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Biogenic Synthesis and Characterization of Gold Nanoparticles from *Morus alba* L. Leaves by Microwave Extraction Method

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Abstract: The aim of this study was to investigate the rapid, simple, and inexpensive biosynthesis and characterization of gold nanoparticles (AuNPs) an extract from leaves of Morus alba L. The generated gold nanoparticles were characterized by UV-Vis spectroscopy, TEM, FT-IR and zeta-sizer. For the biogenesis of gold nanoparticles, Morus alba L. (Mulberry) leaves and $HAuCl4.3H_2O$ solution were utilized as the starting ingredients. Fresh leaves of Morus alba L. were collected from Turkey (Trabzon). 100 mL of distilled water was mixed with 10 g of dried material for 120 minutes. The mixture was then extracted using a laboratory microwave for 4 minutes at 600 W. For the biosynthesis of AuNPs, different amounts 100 ml of (0.5 mM, 1 mM) aqueous $HAuCl4.3H_2O$ solution of leaf extract (0.5 and 1 mL) were mixed with HAuCl4. $3H_2O$ solution, and then the mixture was placed in a household microwave at 90 W for 1 to 30 minutes. UV-vis spectroscopy, TEM, FT-IR and zeta-sizer were performed to characterize the produced gold nanoparticles. UV-Vis absorption spectra was measured using a Shimadzu UV-1240 UV-Vis spectrophotometer with a wave length range of 300 to 800 nm. The development of AuNPs was indicated by the mixture's purple-red colour. From the results of zetasizer study, the average particle size of the AuNPs was 78.95±0.57 nm, the zeta potential was 12.9±0.808 mV, and the polydispersity index was 0.321±0.004. When the AuNP solutions were kept in the refrigerator, their UV-Vis absorption spectra rarely changed and remained stable for around 2 to 2.5 months.

Keywords: Mulberry (*Morus alba* L.) leaf, Au nanoparticle, Microwave extraction, UV-Vis spectroscopy, TEM.

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1. INTRODUCTION

The capacity to translate this structure into applications by introducing a new product in several scientific fields, including materials science, biology, and chemistry, is what makes nanotechnology one of the most promising branches of nanoscience and nanotechnology in the modern era (1). The definition of nanoparticles, which constitute the fundamental units of nanotechnology, may change depending on the application area, studies, and the properties of the corresponding materials, and they are commonly known as particles that range in size from 1 to 100 nm (2). Nanoparticles are particles composed of carbon, metal, metal oxides, or organic substances (3,4). Most crucially, one of the things that distinguish nanotechnology from other technologies is that the surface and volume properties of the material are linked at the nanometer scale, and the ratio of surface to volume rises (5). Surface molecules can cause high hardness in metals and also make electronic devices and drugs highly efficient (1). Nanoparticles in nanotechnology in medicine and pharmaceuticals Electrics/electronics, food, textiles, environment and energy, materials and manufacturing industries (3, 7-9). Nanomaterials can provide solutions to various environmental problems through technological innovations and advances in energy storage, medicine, and wastewater treatment (9-12).

Modern extraction techniques, like microwave-assisted and supercritical fluid extraction, have improved as a result of the shortcomings of conventional approaches, especially when working with medicinal plants, as modern techniques are needed that minimize the use of organic solvents, reduce extraction time, and are environmentally friendly. More recently, "green synthesis" (13,14) has been used to overcome limitations such as expensive chemicals and equipment, extreme reaction conditions, potentially hazardous and negative environmental impact, and biocompatibility (15). "Biogenic synthesis," an alternative production method recognized as an ecological approach for the production of various nanomaterials and plant extracts (16-18), environmentally friendly approaches have been developed and used, including the application of natural and renewable materials. Plant materials containing biological extracts are employed as reducing and capping agents in green synthesis, which is quite inexpensive because it greatly lowers the cost of the synthesis process (19) overall and produces more symmetrical and stable nanoparticles (14, 20). Biological synthesis was preferred due to its environmental and economic advantages (21). Because they are produced using a single approach, biologically produced nanoparticles are one step more convenient and stable and have the characteristic of having dimensions (22).

