Cumhuriyet Sci. J., 43(3) (2022) 416-422
DOI: https://doi.org/10.17776/csj.1077429

Cumhuriyet Science Journal

| csj.cumhuriyet.edu.tr | Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

# Green Synthesis of Gold Nanoparticles Using Aqueous Extract of Asphodelus Aestivus, Coating with Chitosan Biopolymer and Cytotoxicity Studies

**Ersen Yılmaz [1,a,*] Mehmet Ateş [2,b], Muhammed Erbay [3,c]**

[1] *Tunceli Vocational School, Munzur University, Tunceli, Türkiye*
[2] [2]*Rare Earth Elements Application Center, Munzur University, Tunceli, Türkiye*
[3] *Tunceli State Hospital Laboratory, Tunceli, Türkiye*
**Corresponding author*

**Research Article**

*History*
*Received: 23/02/2022*
*Accepted: 09/06/2022*



**ABSTRACT**

The green synthesis of gold nanoparticles (Au-NPs) was carried out by pouring the aqueous extract of East Anatolian origin Asphodelus aestivus plant onto aqueous gold metal ions and reducing them via single-step one-pot method. The absorption peak of the synthesized nanoparticles gave a maximum at 575 nm. All the X-ray diffraction peaks at 2θ = 38.25 , 44.46 , 64.64 and 77.20 that index to (111), (200), (220), and (311) planes verify the successful synthesis of Au-NPs. Mostly spherical shape particles showed a homogeneous distribution with size range 20±5 nm are measured using TEM. From the FTIR spectrum, the peaks are seems to be related to phenolic compounds, flavonoids, benzophenones, terpenoids and anthocyanins which assume that they could act as the reducing agents. The plant extraction, one-pot, single-step method used is environmentally safe without the role of synthetic materials which is highly potential in mild and green synthesis applications. The Au-NPs were coated with chitosan biopolymer in aquatic solution medium and verified by SEM. Then, cytotoxic investigations of the biosynthesized Au-NPs were carried out by HUVEC cells. Au-NPs were showed toxic effects on cell culture, even if in a small amount. However, chitosan biopolymer coating increased cell viability.

***Keywords:*** Gold nanoparticle, Biosynthesis, Asphodelus aestivus, Biopolymer coating, Cytotoxicity

[a] *chemer80@gmail.com* *https://orcid.org/0000-0002-8567-1668*
[b] *atesnrg@gmail.com* *https://orcid.org/0000-0002-2764-6579*
[c] *muhammed.erbay23@gmail.com* *https://orcid.org/0000-0003-2335-1009*

## Introduction

Nanotechnology is a technology that deals with nano-scale materials and applications. It deals with mostly metals, semi-metals and noble metals ranging from 1 to 100 nm [1]. Among these materials, noble-metal nanoparticles have attracted more attention due to their catalytic, optical and opto-electronic properties [2]. Gold nanoparticles have been studied and continue to be investigated especially for their effective properties in medical imaging [3] and drug delivery [4]. The unique optical property of Au-NPs make them very useful in bioimaging by acting as marking agent [5].

It is important to improve the synthesis conditions of such important nanomaterials, to obtain them under conditions that do not contain harmful chemicals, and also to define their physical and chemical properties well. Using naturally derived products such enzyme [6], fungus [7], algae [8] and plant [9] as reducing agents serves this purpose [10]. Plant-mediated synthesis is quick and easy, allowing for size control, and most importantly, allowing chemical reduction in mild conditions, making it stand out from other chemical methods. However, the use of different parts of the plant such as root, stem and leaf makes this method powerful [11].

Fenugreek or Asphodelus aestivus is the common name of the plant species that form the Asphodelus genus from the Asphodelaceae family. While its green leaves are just emerging from the soil in March and April, the grass harvested from the mountains is sold as a vegetable and consumed in large quantities. Using this plant, pastry, soup, stew and rice are made. It has a unique fragrance. Asphodelus aestivus is native to the Mediterranean region, northern Africa, and the Middle East [12]. It is a plant that often grows in mountainous regions in the east of Anatolia. Its taste is between spinach and leek and it is almost impossible to distinguish it from spinach after cooking. It is called ''natural antibiotic'' among local people. Biochemicals such as phenolic acid, flavonoids, alkaloids, and terpenoids that are existed in plant crude extract are play role in the reduction of nanoparticles [13]. The phenolic compounds possess anti-tumor, anti-allergic, and antiviral properties are high potential antioxidants and these kind of compounds are believed to take part in synthesis reactions of different types of gold nanoparticles. Also, different type of flavonoids, benzophenones, [14] and anthocyanins that exist in the plant could be closely related in the reduction synthesis of nanoparticles [15].

