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Eco-friendly Synthesis of Gold Nanoparticles Using *Camellia sinensis* Phytoextracts

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Abstract: Biosynthesis of metal nanoparticles using plant materials involves a fairly rapid reduction of metallic materials. The reduction potential of phytochemicals in a tea extract to reduce gold salt ($NaAuCl_4$) to the highly homogenous gold nanoparticles is presented. Phytoextracts were derived from the mixture of *Camellia sinensis* var. *sinensis* and *Camellia sinensis* var. *assamica* (green tea) leaves. The appearance of the phytoextract's sloping absorption peak with Au salt at the wavelength range 530-550 nm corresponded to the absorption of gold nanoparticles. Obtained nanoparticles were purified from phytoextract excess by centrifugation. Then they were studied by Raman and FTIR spectroscopy and AFM. It was found that the size of produced gold nanoparticles was in the range from 3 nm to 10 nm.

Keywords: Green synthesis; *Camellia sinensis;* Au nanoparticles*;* FTIR; AFM.

1. Introduction

Gold nanoparticles (NPs) have been widely investigated due to their applications in different areas: Au NPs with unique properties have been used in photo-thermal therapy, tissue imaging, immunoassays, biosensors, cancer treatment, and vectors for drug delivery [1-3]. Considering their size and surface functionalization, gold NPs may be divided into three main groups: gold colloids, gold nanoparticles protected by an organic monolayer (MPCs), and small gold clusters. Gold colloids are large metal particles with a size range of 10-100 nm, usually obtained by reducing Au (III) salts in the presence of mild reducing agents, most commonly, trisodium citrate. Monolayer protected clusters (MPCs) are particles with a gold core with a size in the range of 1-10 nm surrounded by a monolayer of strongly chemisorbed ligands; the most studied systems are stabilized by thiols [4]. Thiolated DNA functionalized gold nanoparticles have popularly been used to develop new tools for numerous applications. Researchers demonstrated a facile and fast strategy to prepare mDNA–Au NPs using diblock polyA-DNA. Under acidic pH, polyA can be rapidly adsorbed on AuNPs. This polyA-mediated mDNA–Au NPs exhibited fast hybridization kinetics and high hybridization efficiency [5]. Gold nanoparticles possess notable optoelectronic properties that are dependent on the shape and size of nanoparticles. They have a large surface-to-volume ratio, excellent

biocompatibility, and low toxicity, which are very useful and make them an important tool in nanobiotechnology [6].

The physical properties of gold NPs are strongly influenced by their size because the gold particles' electronic structure changes with size and shape. Gold nanoparticles have a high density of free electrons that result in inherent optical, electrical, and catalytic properties. With the excitation of light, the free electrons on the surface of gold nanoparticles termed as 'plasmons' begin to oscillate from core to one side, which results in the other side being positively charged; this condition is termed as surface plasmon resonance (SPR). SPR of nanoparticles depends mainly on the distance between the particles and shape of nanoparticles, and most of the gold nanoparticles exhibit SPR bands between 510 and 1100 nm [6].

The synthesis of metal nanoparticles (NPs) is a subject of many studies due to its commercial importance and applications. Until now, several methods were employed for their synthesis. The first group of methods is the bottom to top approach; in this case, nanoparticles can be synthesized using chemical reduction by self-assembly of atoms to new nuclei, which grow into a particle of a nanoscale [7]. While in the top to bottom approach, suitable bulk material breaks down into fine particles by size reduction with various lithographic techniques, e.g., grinding, milling, sputtering, thermal/laser ablation [8]. Physical and chemical methods used for the synthesis of NPs are harmful since they require high toxic chemicals that are responsible for various biological risks and are quite expensive [9,10]. Therefore, biogenic approaches are becoming more popular in the field of nanotechnology synthesis. They are non-toxic, providing a single-step technique at ambient temperature and pressure conditions. Biosynthesis of metal nanoparticles (using microorganisms, cell culture, plant tissue, or phytoextracts) acquires more importance due to its simplicity, a rapid rate of formation of metal nanoparticles, eco-friendliness, and safety. Microorganisms could be used to get NPs. For example, the genus *Exiguobacterium* is quite diverse, composed of Gram-positive, facultatively anaerobic, non-sporulating, and motile rods. It is considered a source of enzymes that exhibit a broad range of thermal stability. Au NPs generated by *E. acetylicum* under aerobic and anaerobic conditions were ~ 25 nm and showed irregular morphology. They were composed mainly of carbon and gold [11].