Plant extracts have been suggested in recent years as a straightforward and practical alternative to chemical and physical approaches for producing nanoparticles due to their advantages. One of the most investigated metallic NPs is gold. Due to their good stability, optical properties, biocompatibility, antifungal, antidiabetic and antibacterial effects in nanoparticle research, researchers have become interested in Au nanoparticles in numerous crucial application areas for many chemical, biological, and environmental sectors (21, 23-25). AuNPs with a wide range of sizes and shapes have exceptional properties that can facilitate the development of new devices for a variety of biomedical applications. Important biomedical applications of AuNPs include targeted drug release, cancer therapy, contrast agents in medical imaging, molecular imaging in living cells, antimicrobial and antibacterial activities, antiviral treatments, biosensors and intracellular analysis, photothermal therapy, and hyperthermal effects (23, 24).

In this research, using *Morus alba* L. leaves extract as a coating and reducing agent, it has been reported that gold nanoparticles can be biosynthesised in under 30 minutes. Mulberry, a multifunctional agro-forestry plant from the Moraceae family, is frequently used as a silkworm diet (26). *Morus alba* sometimes referred to as white mulberry, common mulberry, and silkworm mulberry. It is widely cultivated and does well all year long in China, India, Japan, and Korea (27). Mulberry and its parts are frequently utilized to generate a variety of functional foods, including mulberry leaf-carbonated beverages and healthy beverages, due to their high bioactivities. Due to its low toxicity and effective therapeutic properties, it is also consumed in China and Japan as a traditional herbal medicine and substitute tea (28, 29).

This study's objectives were to research the quick, simple, and affordable biosynthesis and characterisation of AuNPs utilizing an extract made from *Morus alba* L. leaves. UV-Vis spectroscopy, TEM, and Zeta-sizer were used to characterize the produced gold nanoparticles.

2. EXPERIMENTAL SECTION

2.1. Preparation of *Morus alba* L. leaves extract

For the biogenesis of gold nanoparticles, *Morus alba* L. leaves and $HAuCl_4.3H_2O$ solution were utilized as the starting materials. *Morus alba* L. leaves were gathered from the Eastern Black Sea region of Turkey (61 m, Trabzon, Turkey) at the end of June 2022. The plant material has been placed at the Herbarium of the Department of Forest Engineering Faculty at Karadeniz Technical University in Trabzon, Turkey (KATO:19271), after Prof. Dr. Salih TERZİOĞLU verified the taxonomic identity of the plant sample. And then cleaned with de-ionized water, dried in the shade and finely ground in a grinder. 10 g of dried material was combined with 100 mL of purified water and left for 120 minutes. The flask was shaken before being placed inside a lab microwave, where it was extracted at 600 W for 4 minutes before being allowed to cool (30). To filter the mixture, Whatman filter paper was employed. The extracted solution was kept for future use in a refrigerator. Analytical grade 99.9% $HAuCl_4.3H_2O$ Sigma Aldrich Chemical Reagent Co. Ltd. was purchased. Using *Morus alba* L. leaf extract as a reducing and stabilizing agent in an aqueous solution, gold nanoparticles were easily created by reducing $HAuCl_4.3H_2O$.

2.2. Production of AuNP

In a typical synthesis procedure, 100 ml of (0.5 mM, 1 mM) aqueous $HAuCl_4.3H_2O$ solution were combined with a specified volume (0,5 mL–1 mL) of mulberry leaves extract. The mixture was then microwaved at 90 W for 1–30 minutes, indicating the creation of gold nanoparticles. There were three iterations of each technique. The gold nanoparticles were then gathered, removed from the mulberry leaf extract using centrifugation, and then dried at 40 °C for an additional 18 hours.