Polymer coatings are being useful in various applications. From regular coatings to nanoparticle incorporated coatings, these polymer coatings ensure a strong functionalities to the selected host materials. It is applicable to wide range of materials from metals, ceramics, polymers to nanoparticles [16]. Some studies revealed that surface modified nanoparticles with

biopolymers like PEG, Chitosan could temporarily avoid the mononuclear phagocyte system and substantially extend the circulation time of the nanoparticles [17]. In addition, the key feature in the preparation of nanoparticles is to prevent agglomeration by using coating agents like carbohydrate based polymers, e.g. dextran, chitosan (CS), starch [18]. Recently, surface optimization of nanoparticles has become a challenge for the researchers.

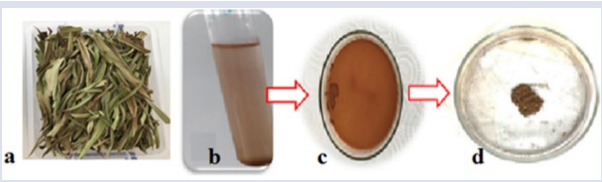


Figure 1. Summary photos of Au-NPs biosynthesis where (a) is dried Asphodelus aestivus stem and leaves, (b) aqueous HAuCl4 and plant extract mixture, (c) Au-NPs left to dry in a petri dish, (d) Au-NPs scraped and collected

In the literature review, we found that Au-NP biosynthesis with the Asphodelus aestivus plant had not been previously performed or reported. Here, we performed the biosynthesis and characterization of Au-NPs by using aqueous gold solution and aqueous extract of Asphodelus aestivus plant extract (See Fig1.). Then, the synthesized Au-NPs were coated with chitosan biopolymer and cytotoxicity experiments were done.

## Materials and Methods

### Chemicals

Asphodelus aestivus were collected from the mountainous regions of Tunceli/Turkey. Analytical grade ($HAuCl_4$, 99.98%) was purchased from Sigma-Aldrich, USA, and used as gold precursor. Ethanol, conc. HCl, Acetone, medium mol weight Chitosan, F-12 nutrient mix, Trypsin-EDTA solution, Fetal Bovine Serum, Phosphate buffered saline (PBS), 3-[4,5-Dimethylthiazol-2-yl] -2,5-di-phenyltetrazoluim bromide (MTT) salt, Trypan Blue and Nitric Acid ($HNO_3$) was purchased from Sigma-Aldrich, USA. All reagents used were of analytical grade. All glassware used was cleaned and washed with distilled water and dried before used.

### Preparation of Asphodelus aestivus Extract

The fresh plant was washed with mains water to remove dirt and washed again with distilled water before being dried in oven (Nuve Laboratory Oven) at 35◦C. All the dried plant (stem and leaves) were ground into fine powder using an electric blender (Sinbo) and stored at room temperature for further use. The extract was prepared by taking 0.2 g of the fine powder and added to 100 mL of distilled boiling water. When the temperature cooled to 70 ◦C in about 10 minutes, the crude extract was filtered with Whatman filter paper No 1.

### Synthesis of Au-NPs

In a Erlenmeyer flask, 10 mL of the filtered extract was reacted with 5 mL, 5 miliMolar of tetrachloroaurate at 25 °C and gently shaken. The time taken for the color change after the reaction and the pH were recorded. The reaction solution changes quickly from pale brown to pale purple. This color change indicated the formation of [Au / Asphodelus aestivus]. This Au-NPs nanoparticle emulsion synthesized was kept at 5 °C.

### Characterization of Au-NPs

The formation of Au-NPs was confirmed by using UV-vis spectroscopy at intervals in the range of 200 to 600 nm and 2 nm resolution (Optima SP-3000 UV-VIS Spectrometer). It was diluted with distilled water before measurement and pure water was used as control solution [24]. All data obtained were converted into graphs using Origin Pro 9.1 SRO software (OriginLab Corp, Northampton, MA, USA). The nanoparticle emulsion was centrifuged for enrichment/concentration and the concentrated emulsion formed was poured into a petri dish. The petri dish was owen dried at 40 °C for one day. The obtained dry sample was collected and analyzed for its crystal structure and composition using X-ray spectroscopy. The XRD spectrum was recorded using the device operating at 40 kV and a current of 15 mA with Cu-Kα1 radiation. The device was operated in the range of 2θ 20-80° with 2/minute (Rigaku MiniFlex 600). The size and shape of the Au-NPs in the study were characterized by using the JOEL-1011 TEM instrument, which provides 0.2 nm resolution with 50-$10^6$ magnification under voltage accelerating from 40-100 kV. The stability of Au-NPs was measured using Zetasizer Malvern Nano ZS and Dynamic Light Scattering (DLS). FT-IR spectra of Ag-NPs were recorded using JASCO-6700 spectrophotometer in the range 4000-400 $cm^{-1}$ wavelength with 16 scans and 4 $cm^{-1}$ resolution.