Actinomycetes and fungi have long been used to synthesize metal nanoparticles, such as *Fusarium oxysporum*, *Verticillium sp.*, and *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium fellutanum*, *Pleurotus ostreatus* [12, 13]. Mycogenesis of NPs is considered a prominent way fungi can be used to produce nanostructures with desirable shape and size intracellularly or extracellularly. Fungi contain a variety of enzymes and proteins as reducing agents, so they can be invariably used to synthesize metal NPs. Fungi usually grow faster than those bacteria under the same conditions. Additionally, fungi secrete many enzymes compared to bacteria; thus, the conversion of metal salts into metal nanoparticles is very fast. So, the fungal cell wall's biopotential is likely to play an important role in the absorption and reduction of metal ions and the formation of metal NPs [12].

Nanoparticles produced by plants are more stable, and the rate of synthesis is faster than in microorganisms. The advantages of using plant and plant-derived materials for biosynthesis of metal NPs focus the research interest to investigate the possible mechanism NPs formation in plants [14].

For instance, plant extract of *Cryptolepis buchanani* L also can be used for green and rapid synthesis of gold nanoparticles [15]. The use of natural resources often provides mild reaction conditions and *in situ* capping abilities. The reaction solution's pH value has a critical

impact on the morphology and size of the synthesized Au NPs. The absorbance increases significantly as pH increases from 2 to 7, followed by a decrease in the pH from 8 to 14. In the acidic to neutral range, λmax was observed at around 530 nm, and the highest intensity was obtained at pH 7 [20]. The morphology of the greenly synthesized Au NPs using the *C. buchanani* extract was examined using a TEM. It was revealed that the dispersed Au NPs were spherical in shape with an average diameter of around 11.1±1.3 nm. Moreover, as prepared Au NPs exhibited efficient antimicrobial activities against Gram-positive and Gram-negative bacteria [15].

Interestingly, it was suggested to synthesize discrete gold nanoparticles by genetically modified tobacco mosaic virus (TMV) [16]. Authors have exploited the use of plants as factories to produce novel virus-based structures that are surface decorated with MBP peptides, which can promote the formation of metal nanoparticles. Using AFM it was found that the sizes of gold nanoparticles were in the range of 9–33 nm. The UV-visible analysis showed that the MBP TMV produces a 500–600 nm absorption band with a peak at 550 nm, indicating gold nanoparticle formation [16].

For the present study, *Camellia sinensis* was chosen as a capping and reducing agent. Fresh leaves contain about 4% caffeine, as well as related compounds, including theobromine. Different leaf ages produce differing tea qualities since their chemical compositions are different [17]. Usually, the tip and the first two to three leaves are harvested for processing. The main components of *C. sinensis* include amino acids, fatty acids, terpenoids, phenolic compounds, and purine alkaloids (xanthines). *C. sinensis* extract is widely used due to its anticancer, antidiabetic, anti-inflammatory, analgesic, and apoptogenic properties [17].

This study suggests a plant-based approach for gold nanoparticles biosynthesis from the mixture of *C. Sinensis* var. *sinensis* and *C. sinensis* var. *assamica* (green tea) leaves, studying their optical and structural properties.

