Watercress-based gold nanoparticles: biosynthesis, mechanism of formation and study of their biocompatibility in vitro

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To avoid the potential adverse effects of chemically-synthesised gold nanoparticles (AuNPs), a new method is developed for their biosynthesis using watercress total extract (WTE) and it is compared it with the citrate-based method. Synthesised AuNPs were characterised using transmission electron microscopy (TEM), particle size analysis and infrared spectroscopy (FTIR), and the mechanism and biocompatibility of WTE-based AuNPs were evaluated by MTT assay. The colour of the WTE-based AuNPs was directly changed from yellow to deep red without any intermediate colour change and spectrophotometric analysis showed significant differences in absorbance as well as peak surface plasmon resonance of synthesised AuNPs as compared with the use of sodium citrate. Formation of AuNPs in the WTE-based method was faster than the method using citrate. TEM pictures showed similar sizes of the AuNPs obtained by both methods, but WTE-based AuNPs were more spherical and the range of sizes provided minor differences with TEM results. FTIR confirmed the involvement of WTE compounds in the structure of AuNPs during biosynthesis and the MTT assay did not show any considerable anti-growth effect of WTE-based AuNPs on target cells. Hence, it seems clear that WTE-AuNPs could be used for various biological-related purposes including drug delivery, diagnosis and treatment of various diseases.

1. Introduction: Gold nanoparticles (AuNPs) are widely used for the sensing and treatment of many diseases such as cancer, and are becoming increasingly important tools in the biomedical sciences. Various methods have been proposed for the synthesis of AuNPs, most of them based on the chemical reduction of Au^{3+} ions [1]. The use of chemical agents for the preparation of AuNPs could affect their biocompatibility and provide environmental side-effects. In addition, these chemicals participate in the structure of the synthesised nanoparticles and could limit their in vivo applications [2]. Therefore safe methods should be used to prepare AuNPs. Most of the safe methods are based on green chemistry and take advantage of the use of plant materials for both reducing and capping AuNPs. Biocompatibility and eco-friendliness are the most significant features of green chemistry-based AuNPs [3].

Watercress (*Nasturtium officinale*) is a common edible plant consumed all over the world. It is rich in potent anti-oxidants, iron, iodine, manganese, calcium, vitamins and isothiocyanates [4–6]. This plant has many medicinal effects including diuretic and anti-hypertensive [7], anti-microbial [8, 9], anti-diabetes and hypoglycaemic [10], anti-hyperlipidemic [11], and anti-cancer properties [12–14]. In this present Letter, considering the various medicinal and nutritional applications of watercress, a simple and rapid biosynthesis of biocompatible AuNPs is proposed using watercress total extracts (WTEs). The characteristics of biosynthesised AuNPs are determined and compared with those of citrate-synthesised AuNPs. Finally, the reaction mechanism of WTE-based AuNPs was elucidated and their biocompatibility was studied by tetrazolium dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.

2. Materials and methods

2.1. Materials and instruments: AuNPs were characterised using an UV-visible spectrophotometer Bioaquarius (CE7250, CECIL Co., UK), a tunneling electron microscope EM 208 (Philips, USA), a Digimizer software (MedCalc Software, Belgium), a particle size analyser (Shimadzu Co., Japan) and a Fourier transform infrared (FTIR) spectrophotometer (Shimadzu Co., Japan). A STATFAX200 Microplate Reader (Diamedix Co., USA) was used for the tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

$HAuCl_4{\cdot}3H_2O$, HCL, NaOH and trisodium citrate were purchased form Merck, Germany. NaCl, glycine and MTT were supplied by Sigma Aldrich, Germany; Whatman filter paper no. 2 was obtained from GE Healthcare, UK; RPMI-1640 powder and heat-inactivated fetal bovine serum were supplied by Gibco, Invitrogen, USA; the T47D breast cancer cell line was purchased from American Type Tissue Culture (code: HTB-133), USA.

Ultrapure water (18 MΩ water) was prepared using a deioniser R.101.D (Absazco, Iran) and all glasswares were treated with aqua-regia solution (3 : 1 HCl: HNO_3) for 3 h, washed three to six times with ultrapure water and dried at 100°C for 2 h before use.

2.2. Methods

2.2.1 Preparation of WTE: Fresh watercress (see inset of Fig. 1*a*) was collected from suburban areas of Julfa city located in north-western Iran. After careful washing, the collected watercress was dried for five days in shadow. Then, the dried plant was ground to a fine powder. For preparation of the total extract, 30 g of watercress powder was boiled in 400 ml of ultrapure water for 20 min. Then, the resulting solution was filtered out thorough Whatman paper no. 2, dried at 50°C during five days and stored at −20°C until further use.