### MTT Assay for Cell Viability

The MTT test, which is used for cell viability testing, is easy to use, has high reliability, reproducibility, and is widely used to determine both cell viability and cytotoxicity tests. The main basis of this technique is experiments involving the use of tetrazolium salts and measuring the color change. In our study, in the MTT cell viability test, Mossman method [19] was followed with some modifications. HUVEC cells were seeded at a density of 5 x $10^3$ cells /hole in a 96-hole plate (Figure 2). After 4 hours of incubation, 5% FBS or 10% FBS containing Au-NPs at concentrations ranging from 6.25 µg / mL to 100 µg / mL were replaced with DMEM and the cells were incubated at 37 ° C for 24 and 48 hours. At the end of the exposure period, the toxicity of Au-NPs was evaluated by a standard colorimetric cellular viability assay using MTT dye. Cytotoxicity analyzes were made as a service purchase from Hacettepe University Advanced Technologies Application and Research Center. Within the scope of this experimental research, ISO 10993 tests and cytotoxicity tests of Au-NPs with standard L929 cell line

were performed for biological evaluation. In the analyzes, absorbance measurements at 570 nm were evaluated using a SpectraMax microplate reader (Molecular Devices, Sunnyvale, CA).

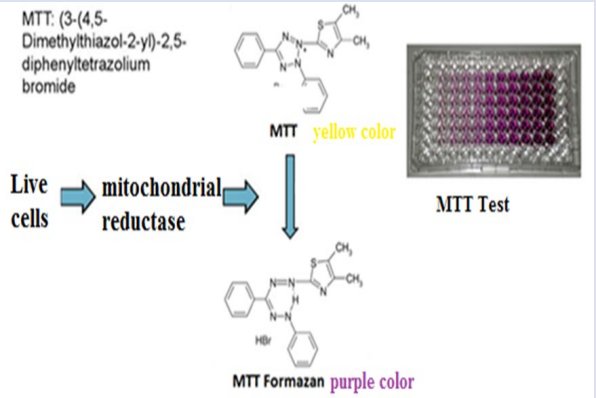


Figure 2. Scheme showing determination of cell viability assay by MTT (Mossman, 1983)

### Use of Chitosan Biopolymer for Coating

The method we used in a previous study was followed for the coating of Au-NPs with chitosan [1]. 1.00 g of 75-85% deacetylated chitosan with medium MW (200-500 kDa) was dissolved in 50 mL of 0.5% (v / v) acetic acid containing solution. 40 mg of Au-NP nanoparticle suspended in 25 mL of ethyl alcohol was added to this solution. This mixture was stirred with a magnetic stirrer for 24 hours at high intensity and centrifuged at 15000 rpm for 20 minutes. The pellet was washed with ethanol and the product was dried. In this study, the effect of coating with chitosan on the stability and cytotoxicity of Au-NPs were analyzed.

### Statistical Analysis

In statistical evaluation, One-Way ANOVA-Dunnet test was applied in SPSS 15.0 computer program and values below P <0.05 were considered significant. In addition, regression analysis was performed using the SPSS 15.0 program in order to reveal the dose-effect relationship in this study.

## Results and Discussion

Asphodelus aestivus extract (0.02 g, 10 mL) acts as both the reducing and stabilizing agent and Chloroauric acid (5 mM) acts as the gold precursor. The reduction of Chloroauric acid was indicated by the colour changes of Asphodelus aestivus extract as shown in Figure 1. The reaction was rapid as the pale greenish colour of the Asphodelus aestivus extract turns into pale purple colour within 5 minutes indicating formation of Au-NPs.

The possible chemical reduction reaction is,

$$HAuCl_4\,(aq) + \text{Asphodelus aestivus} \rightarrow [\text{Au/ A. aestivus}] \quad (1)$$

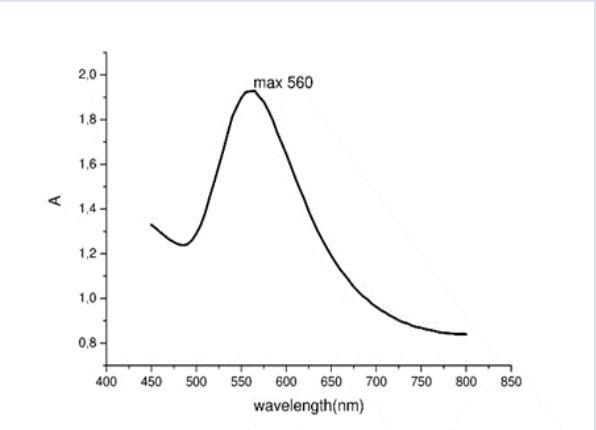


Figure 3. UV-vis absorbance bands for Au-NPs forms using A. aestivus extract

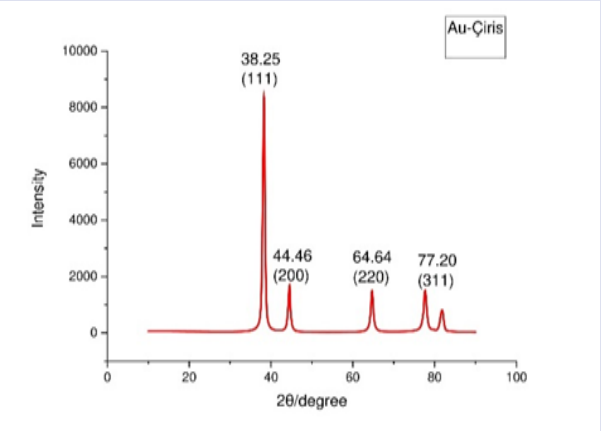


Figure 4. XRD spectra for Au-NPs forms using A. aestivus extract

### UV-Visible Spectroscopy Study

The formation of Au-NPs was confirmed by the UV-vis spectra in Figure 3. After the addition of $HAuCl_4$ solution, a sharp peak occurs in the 555-565 nm range. This peak is confirmed by other characterizations that show the formation of Au-NPs.