2.3. AuNP characterization

UV-Vis spectroscopy makes it simple to see how gold nanoparticles are made by reducing gold ion solution with mulberry leaf extract. Using a Shimadzu UVP-1240 spectrophotometer, the absorption spectra of reaction solutions at various mulberry leaf extract concentrations and $HAuCl_4.3H_2O$ solution concentrations were captured with a resolution at wavelengths between 300 nm and 800 nm. Water that had been double-distilled used as a blank solution.

To determine the shape of nanoparticles, a transmission electron microscope (TEM) was used. A Hitachi HT-7700 Tecnai transmission electron microscopy instrument was used for the TEM observations, and it was run at an accelerating voltage of 40–120 kV. The condition of a nanoparticle in polar liquids is determined by its zeta potential, which also provides specific details about the dispersion mechanisms. Using the DLS technique (Malvern Zetasizer Nano ZSP), light source He-Ne laser 633nm, Max 10mW, 100VA maximum power, temperature range 0°C - 90°C +/ -0.1, zeta potentials, polydispersity indices (PI), and average particle sizes of Au nanoparticles are calculated.

3. RESULTS AND DISCUSSION

3.1. Characterization of Gold Nanoparticles

As stated before, the transition from light yellow to dark purple is a certain indicator that nanoparticle manufacturing has taken place (Figure 1).

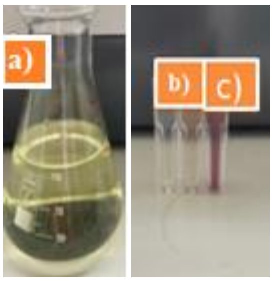


Figure 1. (a) Before microwave treatment, color change of $HAuCl_4.3H_2O$ solution and Mulberry leaf extract mixture, (b) and (c) after microwave treatment, purple pink color indicating the formation of Au-NPs.

The mulberry leaf extract was employed as a capping and reducing agent to create nano gold colloid. Using UV-vis spectroscopy, which is an effective spectroscopic technique for finding metallic nanoparticles, it was possible to confirm the creation of gold nanoparticles.

a)