2. Materials and Methods

2.1. Biological synthesis of gold nanoparticles.

Aqueous dispersions of gold NPs were obtained by adding the phytoextracts derived from the mixture of *Camellia sinensis* var. *sinensis* and *Camellia sinensis* var. *assamica* (green tea) to 0.001 M solution of sodium tetrachloroaurate (III) dihydrate. For these purposes, 100 mg of dry leaves were crushed in a ceramic mortar with 6 mL of bidistilled water and incubated for 7 minutes at 40–55 ^{o}C. Then this solution was poured into a 1.5 mL microtube and centrifuged for 15 min at 10,000 rpm. The supernatant was collected in 15 mL plastic tube using a micropipette for long time storage in the refrigerator at 4 ^{o}C. Synthesis of NPs was performed as follows: 0.1 mL of phytoextract was added to 1 mL of 0.001 M $NaAuCl_4$ solution, mixed by shaking, and incubated for 15 min at 40 ^{o}C. Obtained NPs were washed from other components by centrifugation for 15 min at 10,000 rpm and t = 4^{o}C. The supernatant was taken carefully by micropipette, and the precipitate with nanoparticles was resuspended in 1 mL of bidistilled water.

2.2. Characterization of gold nanoparticles.

Gold NPs were characterized by UV-Vis (Specord 210, Analytik Jena), Raman, and FTIR spectroscopy as well as by AFM microscopy. Raman and FTIR spectroscopy was done by confocal microscope Olympus BX-41 (50X, aperture 0.60) equipped with a Raman

spectrometer Horiba Jobin Yvon T64000 and registered on 1024x256 CCD-detector (Andor). Excitation of micro-Raman and photoluminescence spectroscopy (PL) was carried out by radiation Ar-Kr laser (Spectra-Physics Model 2018-RM with energy exciting radiation E = 2.41 eV (514.5 nm). The frequency of phonon lines was ≤ 0.2 cm^{-1}. Investigation of surface morphology of films with gold nanoparticles was done using an atomic force microscope (AFM)-Nanoscope IIIa (Digital Instruments).

3. Results and Discussion

The phytoextracts of *C. sinensis* with a 0.001 M of $NaAuCl_4$ solution are represented in Fig. 1.

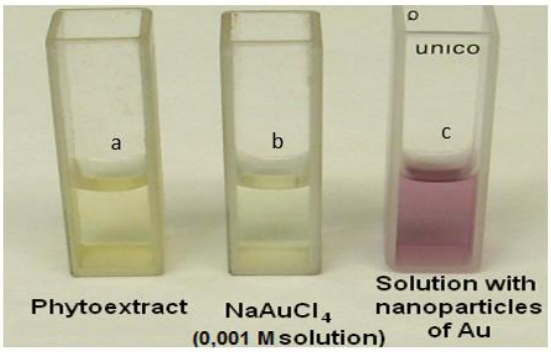

Figure 1. Solutions for preparing gold nanoparticles: initial phytoextract – a; phytoextract with Au-containing salt – b; gold nanoparticles solution – c.

The formation of gold nanoparticles was accompanied by a change in the solution's color to a reddish tinge. It is important to note that there was no need to add external stabilizing agents since phytochemicals themselves act as reducing as well as capping agents. UV-visible spectroscopy is the most commonly used technique for the characterization of noble NPs, which confirm the production of metallic nanoparticles and their stability. We observed the sloping absorption peak of gold NPs was at a wavelength of 530-550 nm (Fig. 2).

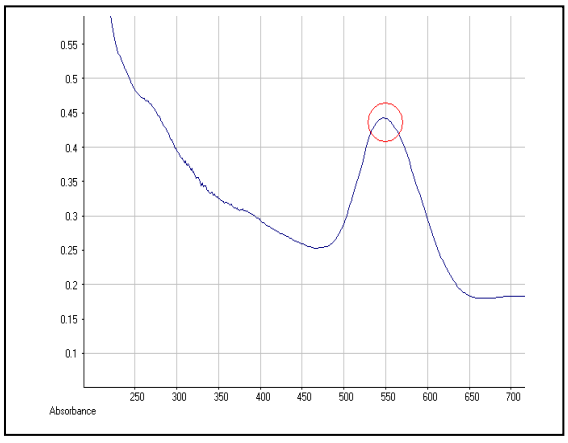

Figure 2. UV-Vis absorption spectrum of produced gold nanoparticles.

