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A Nano-bio-eco Interaction to Synthesis of Gold Nanoparticles Using Tribulus Terrestris Extract and its Antibacterial Activity

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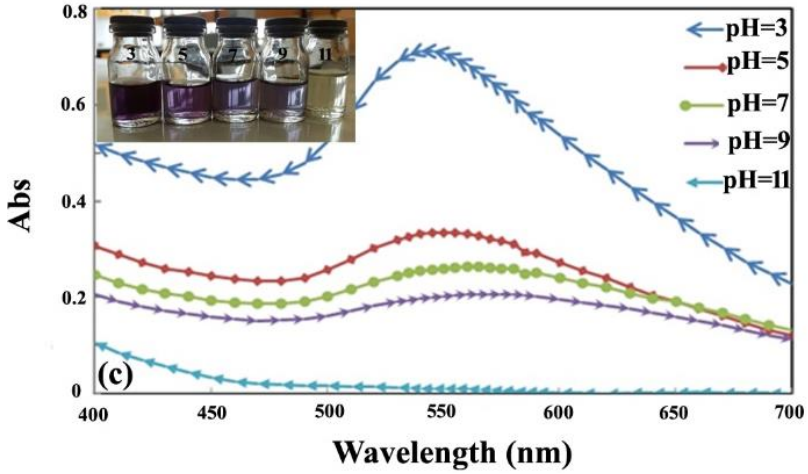
ABSTRACT

A green synthesis of gold nanoparticles (GNPs) was accomplished by aqueous medium of the Tribulus terrestris (*T. terrestris*) extract, without the addition of any external chemical reducing agent. Several factors such as pH of the solution, temperature, and concentration of the added extract greatly influence the synthesized nanoparticles. The reduced GNPs were characterized by energy dispersive X-ray (EDX), ultra-violet–visible absorption spectroscopy (UV–Vis), fourier transform–infrared spectroscopy (FT–IR), and scanning electron microscopy (SEM). Formation of GNPs was approved from the change in color of colorless to violet and strong surface plasmon resonance (SPR) was observed at 555 nm using UV-Vis spectroscopy. The size distribution of the GNPs was also revealed by SEM analysis. The size range of the GNPs was from 6 to 25 nm. Similarly, particles with a distinctive peak of gold were examined with EDX. Moreover, the potential of the nanoparticles for biomedical applications have also been confirmed by their antimicrobial activity against Gram positive and negative bacteria. Actualy, this study provides new possibilities of using *T. terrestris* extract as a capping and reducing agent for the synthesis of GNPs, which may be applicable for the future diagnostic and therapeutic fields.

GRAPHICAL ABSTRACT



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Introduction

Nanobiotechnology is defined as the creation of new devices and systems integrated from the nanoscale using biological principles and materials, and *vice versa* [1]. The bio-based synthesis of nanoparticles makes them an economical, simple, eco-friendly, and viable alternative to the chemical methods. Recently, scientists have paid much attention to the synthesis of NPs, because of their incomparable and excellent properties in biomedical applications such as biolabeling, chemotherapy, cancertherapy, intracellular gene regulation, thermaltherapy and diagnosis of cancer, catalysis, and antioxidant and antibacterial activity [2-16]. Lately, GNPs have been successfully synthesized using many plants such as *Inonotus obliquus* [7], *Pogestemon benghalensis* [8], *Gnidia glauca flower* [10], *Mushrooms* [12, 17], *Red Algae* [18], *Memecylon umbellatum* [19], *Antigonon leptopus* [20], *Cannonball fruit* [21], and *Piper longum* [22].