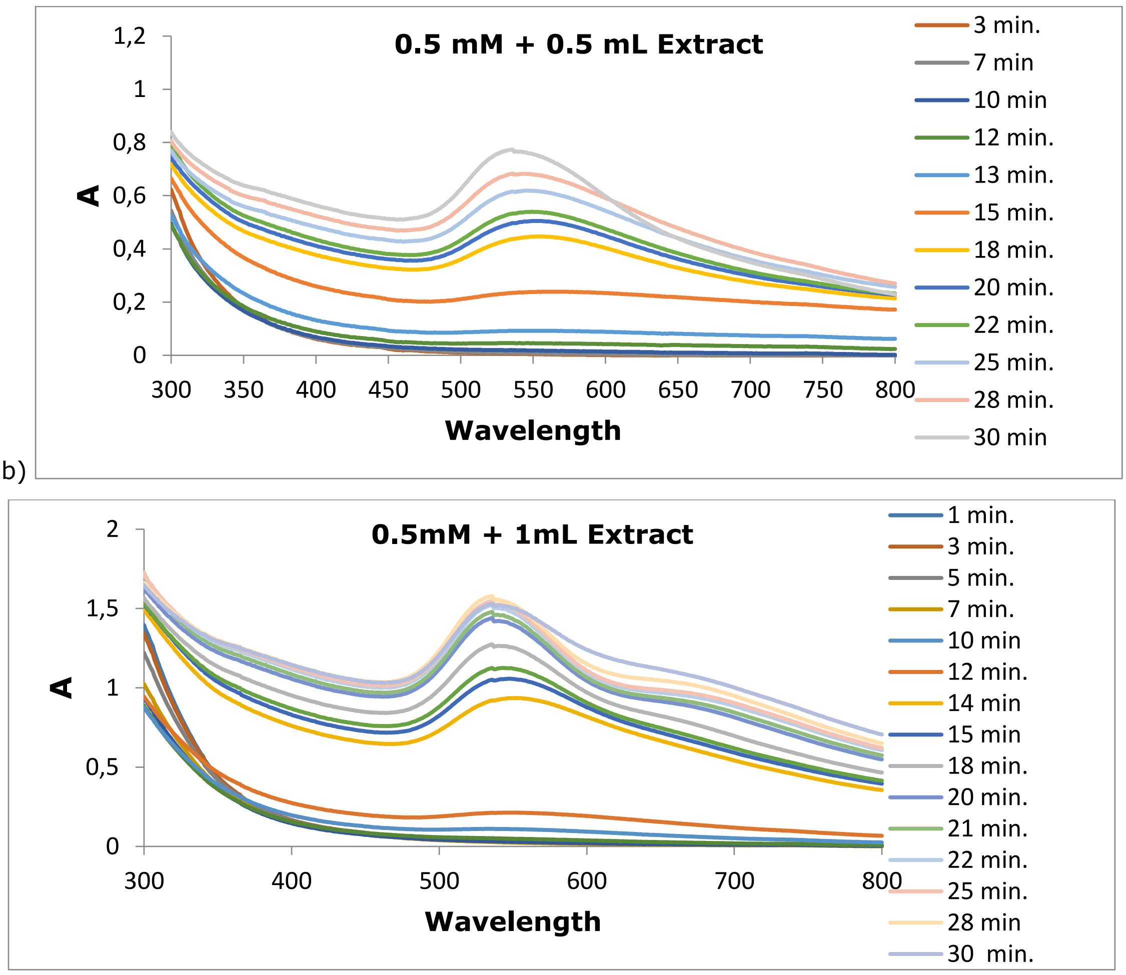
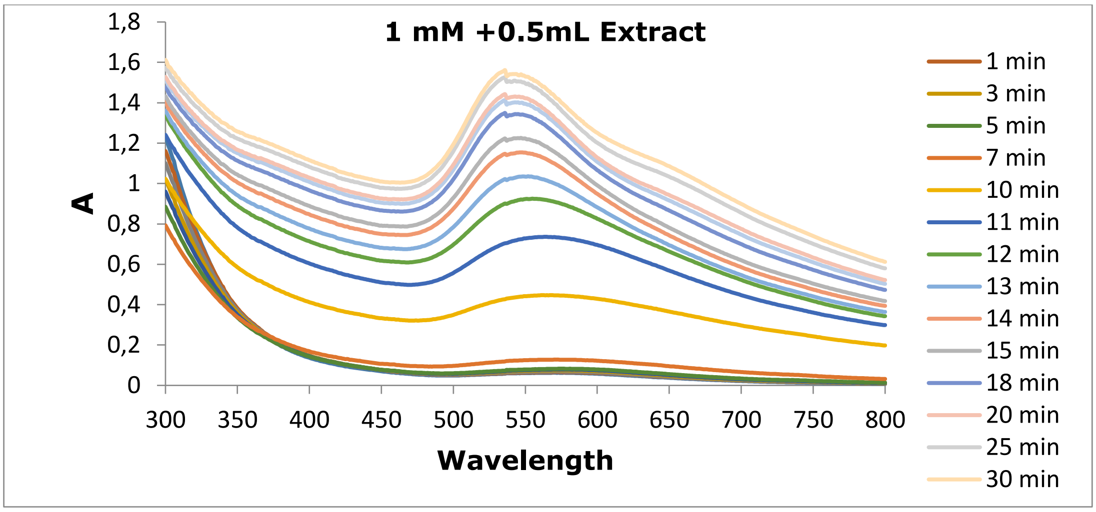


Figure 2. SPR absorption spectra of gold nanoparticles produced at a concentration of 0.5 mM (a [0,5 ml]-b [1 ml]) $HAuCl_4.3H_20$.