### X-Ray Diffraction Analysis

The sharp peaks in the powder X-ray diffraction pattern in Figure 3 show that the synthesized Au-NPs are in crystalline form. All 4 distinct peaks correspond to the standard Bragg reflections (111), (200), (220) and (311) of the surface center cubic (fcc) lattice. The particle size of Au-NPs can be calculated with little error using the Debye-Scherrer equation.

$$d = \frac{k\lambda}{(\beta cos\theta)} \quad (2)$$

where d is the average crystallite size, $k$ Scherrer constant (0.9), $\lambda$ X-ray wavelength (0.154 nm), $\beta$ the expanding line in radians and $\theta$ Bragg angle [18,22]. Using the Debye-Scherrer equation, the average crystallite size of synthesized Au-NPs is calculated as 12 nm. This calculated value is also supported by TEM findings.

### Transmission Electron Microscopy (TEM) Study

In order to determine the size of AgNPs obtained by the biosynthesis method in the laboratory environment, the average particle size and clustering were determined by Transmission Electron Microscope (TEM) analysis. According to the TEM images of the NPs obtained, it can be said that the particles are formed in nano-size (<100 nm). According to the TEM analysis results, it has confirmed that the morphology of Au-NPs is basically global. This finding is also consistent with the sharp shape of the UV-Vis peak. When Au-NPs are examined in terms of size distributions, these particles are in a spherical shape, and the size ranges were found to show a homogeneous distribution in the 5-15 nm range (Figure 5). This result also supports the results calculated from XRD analysis.

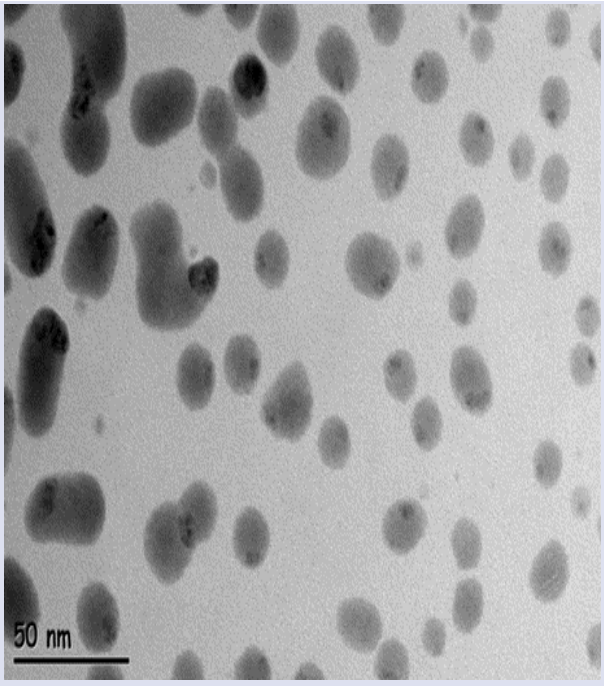


Figure 5. TEM image of Au-NPs forms using A. aestivus extract

### Zeta Potential Study

The stability of the synthesized Au-NPs was measured using zeta potential analysis. The value of the zeta potential provides information about the particle stability. As the zeta potential increases, the particles are prevented from coming together to form aggregates due to a greater electrostatic repulsion between the particles and the stability in the colloidal suspension increases. The behavior of particles in polar liquids is determined by the zeta potential values, not by the electrical charge on their surface.

The zeta potential results of the nanoscale Au particles we synthesized in our research are given in Figure 6. Au-NPs obtained from A. aestivus extract were measured as -24.1 mV and are moderately stable.

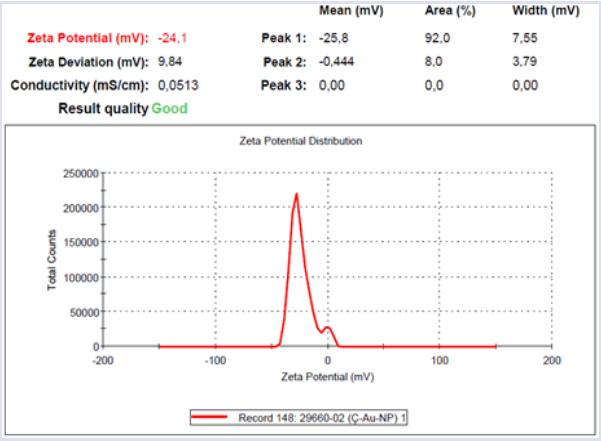


Figure 6. Zeta potential image of Au-NPs form by using A. aestivus extract

### Dynamic Light Scattering (DLS) Study

In order to understand whether the nanoscale materials in the liquid medium are physically stable or not, the distribution of the particles is examined. Particle size and particle distributions can be obtained in dynamic light scattering (DLS) analysis. In this method, the most important parameter is the PdI (Poly dispersity index) value. If the PdI value is between 0.1 and 0.25, it can be said that the desired narrow distribution has been achieved.