Tribulus terrestris (*T. terrestris*) is a natural herb which has been used for protecting against pancreatitis [23], aphrodisiac [24], antihypertensive [25], and antiurolithiatic [26] and has significant antilithiatic efficacy [27]. Besides, inhibitory effect of calcium oxalate crystallization in kidney [28, 29], increasing the number and motility of spermatozoa by increasing testosterone and luteinizing hormone levels is another application of the herb [30]. Lately, Gopinath *et al*. have successfully synthesized a durable and safe spherical structure of antimicrobial Ag nanoparticles using *T. terrestris* extract aqueous [31].

Such interesting properties of GNPs and *T. terrestris* extact encouraged us to synthesize GNPs by using of the extract aqueous. The present study aimed at (a) a simple laboratory set-up to observe the effect of aqueous extract of *T. terrestris* on the synthesis of GNPs under various conditions, and (b) effect of antibacterial activity of the synthesized particles.

Experimental

Chemicals

The dried plant of *T. terrestris* was purchased from local bazaar in Sanandaj, Iran. A 25 gram of the plant powder was boiled in 500 mL of sterile distilled water for 10 minutes. The cooled extract was filtered with filter paper (Whatman no.1). The aqueous extract was stored at 4°C in dark for further use.

$HAuCl_4$ was purchased from Sigma-Aldrich Chemical Company. The multi-drug resistant bacterial pathogens such as Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli) were obtained from Iranian Biological Resource Center. Double distilled Milli-Q water was used throughout the experiments.

Synthesis of Gold Nanoparticles

$HAuCl_4$ without adding the extract was maintained as a control. The change in color of the solution was used for the characterization of the GNPs. Notabley, pH of the solution, concentration of the added extract, and temperature are crucial factors that affect the synthesis of NPs [32]. Hence, the effect of them (*i.e*. pH (3, 5, 7, 9 and 11), reaction temperature (25, 50, 80, and 100 °C), and the extract concentration (0.5, 1, 1.5, 2, 3, 4, 5, 6 and 10% V/V)) were investigated. The extract was added in mentioned conditions to 5 mL of 0.5 mM $HAuCl_4$ solution with rigorous stirring at 200 rpm.

Characterization of Gold Nanoparticles

The effect of the different conditions for the synthesis of the GNPs were studied by observing the product formed using UV–Vis spectroscopy. For getting more information, the purified nanoparticles in the optimized conditions were

freeze-dried, and analyzed by FT–IR, SEM, and EDX.

For each solution, the UV-Vis spectrum of the synthesized nanoparticles was recorded at 380-750 nm, using a spectrophotometer (T90+, PG Instrument, UK) which has a quartz cell with the path length of 1 cm. A relation between the size of various metal NPs and the range of light wavelengths has been confirmed [33]. Due to the excitation of SPR at 500-570 nm, spectrophotometric absorption measurements were applied in the visible region of the electromagnetic spectrum for characterizing the GNPs [2, 5, 8, 18, 34]. Interstingly, by increasing the size of NPs, a larger red-shift would happen [35].

Antibacterial Assay

The antibacterial activity of the GNPs was tested in sterilized deionized water by the disc (agar) diffusion method under sterile conditions in duplicate and repeated twice. In this study gram-positive *S. aureus* and gram-negative *E. coli* were used. These bacteria were grown in a nutrient agar media for 24 h prior to the experiment. A cup was filled with the GNP solution and after incubation at 35 °C for 24 h, the diameter of the growth clear zone (mm) was recorded.

Results and Discussion

UV-Vis absorption spectroscopy analysis

Optical properties of the synthesized GNPs were investigated by UV-Vis spectra. It has been specified that the excitation of the SPR in the aqueous metal NPs causes the coloring of the solution (violet color in the case of GNPs) [7, 8, 10, 12, 17-22, 36]. As *T. terrestris* extract was added into the aqueous solution of Au ions, the reduction of Au ions using the extract was evident by the change from colorless to violet, which indicated the formation of GNPs. In so doing, we investigated the mentioned factors that may directly influence on the phytosynthesis of the GNPs.