The WTE stock solution was prepared by dissolving the dried extract in sterile ultrapure water in the final concentration of 10 mg/ml, and the prepared solution was stored at 4°C and used within eight days.

2.2.2 Biosynthesis of AuNPs with WTE: AuNPs were biosynthesised by reduction of 1 mM of Au^{3+} solution with WTE at different conditions including different ratios, temperatures and pHs while shaking at 115 rpm.

Different ratios of Au^{3+} solution/WTE (3:1, 4:1, 5:1 and 6:1) were treated at four different temperatures (91, 80, 60 and 37°C) while shaking at 115 rpm. Five pHs (3, 4, 5, 6 and 12) were used for the biosynthesis of AuNPs by WTE.

The biosynthesised AuNPs were inspected by the naked eye for colour change from yellow to ruby red. Also, the time point of colour change was recorded for each reaction.

The time needed for completion of the biosynthesis reaction was determined by measuring the absorbance of biosynthesised AuNPs at 1 min intervals. The biosynthesised AuNPs were stored in the dark at 4°C until further analysis.

2.2.3 Chemical synthesis of AuNPs: AuNPs were also synthesised by the citrate-reduction method [15]. This involved the addition of 10 ml of 38.8 mM trisodium citrate to 100 ml of boiling 1 mM Au^{3+} aqueous solution while stirring in the refluxing condition. The synthesised AuNPs were stored in dark at 4°C until further analysis.

2.2.4 Characterisation of synthesised nanoparticles: Synthesised nanoparticles were evaluated using colourimetric analysis, transmission electron microscopy (TEM), particle size analysis and FTIR.

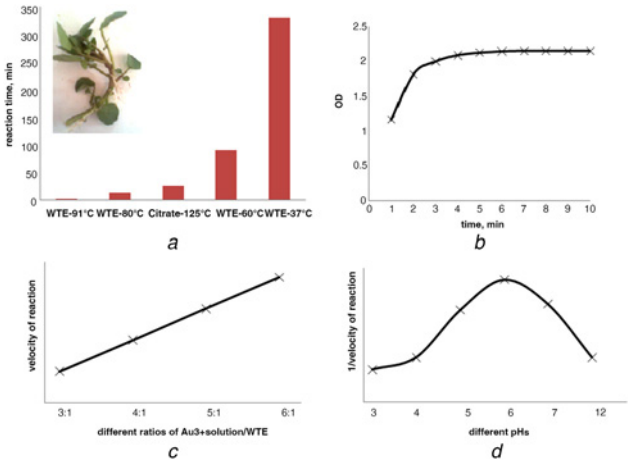

Figure 1 *Reaction time needed for synthesis of AuNPs*
a Comparison between times required for WTE-based method in different experimental conditions and with citrate-based method
Inset: Watercress plant
b Evolution with the time of the absorbance of AuNPs obtained by WTE-based method (91°C at 6:1 ratio)
c Relationship between the velocity of reaction and the Au^{3+}/WTE ratio
d Relationship between the velocity of reaction and pH

After synthesis, the AuNPs were mixed with 6 M of NaCl solution. Any change in colour was recorded between 300 and 800 nm using a quartz cuvette. Peak surface plasmon resonance (SPR) was determined for each condition of extract-based as well as chemical methods.

The size and morphology of the synthesised nanoparticles were studied by TEM according to the literature [16]. A few droplets of the nanoparticle solutions were sonicated, placed on a carbon grid and after drying the pictures were taken with 90 or 100 kV power. Then, the mean size and distribution of approximately 500 particles (citrate-based method), 1400 particles (4:1 ratio of WTE-based method) and 607 particles (6:1 ratio of WTE method) were obtained by measurement of the nanoparticles' diameter in the captured TEM pictures using Digimizer software version 4.

In addition to analysis of the TEM pictures, the size and distribution of AuNPs, prepared by WTE-based method, were confirmed using a particle size analyser and to do this 4–5 ml of each sample was sonicated and analysed.

The possible incorporation of WTE compounds in the structure of biosynthesised AuNPs was evaluated by FTIR [17]. The test sample was centrifuged at 16 000 rpm for 20 min. Then, the supernatant was removed and the pellet was washed once with pure ethanol and two times with ultrapure water by repeating the centrifugation step. Then, the test sample was dried completely at 45°C. The control sample was prepared by drying of WTE at the mentioned temperature. Finally, the dry samples were grounded with KBr and the FTIR spectra were recorded in the range of 4000–400 cm^{-1}.