Different parameters like concentration of salt, the concentration of plant extract and its nature, the origin of initial organisms, pH, exposure time, temperature, etc., will affect the synthesis and rate of production of gold nanoparticles, their quantity, and other characteristics. In particular, at low pH, there would be an agglomeration of gold NPs [14].

A strong surface plasmon absorption band in the range of 500-550 nm is inherent to gold nanoparticles. The photoluminescence (PL) spectra of aqueous solutions of obtained gold NPs are represented in Fig. 3 (a).

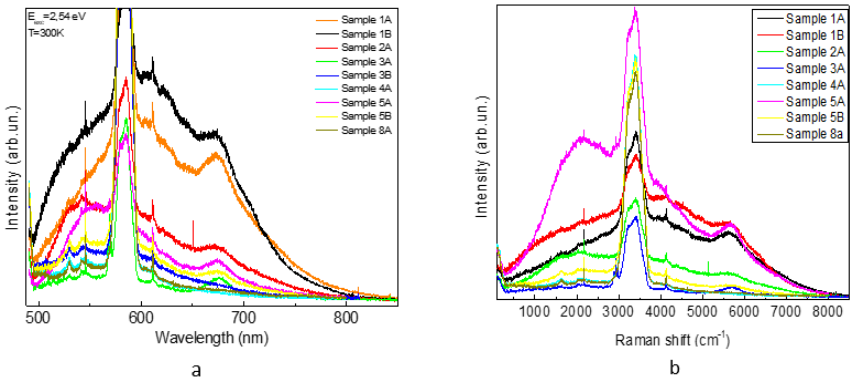

Figure 3. Photoluminescence spectra of gold nanoparticles (a), the same spectra in terms of Raman (b).

The weak broadband with a maximum at 590 nm was detected in the spectral frequency range from 500 nm to 800 nm. This band corresponds to the radiation of isolated gold NPs. The intensity of the luminescence decreases with a gradual dilution of aqueous solutions of gold NPs. We have observed that there is a complex band corresponding to Raman scattering of initial phytoextract in the range from 3000 to 3600 cm^{-1} (Fig. 3 (b). The line consists of two intense bands at ~3206 cm^{-1} and ~3412 cm^{-1} (Fig. 4). The growth of high-intensity bands concerning the low one (~3220 cm^{-1}) may indicate the increase of NaCl content in the solution. In studied samples, the disintegration of $NaAuCl_4$ is coupled with the formation of gold nanoparticles. Triplet with frequencies of about 2883 cm^{-1}, 2930 cm^{-1}, and 2973 cm^{-1} corresponds to Raman scattering from molecules of fatty acids contained in tea.

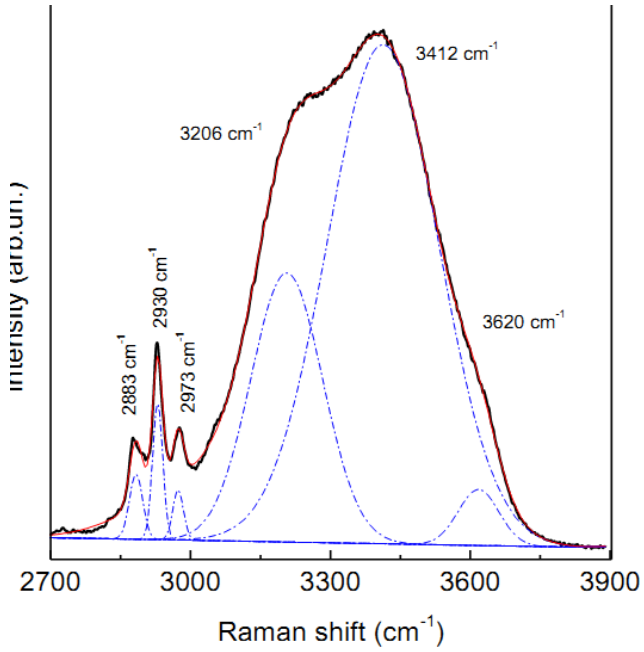

Figure 4. Raman spectrum of phytoextracts used for producing gold nanoparticles.