Effects of T. terrestris Extract Quantity

Different *T. terrestris* extract quantities (varied from 25, 50, 75, 100, 150, 200, 250, 300, 500 µL in 5 mL of 0.5 mM $HAuCl_4$) were already used for the synthesis of GNPs. An increase in peak absorbance was found in the UV–Vis spectrum by adding *T. terrestris* extract quantity from 25 to 300 µL in the solution (Figure 1a). Also, by increasing the extract quantity, a visual observation of the color change of the solutions from colorlress to dark violet can be made. The sharpest, as a criterion for finite distribution size, and highest peak, as a criterion for number of the synthesized nanoparticles, was found for 300 µL of the extract. The sharpest and highest peak was obtained at around 555 nm.

Effect of Temperature on the Preparation of GNPs

The UV-Vis spectra were obtained after 10 min heating of the solution at temperatures ranging from 25 to 100 °C. A relation between reaction temperature and peak sharpness was found in Figure 1b which was due to increasing reaction rate for the GNPs synthesis [37]. With an increase in temperature, the SPR would go up. This finding confirmed a directly proportional between the yield of the nanoparticles and the temperature in the range of 25-80 °C. The sharpest and highest peak was obtained for 80 °C at 555 nm. This sharpness in absorbance peak depends on size of the synthesized nanoparticle. At higher temperature, distribution of particle size has a smaller range.

Effect of pH

The synthesis of the GNP by the extract was tested over a wider pH range of 3-11. It can be concluded that at lower pH, the particle sizes were comparatively smaller than those at the

higher pH, as blue shift was clearly obtained in the SPR spectra (Figure 1c). At low pH growth nucleation and aggregation of the GNP is prevented from forming larger nanoparticles. Our studies confirmed that, formation of the GNPs can be prevented at basis pH. To approve this theory, we put the solution at pH=11 at the first and then decrease pH of the solution to 3 by adding HCl. After adding the acid into the solution, color change was observed. It is thought that at lower pH the cationic functional groups allow the $AuCl_4^-$ ions to approach the binding sites. Hence, Au (III) acts as an oxidizen agenat. Considering this aspect, our findings are in good agreement with the previous observation [38, 39]. A similar pH effect by reducting of Au(III) was also obtaind [21, 40]. This result confirmed that the pH playes a crucial role in controlling of the GNPs synthesis. An inverse relationship between the yield of the NPs and the pH was confirmed by SPR as shown in Figure 1c. The reaction of Au(III) with the extract at pH=3 yielded the nanoparticles with a narrower distribution in diameters. Therefore, the nucleation and subsequent formation of a large number of NPs with smaller diameter was facilitated at lower pH. Hence, the optimum amount of the extract, reaction temperature, and pH were 300 µL, 80 °C, and 3, respectively.