2.2.5 Evaluation of biocompatibility using MTT assay: The biocompatibility of AuNPs, prepared by the WTE-based method, was studied through a tetrazolium dye MTT assay [18]. 3000 T47D breast cancer cells were seeded per well of a 96-well MTT assay plate. After 24 h incubation, different concentrations of AuNPs (0–100%) were added in triplicate. A 0% AuNP solution was used as the control well. The plate was incubated for 24 h at 37°C in the presence of 5% CO_2 in an humidified atmosphere. Then, the media were exchanged with fresh RPMI-1640 containing 10% fetal bovine serum and 25 µl of 2 mg/ml MTT solution was added to each well and incubated for 4 h in the dark. Then, 200 µl of DMSO and 25 µl of Sorensen's glycine buffer were added to the wells. Finally, absorbance of the wells was read at 570 nm with a reference wavelength of 630 nm.

For data analysis, mean absorbance was calculated for each concentration and the viability percentage was established as: % cell viability = (mean absorbance of test/mean absorbance of control) × 100.

3. Results and discussion

3.1. Synthesis of AuNPs: The pattern of the colour change during the formation of the AuNPs was different between the WTE-based method and the citrate-based method. In the WTE-based method, once AuNPs were synthesised, the colour of the solution changed directly from yellow to ruby red or purple without any intermediate grey colour change. However, in the citrate-based method, the colour changed from yellow to intermediate blurred grey and finally to ruby red, indicating the formation of AuNPs.

Although, the colour of AuNPs solutions prepared by the WTE method was similar in all different temperatures and ratios, differences in the colour of AuNPs solutions prepared at different pHs were observed. The colour of the AuNP solution synthesised at pH = 3 was blue (with large aggregates) and that of pH = 12 was grey.

The reactions conducted at 80 and 91°C temperatures using the WTE-based method took less time (10–15 and 1–2 min, respectively) than those made by the citrate-based method (20–30 min) conducted at 125°C (see Fig. 1*a*). Also, biosynthesis reaction was completed within 9 min at 91°C using a 6:1 ratio (see Fig. 1*b*). The reason for the rapid biosynthesis of AuNPs by WTE is the high reduction power of WTE because of the presence of powerful

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anti-oxidants, which are responsible for the reduction of Au^{3+} ions towards the formation of AuNPs, and the reduction capacity of watercress leaf extracts [4].

In addition, when increasing the temperature, the velocity of AuNP formation increased during the conduction of the WTE-method, the most rapid reaction at 91°C and the slowest reaction at 37°C being observed (Fig. 1*a*). At all temperatures assayed, the velocity of AuNPs formation increased with increasing of the Au^{3+} solution/WTE ratio (see Fig. 1*c*). In fact, high temperatures and high concentrations of the Au^{3+} increase the chance of reaction between the reducing agents of WTE and free Au^{3+} and provided a fast formation of AuNPs.

Similarly, reactions conducted at low pHs were faster compared with high pHs (see Fig. 1*d*). However, changes in pH have no positive effects on the velocity and stability of biosynthesised AuNPs (the best pH being about 4.75). The biosynthesised AuNPs formed macroscopic aggregates at pH 3. This finding is in accordance with results of a recent study in which AuNPs produced from red cabbage extract showed similar aggregations in the extreme acidic pH [19].

Based on our results, the best conditions for the biosynthesis of AuNPs with WTE concern the Au^{3+}/WTE ratio of 6:1, a temperature of 91°C and a pH of 4.75.

Both the citrate-based and the WTE-based methods resulted in stable solutions of AuNPs (at least 6 months) without any visible aggregation after storage at 4°C.

3.2. Characterisation of AuNPs

3.2.1 Optical and colourimetric methods: Citrate-based and WTE-based AuNPs provided differences in their absorbance and λ_{SPR} (see Fig. 2). Furthermore, there was an increasing trend in the absorbance of AuNP solutions when increasing the reaction temperature, but were no direct associations between absorbance as well as λ_{SPR} of the biosynthesised AuNPs solution with the different reaction ratios (Fig. 2*b*).

