bacteria. ACS nano 2014;8(10):10682-10686.
45. Ashokkumar S, Ravi S, Kathiravan V, Velmurugan S. Synthesis, characterization and catalytic activity of silver nanoparticles using *Tribulus terrestris* leaf extract. Spectrochim Acta Mol Biomol Spectrosc 2014;121:88-93.
46. Ahluwalia V, Kumar J, Sisodia R, Shakil NA, Walia S. Green synthesis of silver nanoparticles by Trichoderma harzianum and their bio-efficacy evaluation against *Staphylococcus aureus* and *Klebsiella pneumonia*. Indust Crop Prod 2014;55:202-206.
47. Mata R, Nakkala JR, Sadras SR. Catalytic and biological activities of green silver nanoparticles synthesized from *Plumeria alba* (frangipani) flower extract. Mater Sci Eng C 2015;51:216-225.
48. Ajitha B, Reddy YAK, Reddy PS. Biogenic nano-scale silver particles by *Tephrosia purpurea* leaf extract and their inborn antimicrobial activity. Spectrochim Acta Mol Biomol Spectrosc 2014;121:164-172.

49. Narayanan KB, Sakthivel N. Green synthesis of biogenic metal nanoparticles by terrestrial and aquatic phototrophic and heterotrophic eukaryotes and biocompatible agents. Adv Colloid Interface Sci 2011;169(2):59-79.
50. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang Y, Shao W, He N. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomum camphora* leaf. Nanotechnology 2007;18(10):105104.
51. Sastry M, Ahmad A, Khan MI, Kumar R. Biosynthesis of metal nanoparticles using fungi and actinomycete. Curr Sci 2003;85(2):162-170.
52. Elia P, Zach R, Hazan S, Kolusheva S, Porat Ze, Zeiri Y. Green synthesis of gold nanoparticles using plant extracts as reducing agents. International journal of nanomedicine 2014;9:4007.
53. Fazal S, Jayasree A, Sasidharan S, Koyakutty M, Nair SV, Menon D. Green synthesis of anisotropic gold nanoparticles for photothermal therapy of cancer. ACS Appl Mater Interface 2014;6(11):8080-8089.
54. Alam MN, Das S, Batuta S, Roy N, Chatterjee A, Mandal D, Begum NA. *Murraya koenegii* Spreng. leaf extract: an efficient green multifunctional agent for the controlled synthesis of Au nanoparticles. ACS Sustainable Chemistry & Engineering 2014;2(4):652-664.
55. Lee J, Kim HY, Zhou H, Hwang S, Koh K, Han D-W, Lee J. Green synthesis of phytochemical-stabilized Au nanoparticles under ambient conditions and their biocompatibility and antioxidative activity. J Mater Chem 2011;21(35):13316-13326.

56. Muthukumar T, Sambandam B, Aravinthan A, Sastry TP, Kim J-H. Green synthesis of gold nanoparticles and their enhanced synergistic antitumor activity using HepG2 and MCF7 cells and its antibacterial effects. Process Biochem 2016;51(3):384-391.
57. Hajipour MJ, Fromm KM, Ashkarran AA, de Aberasturi DJ, de Larramendi IR, Rojo T, Serpooshan V, Parak WJ, Mahmoudi M. Antibacterial properties of nanoparticles. Trend Biotechnol 2012;30(10):499-511.
58. Kim JS, Kuk E, Yu KN, Kim J-H, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang C-Y. Antimicrobial effects of silver nanoparticles. Nanomed Nanotechnol Biol Med 2007;3(1):95-101.
59. Bauer LA, Birenbaum NS, Meyer GJ. Biological applications of high aspect ratio nanoparticles. J Mater Chem 2004;14(4):517-526.
60. Rai A, Prabhune A, Perry CC. Antibiotic mediated synthesis of gold nanoparticles with potent antimicrobial activity and their application in antimicrobial coatings. J Mater Chem 2010;20(32):6789-6798.
61. Srivastava N, Mukhopadhyay M. Biosynthesis and characterization of gold nanoparticles using *Zooglea ramigera* and assessment of its antibacterial property. J Cluster Sci 2015;26(3):675-692.
62. Phull A-R, Abbas Q, Ali A, Raza H, kim SJ, Zia M, Haq I-u. Antioxidant, cytotoxic and antimicrobial activities of green synthesized silver nanoparticles from crude extract of *Bergenia ciliata*. Future J Pharm Sci 2016;2(1):31-36.
63. Sivaraman SK, Elango I, Kumar S, Santhanam V. A green protocol for room temperature synthesis of silver nanoparticles in seconds. Curr Sci 2009;97(7):1055-1059.