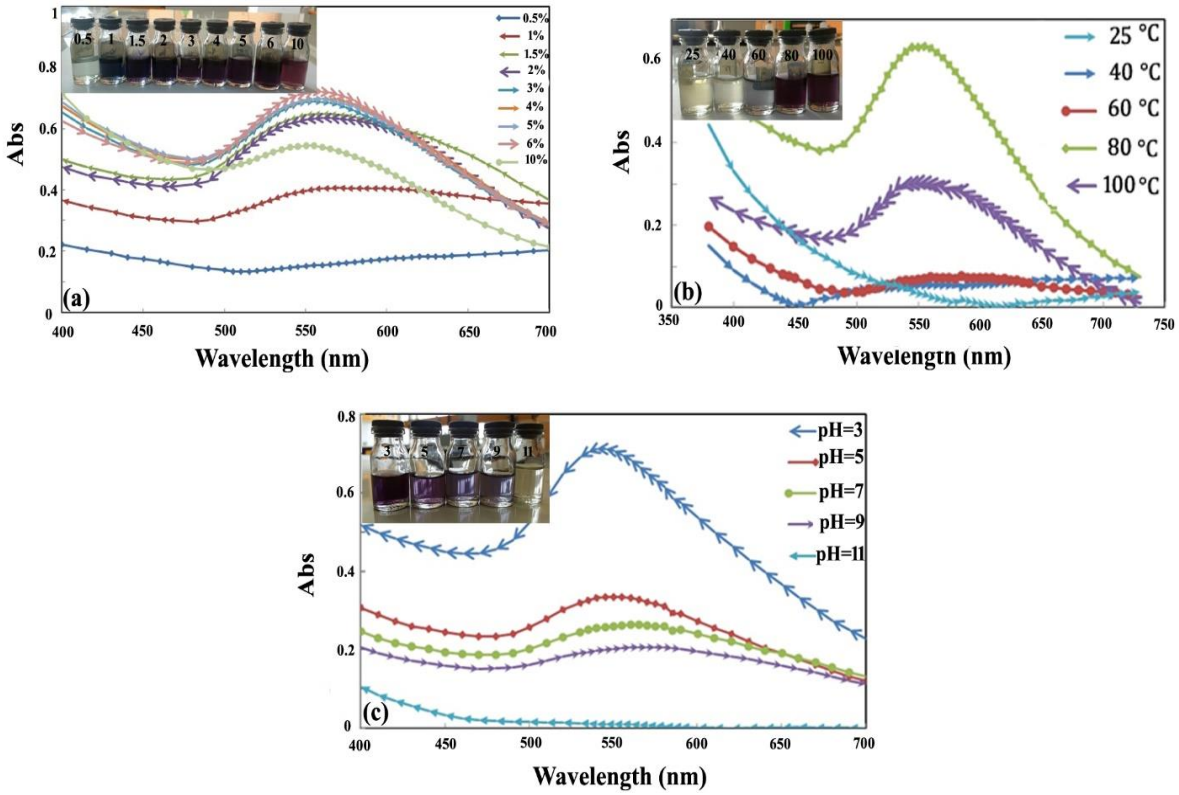

Figure 1. UV–Vis spectra of the GNPs synthesized from *T. terrestris* at different (a) concentration of the added extract, (b) temperature of the solution, and (c) pH of the solution

FT–IR Spectroscopy Analysis

The different phytochemicals in *T. terrestris* extract play a significant effect in the reduction of the Au(III) and capping of the NPs. The FT–IR spectra for the extract and the solution with the NPs revealed several characteristic peaks in Figure 2. The peak at 3434 cm^{-1} is related almost