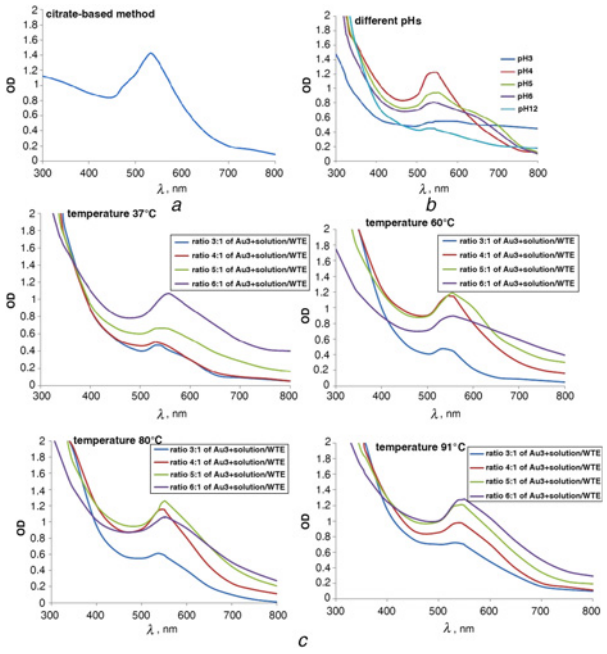

Figure 2 *Spectrophotometry analysis of the synthesised AuNPs with citrate-based and WTE-based methods*
a Absorbance and λ_{max} of citrate-based AuNPs' solution
b Absorbance and λ_{SPR} of AuNPs solutions prepared by WTE – at different pHs
c Absorbance and λ_{SPR} of WTE-based AuNPs' solutions prepared at different temperatures and different Au^{3+}/WTE ratios

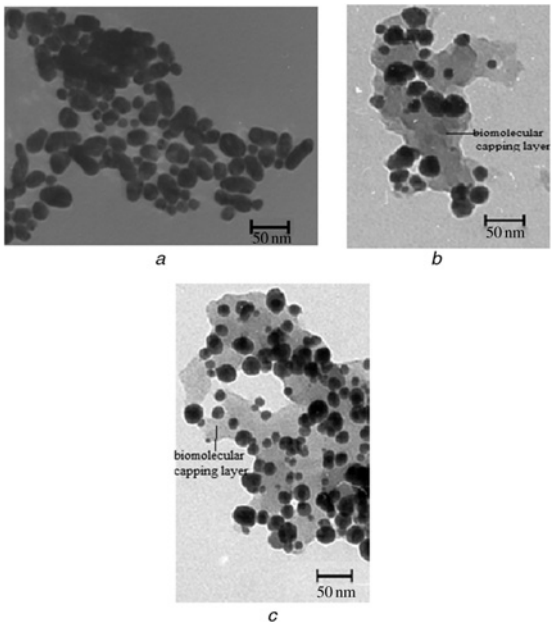

Figure 3 *TEM images of AuNPs*
a Citrate-based AuNPs
b WTE-based AuNPs prepared at a ratio 4:1
c WTE-based AuNPs prepared at 6:1 ratio
In the last two cases at a temperature of 91°C

Changing of the reaction pH had different effects on the absorbance and λ_{SPR} of the AuNPs. Maximum absorbance was obtained at pH = 4.75 and minimum absorbance at pH = 12. In addition, there was no direct relationship between the changing of pH and λ_{SPR} (see Fig. 2*c*).

The addition of 6 M of NaCl to the solution of citrate-based AuNPs resulted in a rapid colour change from ruby red to blue. However, the colour of the WTE-based AuNPs' solution remained almost the same after the addition of 6 M of NaCl. The difference in salt-induced colour change originates from the difference in the surface chemistry of the prepared AuNPs. In fact, citrate-based AuNPs have net negative charge on their surfaces which are neutralised by Na ions, leading to the aggregation and changing of colour from red to blue/purple [20]. However, WTE-based AuNPs do not have net charges on their surface because of the capping by large biomolecules which are present in the WTE. These capping biomolecules surround the Au cores, preventing AuNPs from aggregation and therefore make WTE-based AuNPs stable (see Figs. 3*b* and *c*).

In short, after trying different synthesis conditions (different ratios of Au^{3+} solution/WTE, different temperatures and different pHs), 4:1 and 6:1 ratios at a temperature of 91°C and pH = 4.75 provided the best reaction conditions of the WTE-based method (see Fig. 2). Hence, further characterisations were carried out on particles produced under these conditions.

3.2.2 TEM analysis: The mean size with its standard deviation (± SD) of AuNPs prepared by the citrate-based method was 15 ± 3 nm based on the statistical analysis of TEM data, and it was confirmed that most of the AuNPs were morphologically spherical (see Fig. 3*a*).