PdI values of AuNPs obtained from A. aestivus extract were obtained as 0.386. When the data obtained in this context are evaluated; Since the PdI value of the Au-NPs obtained by the biosynthesis method is between 0.25 and 0.5, the particles are in narrow and wide distribution range. When the DLS values are read on Figure 7, it is seen that the AuNPs obtained from the A. aestivus plant are formed in the range of 130-135 nm at 2 % and between 6-15 nm at 98 %.

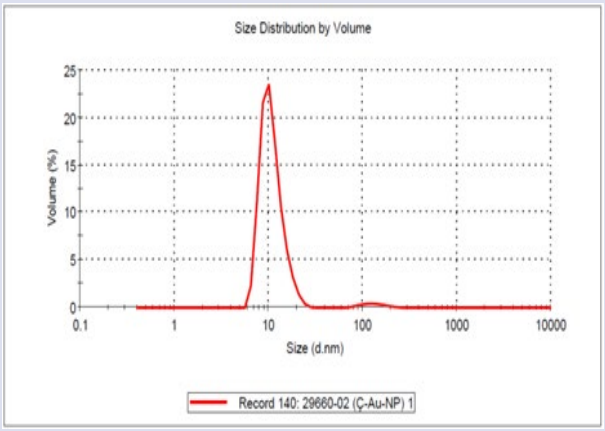


Figure 7. DLS image of Au-NPs form by using A. aestivus extract

### Scanning Electron Microscopy (SEM) Study

After conducting TEM, DLS, Zeta Potential analyzes of the synthesized Au-NPs, the particles in liquid form were dried and turned into powder form for SEM, XRD and FT-IR analyzes. Scanning Electron Microscope (SEM) images

of AuNPs obtained from A. aestivus extract are given in Figure 8.

Powdered metal-based nano-sized particles generally tend to show aggregation [1] . A clustering is clearly seen in SEM images obtained at different magnifications of Au-NPs obtained by the biosynthesis method.

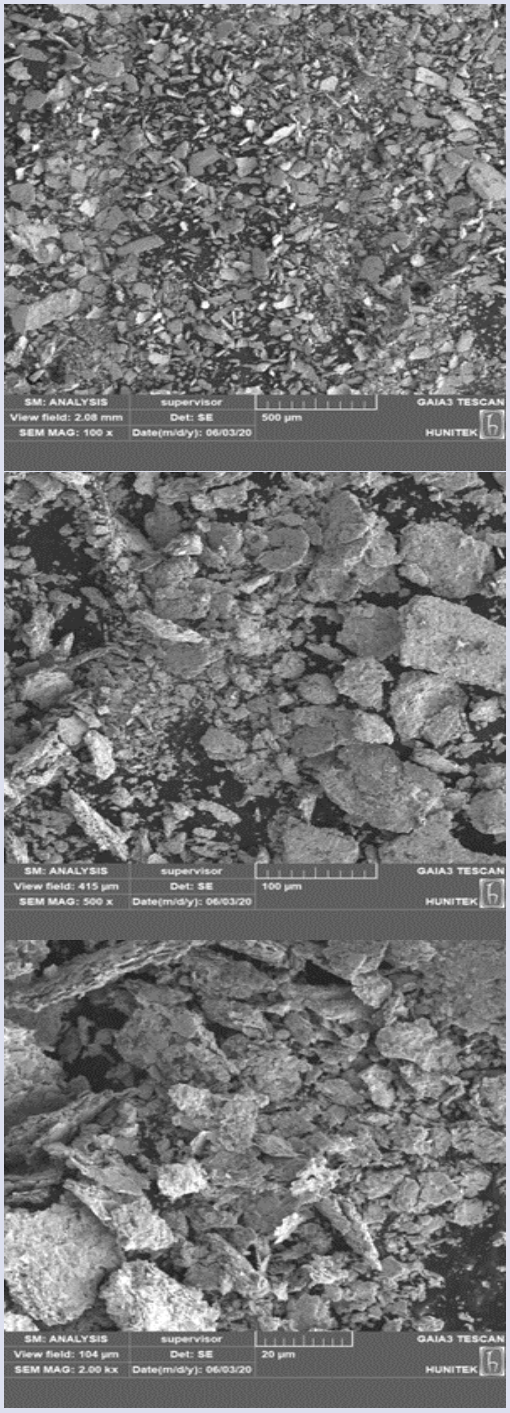


Figure 8. SEM images of the Au-NPs at three different (100x, 500x, 2kx) magnifications

### *Fourier Transform Infrared Spectroscopy Study*

FTIR spectroscopy was performed to identify potential functional groups that allow the reduction of gold nanoparticles. Figure 9 shows the spectra from Au-NPs synthesized using the A. aestivus extract.