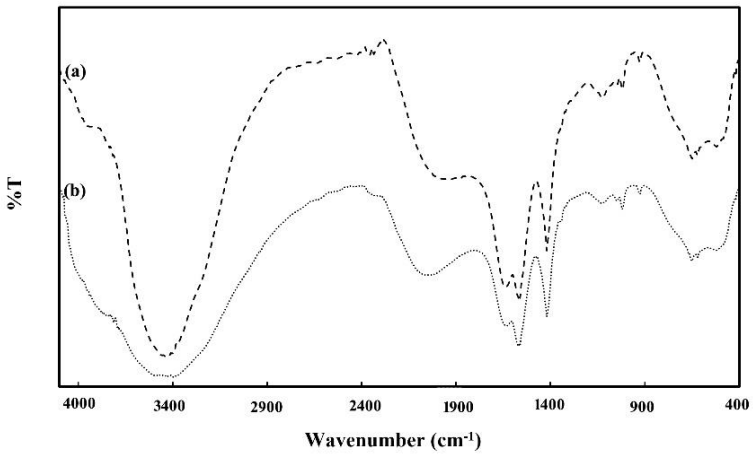

Figure 2. FTIR spectra of (a) *T. terrestris* extract and (b) as-synthesized GNP

entirely to the O-H stretching of the alcoholic groups [41, 42] which are significantly reduced and become broader upon coordination with GNPs, suggesting the role of these groups in the reduction of $HAuCl_4$ to GNPs. The broad band appearing at 3400 cm^{-1} may be due to the existence of water molecules in the GNPs as shown in Figure b [43]. The band at 1605 and 1703 cm^{-1} corresponds to polysaccharides and carbonyl peak (C=O stretching). The peaks at 1560 cm^{-1} to 1577 cm^{-1} are attributed to C=C stretching vibrations of aromatic compounds [42]. The bands between 1019 to 1126, 927, 651, and 518 cm^{-1} are related to C-X (X=halogen atoms) [42]. The pH variations can affect hydroxyl group (–OH) and this group acts as an active site for the reduction of metal salts and capping the NPs [44]. The hydroxyl groups prefer to protonate at acidic condition. The overall positively charged surface could promote the interaction between the protonated functional groups and the negatively charged ($AuCl_4^-$) through electrostatic attraction or electrovalent bond. Thus, we believe that presence of hydroxyl group in the extract might be a plausible reason for the formation and capping of the GNPs.

SEM and EDX Studies

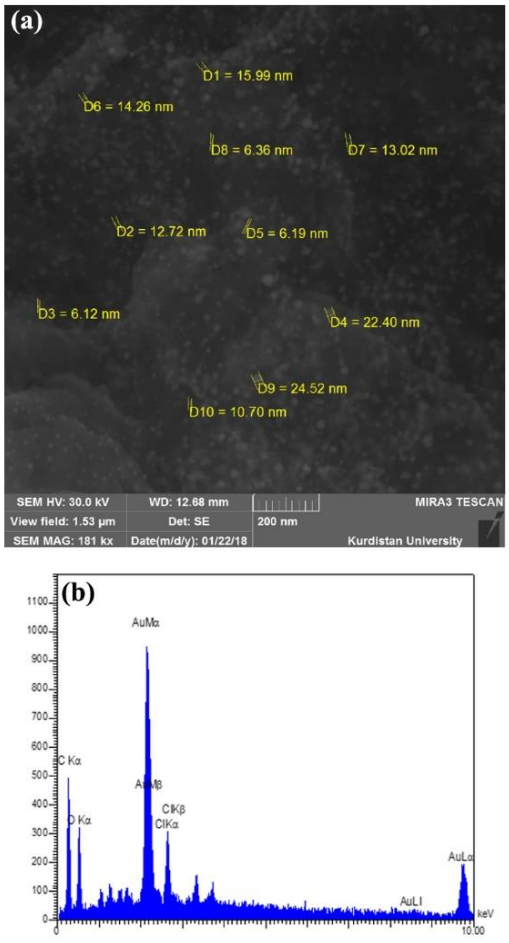

Figure 3. (a) SEM images of the GNPs after bioreduction with *T. terrestris* extract. (b) EDX profile of the green synthesized GNPs

SEM images show uniformly distributed GNPs that indicate the stabilization of nanoparticles by capping agents as shown in Figure 3a. The GNPs

are spherical with particle size range from 6 to 25 nm. The representative EDX spectrum presented in Figure 3b shows a strong Au signals with weak signals for carbon, oxygen, and chloride which may have originated from the biomolecules bound to the surface of the GNPs [45]. The EDX and FT-IR spectra indicated the presence of biomolecules on the surface of the biosynthesized GNPs.

Antimicrobial Activity

The antibacterial activity of the synthesized GNPs was evaluated against the bacteria *E. coli* and *S. aureus* at different concentrations are summarized in Table 1. A direct relation was observed between concentration of the NPs and inhibitor activity. As revealed in Figure 4, the gram negative bacterium showed larger diameter of the inhibiting zones than the gram positive one which may be due to the presence of thick peptidoglycan layer in cell wall of gram positive bacteria thus forming rigorous structure leading to hard diffusion of the GNPs compared with the gram negative bacteria where the cell wall possesses the thinner one [46]. The mechanism of the inhibitory action of GNPs has not been fully explained. One possible mechanism of inhibition action of the inhibitors is the strong electrostatic interaction of the surface charged particles with the outer wall cell of the bacteria [31, 47]. Some functional groups attach to the surface of the NPs. These agents may have a direct effect on the toxicity of the NPs, likely due to their ability to reduce NP agglomeration [48-50]. Thus, the synthesized GNPs from the extract inhibited the growth of all the tested bacteria, suggesting the NPs could be efficiently used as strong antimicrobial agent against the bacteria.