In addition, the mean size of AuNPs prepared by the WTE-method was 17 ± 5 and 16 ± 4 nm for 4:1 and 6:1 ratio conditions, respectively. The morphology of the biosynthesised particles using both ratios were mostly spherical (see Figs. 3*b* and *c*). Although there were similarities in the size and morphologies of AuNPs synthesised by both assayed methods, the WTE-based AuNPs were more spherical than the citrate-based ones, being gold nanocores located inside the capping and stabilising bio-molecular

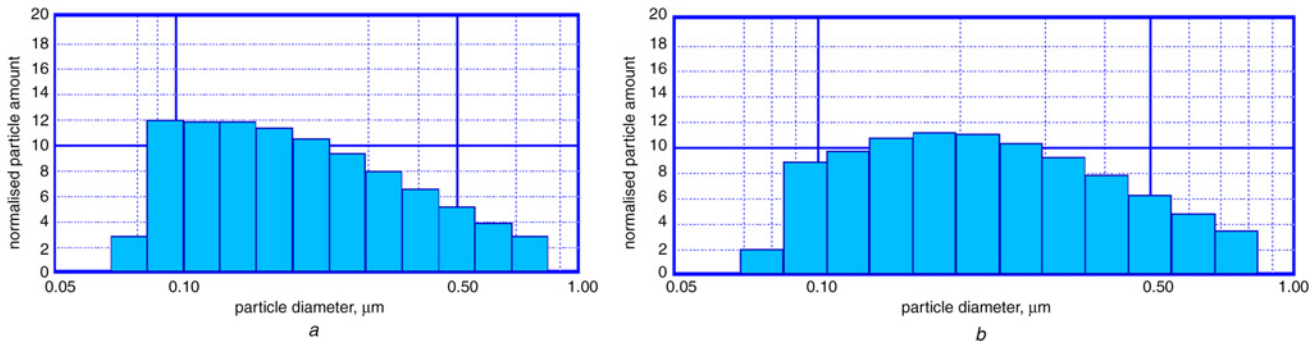

Figure 4 *Size distributions of WTE-based AuNPs*
a Prepared in the Au^{3+}/WTE ratio 4:1
b Prepared in the Au^{3+}/WTE ratio 6:1
In both cases at a temperature of 91°C

layer, which is responsible for the stability as well as the resistance to salt-induced aggregation of AuNPs derived from natural compounds of WTE.

3.2.3 Particle size analysis: In addition to TEM analysis, the size distribution of WTE-based AuNPs was studied by a particle size analyser. AuNPs prepared from an Au^{3+} solution/WTE ratio 4:1 showed smaller size distributions compared with those obtained from a ratio 6:1. Most of the particles obtained from a Au^{3+}/WTE ratio 4:1 were distributed between 50 and 250 nm (see Fig. 4*a*), and this range was 150–350 nm for particles obtained in Au^{3+}/WTE ratio 6:1 (see Fig. 4*b*). The difference between results obtained by TEM and particle analysis may be because of the presence of some aggregates which may be counted as a single large particle. Also, based on TEM pictures, nanoparticles are located inside the transparent stabilising layer which may be counted as a single particle by the particle size analyser. In addition, the TEM picture gives the size at one dimension, but the particle size analyser determines the size regarding all dimensions of the analysed particle.

3.2.4 FTIR analysis: Analysis of WTE-based AuNPs evidenced the incorporation of organic capping/stabilising biomolecules into the chemical structure of AuNPs during the biosynthesis reaction. Fig. 5 shows that the FTIR spectra of WTE (control sample) and biosynthesised AuNPs are almost the same, with some minor differences. Assignment of functional groups to the corresponding spectra bands was carried out based on the literature [12, 13, 21, 22]. For a WTE sample (see Fig. 5*a*), bands include 3410 cm^{-1} (–OH and –NH stretching of alcohols, proteins, phenolics and polysaccharides); 2955 and 2920 cm^{-1} (–CH stretching of alkanes); 2375 and 2350 cm^{-1} (–NH stretching and –C=O vibration of proteins); 1625 cm^{-1} (–NH bending, –C=C stretching, –NO, –N=N, –C=N and –C=O stretching of proteins); 1520 cm^{-1} (–CH of alkanes, –NO of nitro-compounds such as glucosinolates and proteins); 1405 cm^{-1} (–CH scissoring and bending of hydrocarbons; –OH bending and –C=O of phenolics, alcohols, proteins and polysaccharides; organic sulphates of thiocynates and glucosinolates); 1100 cm^{-1} (–CO stretching of proteins; –CN stretching, –CC stretching, –OCN and –COCN stretching, $–NO_2$ and $–SO_2$ stretching of thiocyanates, terpens and glucosinolates); 900 cm^{-1} (–CH bending and –C–C vibrations of hydrocarbons; –P–O–C stretching of nucleic acids); 835 cm^{-1} (–CH bending of hydrocarbons; –C–O–O stretch of proteins); 660 cm^{-1} (–CH bending of hydrocarbons; $–CH_3$–S–(C–S) stretch of compounds such as thiocynates and glucosinolates, –OH bending of phenolics, alcohols, proteins and polysaccharides); 625 cm^{-1} (–CH bending of hydrocarbons, –C–S stretching of thiocynates and glucosinolates, –OH bending of phenolics, alcohols, proteins and polysaccharides); and 540 cm^{-1} (–C–I stretching of organic iodine compounds).