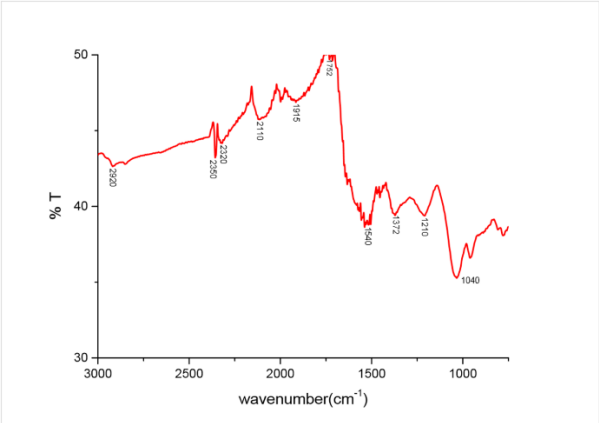


Figure 9. FT-IR graph of Au-NPs form by using A. aestivus extract

At the region of 2920 $cm^{-1}$ the presence of C-H bond in xanthone [20] and other compounds in the plant extract is significant. Peak at 1752 $cm^{-1}$ shows the C=O strechings. Peaks between 1500 and 1600 $cm^{-1}$ are C-C aromatic bond peaks indicating the presence of aromatic components in the extract. Peaks seen in the region between 1400-1500 $cm^{-1}$ are related to the aromatic backbone in the extract. Peaks between 1300-1000 $cm^{-1}$ were attributed to C-O-C stretch. When all the IR findings are evaluated together, it can be said that aromatic compounds such as xanthones, flavonoids, benzophenones are involved in the reduction and stabilization of gold nanoparticles.

### *Chitosan Capping Study*

The zeta potential plot of nanoparticles obtained via biosynthesis is given in Figure 10.

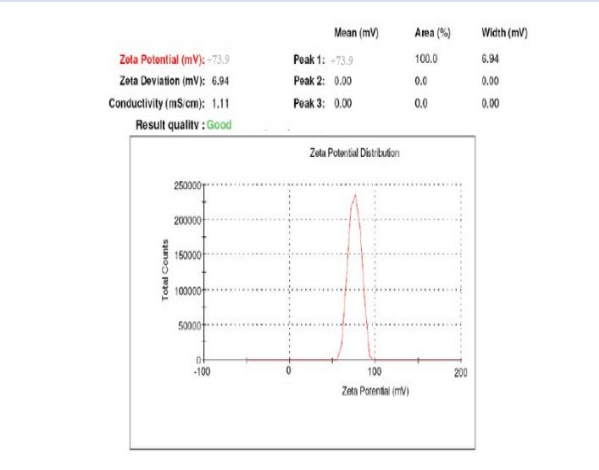


Figure 10. Zeta potential image of Au-NPs capped with Chitosan biopolymer

After biopolymer capping, the surface properties of the biosynthesized gold nanoparticles and the associated zeta potential have been changed from negative to positive and increased in absolute value. In the light of these data, it can be said that the stability of nanoparticles increased after chitosan capping.

In the SEM image given in Figure 11 below, the biosynthesized gold nanoparticles coated with chitosan

are seen. As with the synthesized uncoated powder particles, it can be said that polymer coated Gold nanoparticles also tend to form clusters.

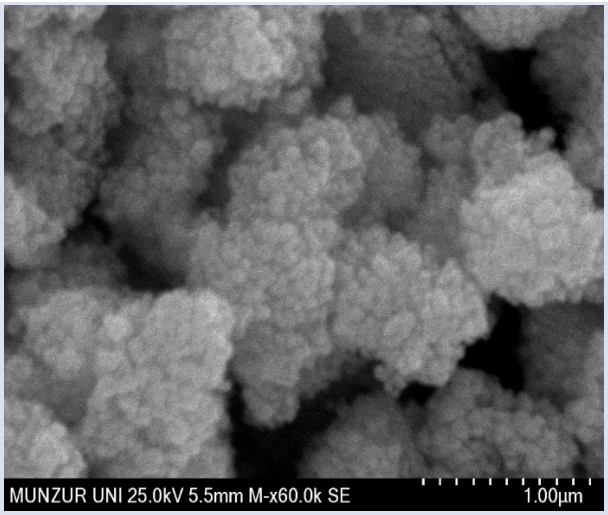


Figure 11. SEM image (60k.x) of the biosynthesized Au-NPs coated with Chitosan biopolymer

### *Cytotoxicity Study*

HUVEC cells have been accepted as a general model for toxicity determination of NPs [21]. Because these tests are important methods for colorimetric cell viability experiments especially in the study of eukaryotic cell activity and they are widely used in cell proliferation and cytotoxicity analyzes. In this context in the study, the in-vitro cytotoxicity of AuNPs and their polymer coated derivatives obtained from A. aestivus plants was evaluated using the MTT test on HUVEC cells for 24 and 48 hours. First, HUVEC cells were incubated depending on the dose (µg / ml) of AuNPs and the polymer coated forms. Results were evaluated as % viability ± confidence interval. The obtained results were given in the form of a table with percentage (%) against concentration of live cells (Figure 12 and 13).