The MIC and MBC Results

The minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) results were shown in Table 2 and 3. According to Aligiannis *et al.* [51], plant materials with MIC up to 500 μg mL^{-1} are considered strong inhibitors of bacterial activity. Moderate inhibition is given by plant extract with MIC values between 600 and 1500 μg mL^{-1}, whereas MIC above 1600 μg.mL^{-1} is classified as weak inhibition. The GNPs prepared by *T. terrestris* extract showed a pronounced antimicrobial effect.

Table 1. Antimicrobial activity of the synthesized GNPs using *T. terrestris* extract

Bacteria	***S. aureus***			***E. coli***		
Volume of the added solution (μL)	20	40	60	20	40	60
Zone of inhibition (mm)	9	11	15	11	15	16

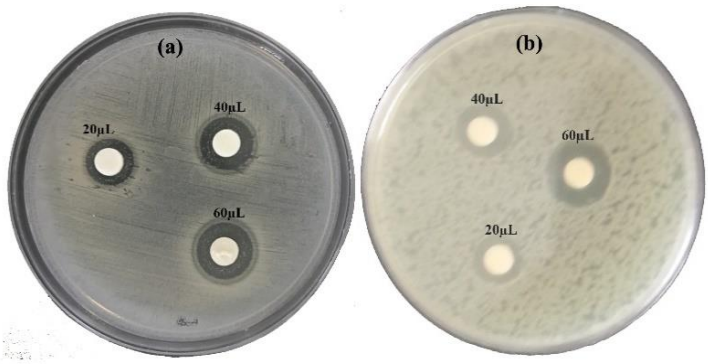

Figure 4. The inhibition zones of the synthesized GNPs against (a) *E. Coli* (b) *S. aureus* on nutrient agar plates with different volumes of the solution

Table 2. Minimum inhibitory concentration (MIC) of synthesized gold nanoparticles (0.0005 mol.L^{-1}) prepared by *T. terrestris* extract against different microorganisms after 24 h. Positive (+): indicating growth, Negative (–): indicating absence of growth.

Dilution of GNPs (µL)	500.0	250.0	125.0	62.5	31.2	15.6	7.8
S. Aureus	–	–	–	–	+	+	+
E. Coli	–	–	–	–	–	+	+

Table 3. Minimum Bactericidal Concentrations (MBC) of silver nanoparticles after 24 h; Positive (+): Indicating growth; Negative (–): Indicating absence of growth.

Dilution of GNPs (µL)	500.0	250.0	125.0	62.5	31.2	15.6	7.8
S. Aureus	–	–	–	+	+	+	+
E. Coli	–	–	–	–	+	+	+

Conclusion

Biosynthesis and characterization of GNPs from the aqueous extract of the *T. terrestris* have been achieved. In this study, different factors influencing the synthesis of NPs and various techniques used to characterize them were described in detail. The GNPs were characterized by UV–Vis and FT-IR spectroscopy; also, SEM and EDX showed that most of the particles are spherical in shape and particle sizes are in the range of 6-25 nm. FTIR spectroscopic analysis indicated that the interaction between hydroxyl functional groups of the extract may be chargable for the reduction of Au(III) to form stable GNPs. The biosynthesized GNPs exhibited excellent antibacterial activities. The GNPs possess potentials as therapeutic agents against pathogens.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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