The AuNP sample (see Fig. 5*b*) showed bands at 3410 cm^{-1} (no shift); 2925 cm^{-1} (shift from 2955 cm^{-1}); 2865 cm^{-1} (shift from 2920 cm^{-1}); 2375 cm^{-1} (no shift); 2350 cm^{-1} (no shift); 1650 cm^{-1} (shift from 1625 cm^{-1}); 1520 cm^{-1} (no shift); 1450 cm^{-1} (shift

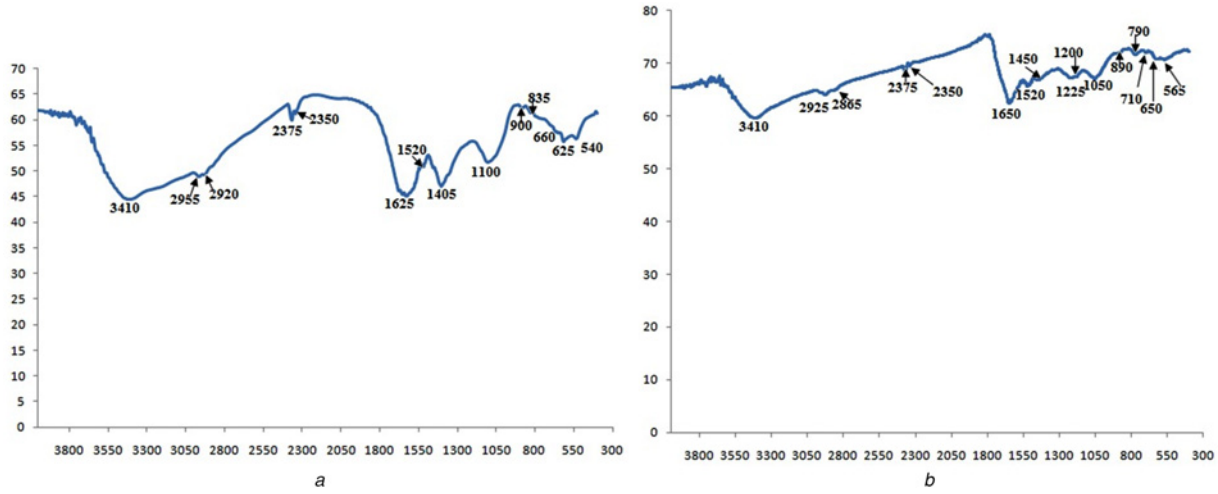

Figure 5 *FTIR spectra of WTE and of WTE-based prepared AuNPs*
a FTIR spectra of WTE
b WTE-based prepared AuNPs

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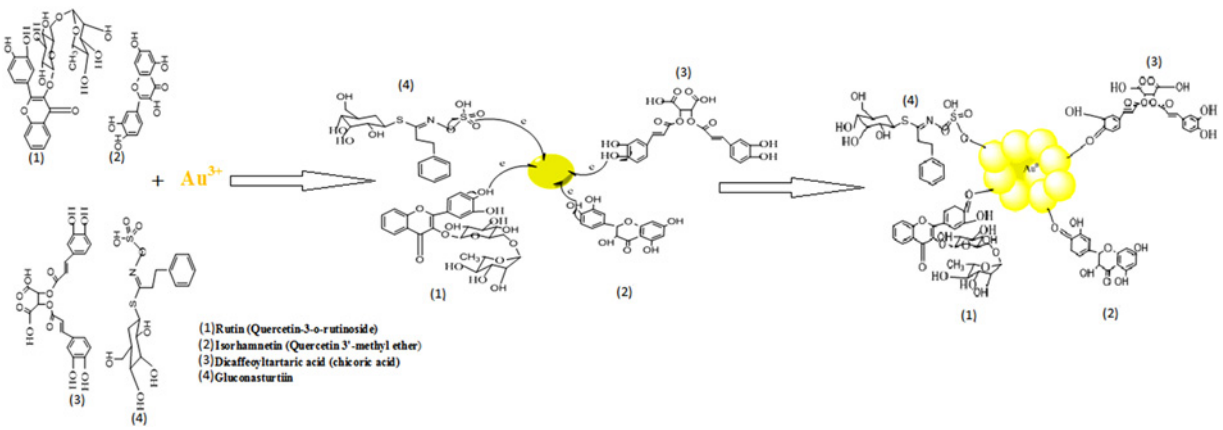