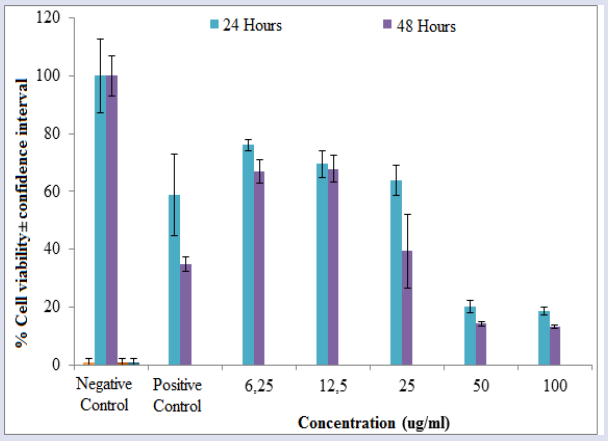


Figure 12. HUVEC viability in the presence of Au-NPs assessed by MTT assay [(% viability ± confidence interval (95%, absolute)]

Percentage viability values of both plant extract and biopolymer-coated AuNPs on the HUVEC cell decreased due to the increase in both time and concentration. However, by coating Au-NPs with Chitosan, cell viability appears to be higher than the uncoated forms in terms of both time and concentration.

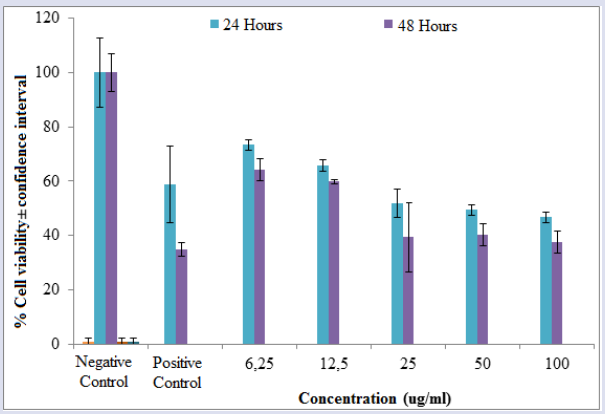


Figure 13. HUVEC viability in the presence of Chitosan coated Au-NPs assessed by MTT assay [(% viability ± confidence interval (95%, absolute)]

This is an expected result for a coating made with a biocompatible polymer. Cell viability increased significantly with chitosan coating, especially at high concentrations.

## Conclusion

In this study, an economical, green, sustainable, rapid, mild and efficient method for synthesis of Au-NPs using A. aestivus extract has been demonstrated. At the same time, coating with Chitosan biopolymer was done by similar methods. Based on UV-visible spectrum, DLS and TEM analysis results, Au NPs of a small size were produced. While ± 30mV zeta value is required for a suspension to be physically stable [23], the measured -24mV value of the synthesized Au-NPs can be said to be stable. It is understood that the groups determined by FT-IR are favorable reducing and stabilizing agents. The synthesized nanoparticles were further stabilized by coating with chitosan biopolymer. The two types of nanoparticles synthesized showed little cytotoxic effect. Coating with chitosan reduced cytotoxicity, especially at high particle doses. It was determined that the coating with Chitosan increased the stability and decreased cytotoxicity. Of these particles synthesized and identified it is expected to have extensive other applications.

## Acknowledgment

This master thesis research procect was supported by the grant funded by the Munzur University Scientific Research Projects Coordination Unit (MUNIBAP Reference Grant no. YLMUB019-03).

## Conflicts of interest

The authors state that did not have conflict of interests

## References


[1] Yilmaz E., Ates M., Sahilli Y.Ç., Synthesizing, chitosan coating and detecting the nanotoxic effect of the lead selenide (PbSe) quantum dots, *Digest Journal of Nanomaterials and Biostructures,* 13(4) (2018) 1173-1182.

[2] Rao K.J., Paria S., Aegle marmelos Leaf Extract and Plant Surfactants Mediated Green Synthesis of Au and Ag Nanoparticles by Optimizing Process Parameters Using Taguchi Method, *ACS Sustainable Chemistry and Engineering*, 3(3) (2015) 483–491.

[3] Wu Y., Ali M.R.K., Chen K., Fang N., El-Sayed M.A., Gold nanoparticles in biological optical imaging, *Nano Today,* 24 **(**2019) 120-140.

[4] Male D., Gromnicova R., McQuaid C., In: Al-Jamal K.T. (Eds). Gold Nanoparticles for Imaging and Drug Transport to the CNS, International Review of Neurobiology. Massachusetts: Academic Press (130) (2016) 155-198.