Fig. 5 shows the Raman spectra of aqueous extract of initial plant material (green tea leaves). In the spectrum, there are many narrow lines, which correspond to lipids and proteins. For example, the bands at 1035 cm^{-1}, 1169 cm^{-1}, and 1530 cm^{-1} correspond to carotenoids

present in tea. It is known that tea may contain up to 30% of a protein. The narrowband at 1446 cm-1 corresponds to CH_2 bending modes of the tea protein (Fig. 5).

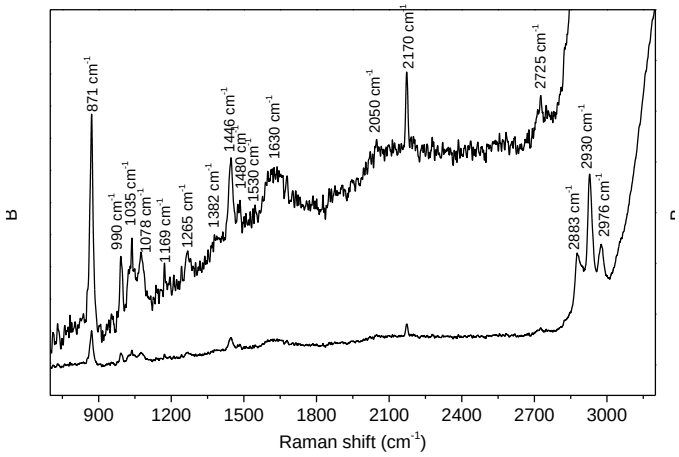

Figure 5. Raman spectrum of an aqueous extract of green tea leaves.

AFM study of obtained gold nanoparticles indicates that they are very homogeneous and have a size distribution from 3 nm to 10 nm with an average of 5 nm (Fig. 6).

Extracts of plants or their parts, such as roots, leaves, flowers, bark, and seeds, have been extensively screened for their ability to promote the biosynthesis of different kinds of nanomaterials [17, 18]. The synthesis of metal NPs by plants is clean, non-toxic, and biocompatible, suggesting the synthesis of nanoparticles with a wide range of size, shape, composition, physiochemical, and pharmacological properties. Certain organic agents of plant extracts have antioxidant properties that are responsible for Au metallic nanostructures formation.