According to Figure 2a, which shows a distinctive resonance band about 530-580 nm after 18 minutes of exposure, which is the typical absorption band of spherical gold nanoparticles, it appears that AuNPs were successfully produced with 0.5 mL mulberry leaves extract. After 30 minutes of microwave treatment at 90 W power, the band reached its highest point. The size and form of the gold nanoparticles are related to the absorption band, which is the surface plasmon absorption of gold nano colloids.

a)



b)

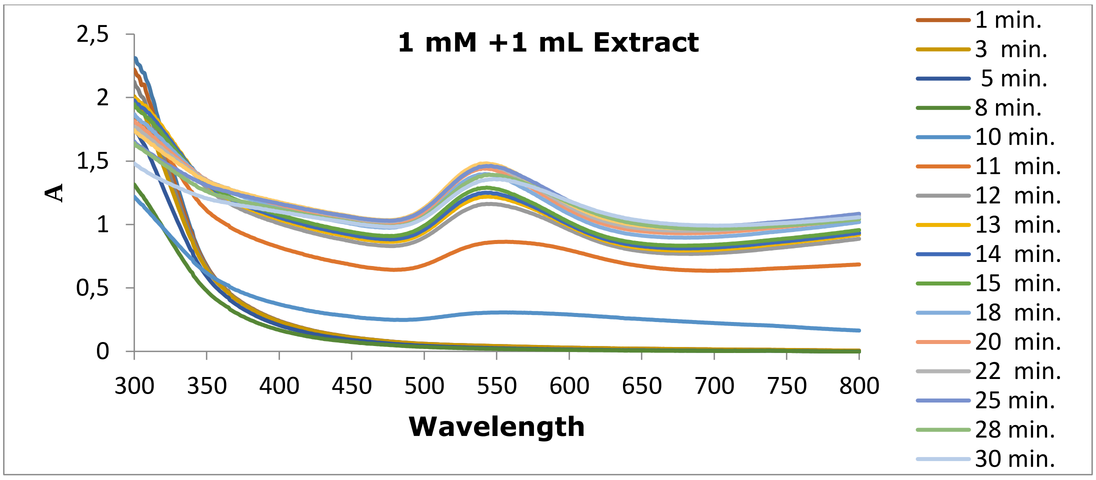


Figure 3: SPR absorption spectra of gold nanoparticles produced at a concentration of 1 mM (a [0.5 ml]-b [1 ml] $HAuCl_4.3H_20$.

The amount of nanoparticles was undoubtedly increased by the larger extract volume (1 mL). For 14 and 30 minutes, the surface plasmon resonance (SPR) absorption band was very strong and wide. However, significant agglomeration caused the particle size to be high. The hue of the reaction mixture revealed it. After the exposure period, the colloidal solution had turned dark purple and cloudy. A higher concentration of $HAuCl_4.3H_2O$ led to more AuNP generation (Figure 3a and b). There must be a limit to the bioactive components reductive power. On the other hand, even after an 11-minute microwave treatment, 1 mL of mulberry leaves extract appears to yield AuNPs (Figure. 3a and 2b). A notable band peak shift to the 600 nm region was also noted (Figure 3).

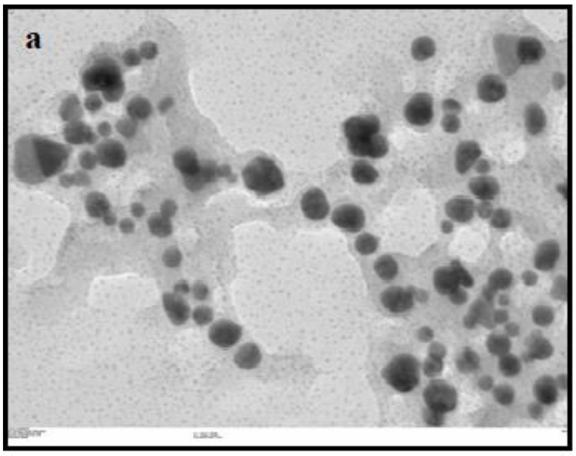


Figure 4. TEM images of Au nanoparticles were produced using the microwave extraction method with 0.5 mM $HAuCl_4.3H_2O$ (100 nm).