Figure 6 *Mechanism of AuNP formation by WTE*
Chemical structure of main reducing (anti-oxidant) molecules in WTE which reduce and stabilise free Au^{3+} ions

from 1405 cm^{-1}); 1225 cm^{-1} (not observed for WTE sample); 1200 cm^{-1} (not observed for WTE sample); 1050 cm^{-1} (shift from 1100 cm^{-1}); 890 cm^{-1} (shift from 900 cm^{-1}); 790 cm^{-1} (shift from 835 cm^{-1}); 710 cm^{-1} (shift from 660 cm^{-1}); 650 cm^{-1} (shift from 625 cm^{-1}); and 565 cm^{-1} (shift from 540 cm^{-1}).

From shifts in the FTIR spectra as well as from the presence of their corresponding functional groups, it can be indicated that the main phenolics of WTE comprise flavonoids [quercetin 3′-methyl ether (isorhamnetin), hydroxycinamic acid, flavones apigenin, luteolin, flavonols kaempferol and chicoric acid] and carotenoids [beta-carotene, lutein and rutin (quercetin-3-o-rutinoside)] [12, 22, 23]. It has been shown that these phenolic compounds have potent anti-oxidant activities [14, 23], and probably, they were responsible for the reduction of free Au^{3+} ions to AuNPs. Other main phytochemicals of WTE are known as gluconasturtiin and glucosinolates which provided bands at 3410, 1405, 660, 625 and 1100 cm^{-1}. These sulphur-containing saccharides are degraded to isothiocyanates [such as phenylisothiocyanate], sulphoraphene, indoles (such as indole-3-carbinol) and methylsulphinylakyl isothiocyanates inside watercress; and they have potent anti-cancer and anti-microbial properties against colon and breast cancers [12, 13]; as well as mycobacterium and low-respiratory tract pathogens [8, 9], respectively. Also, with regard to the absorbing of high amounts of iodine by watercress, the WTE sample showed a band at 540 cm^{-1}, confirming the previous findings about the presence of iodine in watercress [24]. Most of the compounds observed from the crude extract of watercress (WTE), were also detected with some shifts in the AuNP sample (Fig. 5*b*). The spectral shifts in 1405, 660, 625 and 1100 cm^{-1} indicate possible capping of formed AuNPs by sulphur-containing phytochemicals of WTE and the shifts in 2955, 2920, 1625 and 900 cm^{-1} spectra of WTE (compared with those of AuNP sample) show incorporation of alkanes, proteins and nucleic acids and accordingly, the shift in 565 cm^{-1} indicates the integration of iodinated compounds with the chemical structure of AuNPs during formation. Regarding the obtained results, it can be concluded that most of the WTE compounds have taken part in the structure of AuNPs; also, WTE phenolic compounds (flavonoids and carotenoids) have acted as both reducing and stabilising agents during the biosynthesis. Hence, it could be concluded that the incorporation of biological compounds in the structure of AuNPs increases the biocompatibility of these nanoparticles.