[5] Yong K.T., Swihart M.T., Ding H., Prasad P.N., Preparation of Gold Nanoparticles and their Applications in Anisotropic Nanoparticle Synthesis and Bioimaging, *Plasmonics,* 4(2) (2009) 79–93.

[6] Sanket S., Das S.K., Role of Enzymes in Synthesis of Nanoparticles. In: Thatoi H., Mohapatra S., Das S.K. (Eds). Bioprospecting of Enzymes in Industry, Healthcare and Sustainable Environment. Berlin: Springer, (2021) 139-153.

[7] Guilger-Casagrande M., de Lima R., Synthesis of Silver Nanoparticles Mediated by Fungi: A Review, *Frontiers in bioengineering and biotechnology*, 7 (2019) Article: 287.

[8] Khanna P., Kaur A., Goyal D., Algae-based metallic nanoparticles: Synthesis, characterization and applications, *Journal of Microbiological Methods,* 163 (2019) 105656.

[9] Kumar, S.V., Rajeshkumar, S., Plant-Based Synthesis of Nanoparticles and Their Impact. In: Tripathi D.G., Ahmad P., Sharma S., Chauhan D.K., Dubey N.K., (Eds). Nanomaterials in Plants, Algae, and Microorganisms. Massachusetts: Academic Press, 1 (2018) 33-57.

[10] Mittal A.K., Chisti Y., Banerjee U.C., Synthesis of Metallic Nanoparticles Using Plant Extracts, *Biotechnology Advances,* 31/2 (2013) 346-356.

[11] Lee K.X., Shameli K., Miyake M., Kuwano N., Khairudin N.B.A., Mohamad S.E.B., Yew Y.P., Green synthesis of gold nanoparticles using aqueous extract of Garcinia mangostana fruit peels, *Journal of Nanomaterials*, (2016) Article ID 8489094.

[12] Davis P.H., Flora of Turkey and the East Aegean Islands. Edinburgh: Edinburgh University Press, 8 (1984) 543-544.

[13] Chung IM., Park I., Seung-Hyun K., Thiruvengadam M., Rajakumar G., Plant-Mediated Synthesis of Silver Nanoparticles: Their Characteristic Properties and Therapeutic Applications, *Nanoscale Research Letter,* 11 (2016) Article 40.

[14] Shakeel A., Annu S.I., Salprima Y.S., Biosynthesis of gold nanoparticles: A green approach, *Journal of Photochemistry and Photobiology B: Biology*, 161 (2016) 141-153.

[15] Singh P., Kim Y.-J., Zhang D., Yang D.-C., Biological Synthesis of Nanoparticles from Plants and Microorganisms, *Trends in Biotechnology*, 34 (7) (2016) 588-599.

[16] Nathanael A.J., Oh T.H. Biopolymer Coatings for Biomedical Applications, *Polymers*, 12(12) (2020) 3061.

[17] Wang Y., Dave R. N., Pfeffer R., Polymer coating/encapsulation of nanoparticles using a supercritical anti-solvent process, *The Journal of Supercritical Fluids*, 28(1) (2004) 85-99.

[18] Neuberger T., Schopf B., Hofmann H., Hofmann M., von Rechenberga B., Superparamagnetic Nanoparticles for Biomedical Applications: Possibilities and Limitations of a New Drug Delivery System, *Journal of Magnetism and Magnetic Materials*, 293 (2005) 483–496.

[19] Mossman T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *Journal of Immunological Methods,* 65 (1983) 55-63.

[20] Shameli K., Ahmad M.B., Shabanzadeh P., Effect of curcuma longa tuber powder extract on size of silver nanoparticles prepared by green method. Research on Chemical Intermediates, *Research on Chemical Intermediates*, 40 (2014) 1313–1325.

[21] Cao Y., Gong Y., Liu L., The use of human umbilical vein endothelial cells (HUVECs) as an in vitro model to assess the toxicity of nanoparticles to endothelium: a review, *Journal of Applied Toxicology*, 37(12) (2017) 1359–1369.

[22] Mahdavi M., Namvar F., Ahmad M.B., Mohamad R., Green biosynthesis and characterization of magnetic iron oxide ($Fe_3O_4$) nanoparticles using seaweed (Sargassum muticum) aqueous extract, *Molecules (Basel, Switzerland)*, 18**(**5) (2013) 5954–5964.

[23] Faried M., Shameli K., Miyake M., Hajalilou A., Synthesis of silver nanoparticles via green method using ultrasound irradiation in seaweed Kappaphycus alvarezii media, *Research on Chemical Intermediates*, 42 (2016) 7991–8004.

[24] Sharma R.K., Tahiliani S., Jain N., Priyadarshi R., Chhangani S., Purohit S.D., Joshi P., Cynodon dactylon Leaf Extract Assisted Green Synthesis of Silver Nanoparticles and Their Anti-Microbial Activity, *Advanced Science, Engineering and Medicine,* 5(8**)** (2013) 858-863.