Through TEM analysis, the morphology of the biosynthesized AuNPs was further confirmed. Figures 4. demonstrated the creation of a few irregularly shaped particles as well as three different magnifications of almost spherical AuNPs.

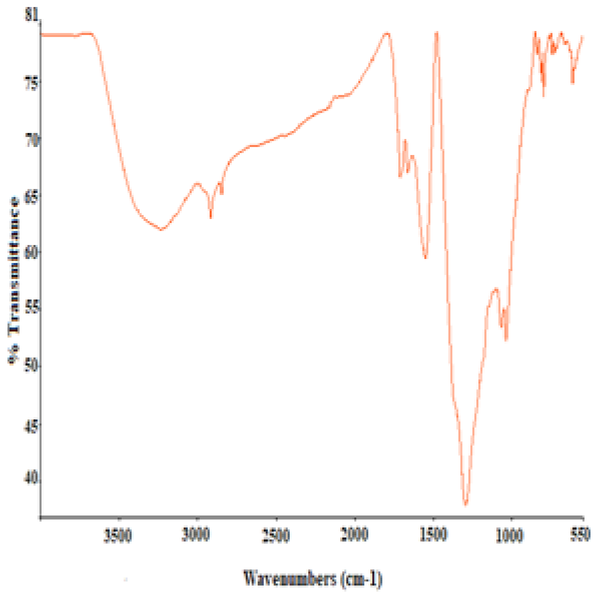


Figure 5. FT-IR spectrum of synthesized AuNPs.

The potential biomolecules responsible for capping and effective stabilization of the Au-NPs produced with mulberry leaf extract were determined using FT-IR studies. The biosynthesized AuNPs demonstrate strong bands at 3228 cm^{-1}, 2917 cm^{-1}, 1551 cm^{-1}, 1290 cm^{-1}, 1071 cm^{-1}, 1041 cm^{-1}, 616 cm^{-1}. This shows the various functional groups of biomolecules that have been adsorbed on the surface of the nanoparticles and also shows the impact of organic moieties on the synthesis of AuNPs and their ability to withstand the aqueous medium. The strong band seen at 3228 cm^{-1} is due to amine group stretching vibrations on the side of the hydroxyl group. It is possible to attribute the signal at 2917 cm^{-1} to C-H stretching vibrations of the methyl, methoxy, and methylene groups. The properties of C-OH protein vibrations and the -C-O-C bending mode are represented by the peaks at 1071 cm1 and 1041 cm^{-1}, respectively. The N-H groups of proteins' plane-bending vibrations could be the cause of the band at 661 cm-1. According to the findings of FTIR spectral analysis, the biomolecules in the extract of Mulberry leaf served as the agents in reducing, stabilizing of AuNPs.