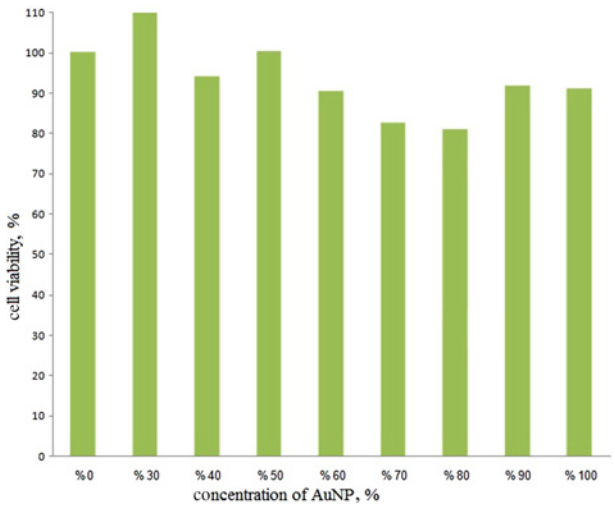

Figure 7 *Biocompatibility test (MTT assay) of WTE-based AuNPs*
Different concentrations of AuNPs were incubated with T47D cell line for 24 h

3.3. Mechanism of AuNP formation by WTE: The possible molecular mechanism of AuNP formation in the WTE-based method is illustrated in Fig. 6. Based on the FTIR results and previous researches [12, 22, 23], the main reducing compounds of watercress include chicoric acid, quercetin-3-O-rutinoside, isorhamnetin and glucosnastrutiin. These compounds reduce free Au^{3+} to Au^{0} to form AuNPs; this reduction reaction is faster than the reduction of Au^{3+} by citrate ions because of the high concentration and reducing power of the compounds present in WTE compared with citrate (the colour change is very fast in the WTE-based method, taking only about 1–2 min). In the WTE-based method, –OH groups of reducing molecules act as electron donors to Au^{3+} ions; and the rest of the reducing molecules act as capping moieties during the AuNP formation. Furthermore, other molecules may cap the formed AuNPs. These molecules include alkanes, proteins, nucleic acids and iodinated compounds, as inferred from the FTIR spectra of WTE-based AuNPs.

3.4. Evaluation of biocompatibility of WTE-based AuNPs using MTT assay and their potential applications in nanomedicine: The 24 h exposure of the T47D cell line to different concentrations of WTE-based AuNPs did not show any significant anti-growth effects (see Fig. 7) and thus indicates the biocompatibility of WTE-based AuNPs, which makes them attractive tools in the nanomedicine field. The produced AuNPs could be used in the following cases.

3.4.1 Delivery of both hydrophilic and hydrophobic agents to the target cells: Regarding to the presence of different capping residues

on the surface of WTE-based AuNPs (see Fig. 6), different types of hydrophilic and hydrophobic molecules including drugs, peptides, nucleic acids, proteins, dyes and so on could be delivered to the target cells. The capping molecules coming from the WTE have both hydrophilic and hydrophobic chemical groups in their structures. Therefore hydrophilic and hydrophobic molecules can react with them, and various ranges of molecules could be loaded on the surface of the produced WTE-based AuNPs via interaction with these groups.

3.4.2 Application as imaging agents: Biocompatible AuNPs can be conjugated with dye molecules and used as imaging tools. In addition, they can be used purely as contrast-enhancement agents in the photoacoustic/CT imaging methods. Also, the high atomic weight of the produced AuNPs allows them to be used in immunostaining at the subcellular level. Furthermore, various diseases could be diagnosed by bioconjugation of biomarker-specific monoclonal anti-bodies and aptamers to the AuNPs surface [25].

3.4.3 Treatment of diseases: The produced AuNPs could be used to treat various diseases, particularly cancer. Photothermal therapy is the main approach of cancer therapy using AuNPs. In photothermal therapy, target cancerous cells are disrupted through heating up the nanoparticles [26]. Accordingly, the produced WTE-based AuNPs could be used as photothermal therapy agents.

4. Conclusion: Based on a literature review, our study is the first report on the successful biosynthesis of AuNPs with WTE. Application of watercress in the biosynthesis of AuNPs has significant advantages over the currently used chemical methods: (i) the produced AuNPs are eco-friendly with minimum toxicity in comparison with chemically synthesised AuNPs, (ii) the overall time taken for synthesis is lower compared with the citrate-based method, (iii) the produced WTE-based AuNPs have good shape and sizes compared with the chemically-produced AuNPs, (iv) the availability of watercress in high amounts, especially from industrial cultivation, makes it a low-cost natural resource for large-scale affordable production of AuNPs compared to the chemical sources and (v) because of the biocompatibility of the WTE-based method, AuNPs can be used as nanomedical tools for the targeted delivery of various molecules, disease diagnosis and therapy. considering the fact that watercress has been shown to have very benefits on human health in view of its anti-cancer [12–14], anti-microbial [8, 9], anti-diabetes [10] and anti-hyperlipidaemic [11] properties, and it can be speculated that biosynthesised AuNPs from this super-food can render similar effects, although further studies are needed regarding this issue.

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