64. Ramalingam V, Revathidevi S, Shanmuganayagam T, Muthulakshmi L, Rajaram R. Biogenic gold nanoparticles induce cell cycle arrest through oxidative stress and sensitize mitochondrial membranes in A549 lung cancer cells. RSC Advances 2016;6(25):20598-20608.
65. Chahardoli A, Karimi N, Sadeghi F, Fattahi A. Green approach for synthesis of gold nanoparticles from Nigella arvensis leaf extract and evaluation of their antibacterial, antioxidant, cytotoxicity and catalytic activities. Artif Cell Nanomed Biotechnol 2017:1-10.
66. Park MV, Neigh AM, Vermeulen JP, de la Fonteyne LJ, Verharen HW, Briedé JJ, van Loveren H, de Jong WH. The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. Biomaterials 2011;32(36):9810-9817.
67. Alkilany AM, Murphy CJ. Toxicity and cellular uptake of gold nanoparticles: what we have learned so far? J Nanopart Res 2010;12(7):2313-2333.
68. Small JM, Hintelmann H. Methylene blue derivatization then LC–MS analysis for measurement of trace levels of sulfide in aquatic samples. Anal Bioanal chem 2007;387(8):2881-2886.
69. Burhenne J, Riedel K-D, Rengelshausen J, Meissner P, Müller O, Mikus G, Haefeli WE, Walter-Sack I. Quantification of cationic anti-malaria agent methylene blue in different human biological matrices using cation exchange chromatography coupled to tandem mass spectrometry. J Chromatogr B 2008;863(2):273-282.
70. Kavitha D, Namasivayam C. Experimental and kinetic studies on methylene blue adsorption by coir pith carbon. Bioresource Technol 2007;98(1):14-21.

71. Hvolbæk B, Janssens TV, Clausen BS, Falsig H, Christensen CH, Nørskov JK. Catalytic activity of Au nanoparticles. Nano Today 2007;2(4):14-18.
72. Haruta M, Yamada N, Kobayashi T, Iijima S. Gold catalysts prepared by coprecipitation for low-temperature oxidation of hydrogen and of carbon monoxide. J Catal 1989;115(2):301-309.
73. Mallick K, Witcomb M, Scurrell M. Silver nanoparticle catalysed redox reaction: an electron relay effect. Mater Chem Phys 2006;97(2):283-287.
74. Bonnia N, Kamaruddin M, Nawawi M, Ratim S, Azlina H, Ali E. Green biosynthesis of silver nanoparticles using '*Polygonum Hydropiper*'and study its catalytic degradation of methylene blue. Proced Chem 2016;19:594-602.
75. Vanaja M, Paulkumar K, Baburaja M, Rajeshkumar S, Gnanajobitha G, Malarkodi C, Sivakavinesan M, Annadurai G. Degradation of methylene blue using biologically synthesized silver nanoparticles. Bioinorg Chem Appl 2014;2014.
76. Daniel M-C, Astruc D. Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. Chem Rev 2004;104(1):293-346.

Figure legends

Figure 1. UV–Vis spectra of AuNPs biosynthesized from *Dracocephalum kotschyi* leaf extract.

Figure 2. XRD pattern of biosynthesized AuNPs using *D. kotschyi* leaf extract.

Figure 3. FT-IR spectra of *D. kotschyi* leaf extract and biosynthesized AuNPs.

Figure 4. Particle size analysis. a) Size distribution and b) zeta potential distribution of biosynthesized AuNPs using *D. kotschyi* leaf extract.

Figure 5. TEM imageof biosynthesized AuNPs at different magnifications.

Figure 6. FE-SEM analysis of AuNPs biosynthesized from *D. kotschyi* leaf extract.

Figure 7. EDX analysis of AuNPs biosynthesized from *D. kotschyi* leaf extract.

Figure 8. percent of DPPH scavenging activity of *D. kotschyi* extract and biosynthesized AuNPs at various concentrations. **(**Data represent as Mean ± S.E (n = 3). Different letters on each error bar are statistically significant at $p < 0.05$ level).

Figure 9. UV–vis spectra showing degradation of MB on addition of biosynthesized AuNPs in varying times.

Figure 10. *In vitro* cytotoxic activity of biosynthesized AuNPs against: a) H1299 cell line and b) MCF-7 cell line at various concentrations.

Figure 11. (a)Total phenolic and (b)Total flavonoid content of *D. kotschyi* extract and biosynthesized AuNPs at various concentrations (µg/ml).

Table 1 Antibacterial activity of biosynthesized AuNPs and extract of *D. Kotschyi* by MIC method.

Bacteria strains	**AuNPs (µg/ml)**	**Plant extract (µg/ml)**
E. coli	250	-
P. aeruginosa	250	-
S. marcescens	250	250
S. aureus	-	-
B. subtilis	62.5	250
S. epidermidis	-	-