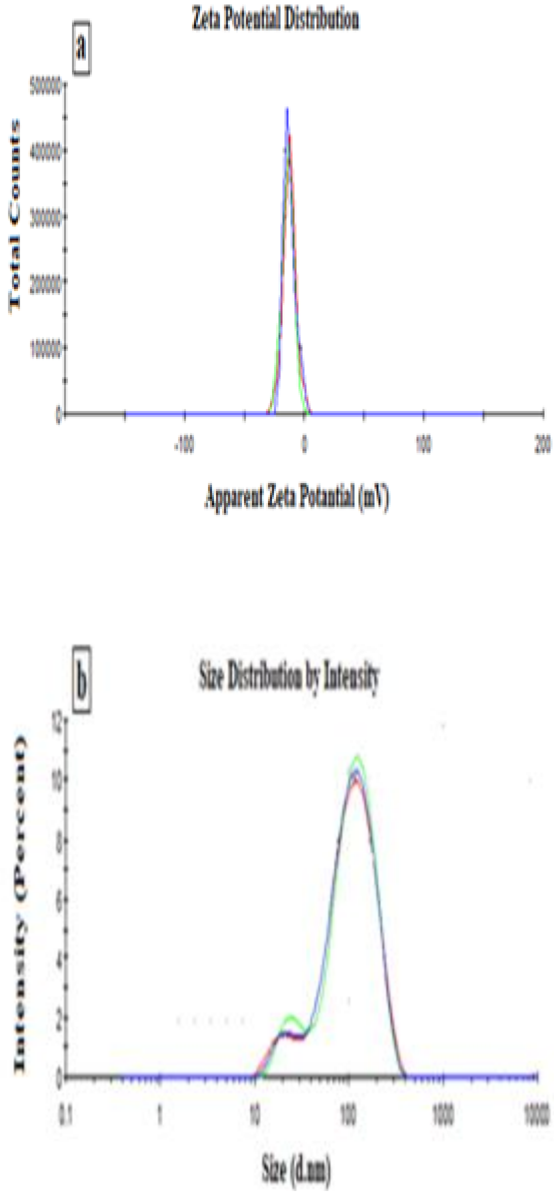


Figure 6. Zeta potential (a) and size distribution (b) of Au nanoparticles produced by microwave extraction using 0.5 mM $HAuCl_4.3H_2O$ and 0.5 mL mulberry leaves extract, respectively.

The size of the metallic core and the thickness of the capping or stabilizing substance surrounding the metallic nanoparticles are determined using the dynamic light scattering (DLS) technique (31). Using DLS measurements, Figure 6(a) displays the particle size distribution of the biosynthesized AuNPs. Au-NPs were discovered to have an average particle size of 78.95 ± 0,57 nm. The ZP value of -12,9 ± 0,808 mV and a polydispersity index of 0,321±0,004 are shown in Figure 6(b), which points to colloidal AuNPs' increased stability. These findings were found to be comparable with the literature carried out by Adavallan and Krishnakumar (2014) (31). Adavallan and Krishnakumar (2014) synthesized gold nanoparticles from Mulberry leaf extracts using chemical synthesis and biosynthesis and used the zeta-sizer method for their characterization. They found the zeta potential and the average particle size of the biosynthesized AuNPs gold nanoparticles were -16 mV, 50 nm, respectively. Zeta potential (ZP) values provide details on the stability and surface charge of biosynthesised Au-NPs (32). It's possible that the mulberry leaf extract's abundant protein content is what causes the metal ions to be reduced and the biosynthesized nanoparticles to be stabilized effectively (32).

4. CONCLUSION

The study describes a straightforward, innovative, and productive method for creating gold nanoparticles that uses *Morus alba* L. leaf extract as a reducing and stabilizing agent for gold. The surface plasmon resonance of biosynthesized AuNPs was validated by UV-visible spectrum spectroscopy. For bio-synthesized AuNPs, the zeta potential value was −12,9 ± 0,808 mV, confirming the stability of the nanoparticles. Gold nanoparticle preparation is illustrated utilizing a straightforward, quick, and environmentally friendly method that uses mulberry leaf extract as a capping and reducing agent. In the proposed technique, when the extract volume is 0,5 mL and the $HAuCl_4.3H_2O$ concentration is 0,5 mmol/L, it takes 30 minutes to synthesis gold nanoparticles in MAE. The production of size particles depends on several factors, including the quantity of extract, the concentration of $HAuCl_4.3H_2O$, and time.

5. CONFLICT OF INTEREST

There are no disclosed conflicts of interest for this author. The research study complies with publication and research ethics.

6. ACKNOWLEDGMENTS

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