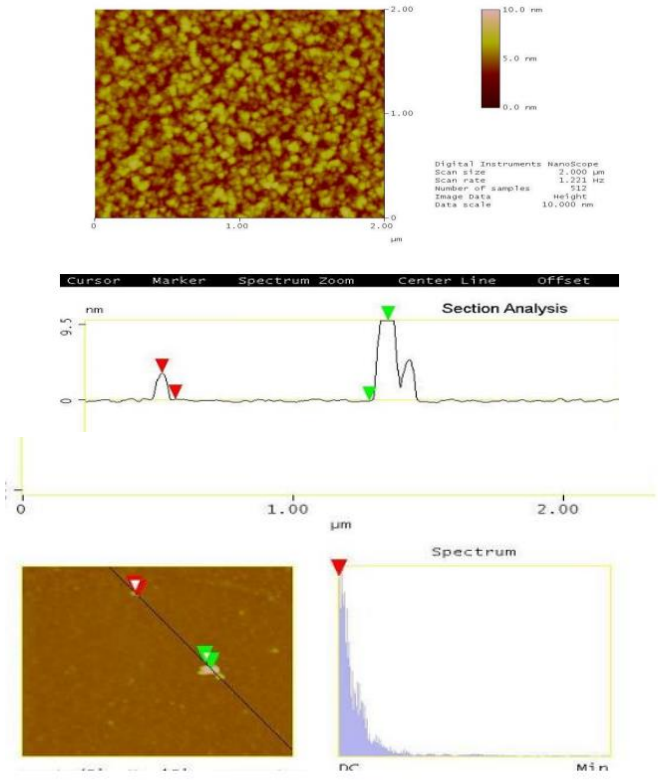

Figure 6. AFM microscopy of gold nanoparticles.

Phenolic compounds act as active reduction agents to reduce Au^{3+} ion to Au^{0} and stabilize the Au NPs through their surface interaction with carbonyl and hydroxyl groups of phenols. Given some plants' pharmaceutical properties, NPs can be synthesized by *Hibiscus sabdariffa,* for instance. Such NPs have uniform spherical morphology and diameters of 15-45 nm [19]. *Abies spectabilis* have been used to produce Au NPs and study their anticancer activity [20]. Absorption properties showed a surface plasmon resonance peak at 510–550 nm. Au NPs possess spherical morphology with a diameter of 20–200 nm. FT-IR analysis confirmed the presence of phytochemicals interaction in the reduction and stabilization of nanoparticles. Au NPs induced apoptosis in cancer cells [20]. Another research suggests «green» synthesis of Au NPs using *Mimosa tenuiflora* extract. Au NPs exhibit multiple shapes with sizes between 20 and 200 nm. The characteristic absorption spectra of Au NPs were found in the region comprised of 250–875 nm. SPR for Au NPs shows a symmetric band with maximum absorption in 550 nm and broad of 200 nm. The polyphenolic molecules of the extract act as a stabilizer of the Au NPs [21]. Also, *Marsdenia tenacissima* leaf extract was used to synthesize gold NPs. It was found that this medicinal plant has anti-cancer properties. Previous studies have revealed that *M. tenacissima* has capable antihepatoma property when used only or combined with chemotherapeutic drugs [22]. Au NPs exposed the size of the nanoparticles within 30 to 50 nm. The crystalline structure of the synthesized Au NPs was observed by using XRD measurements. FT-IR analysis was confirmed by the occurrence of phytochemicals interaction in the stabilization and reduction of NPs. Moreover, plant-mediated Au NPs induce apoptosis through inhibiting anti-apoptotic and enhancing proapoptotic pathway proteins [22].

Furthermore, "green" synthetic route of gold nanoparticles is described in [22] by using *Corchorus olitorius* as a reducing and stabilizing agent. A low concentration of the *C. olitorius* extract yielded mixed triangular and hexagonal shapes; in contrast, quasi-spherical shapes of Au NPs with an average size of 37–50 nm were obtained at a higher extract concentration. The Au NPs displayed SPR bands at 535 nm. An *in vitro* cytotoxic assay of the biocompatible Au NPs revealed a strong cytotoxic activity in three human cancer cell lines: colon carcinoma, hepatocellular carcinoma, and breast adenocarcinoma. The obtained biosynthesized nanoparticles exhibited stability for up to two weeks [23].

Juniper extract (*Juniperus communis* L.) as a reducing and stabilizer agent was used to synthesize gold nanoparticles [24]. Absorption measurements showed that the plasmon resonance wavelength appears around 530 and 700 nm. TEM analysis showed spherical Au NPs and gold triangular nanoprisms. The size of the triangular nanoprisms varies from 10 up to 50 nm, and the thickness is up to 0.8 nm. The authors established that different ratios of the aqueous ethanol extract of *Juniperus communis* L. and $HAuCl_4$ were applied to obtain the prismatic shaped Au NPs. However, only the spherical Au NPs can be synthesized within a low concentration of reactants. At higher concentrations, the triangle-shaped AuNPs with a size up to 50 nm can be prepared [24].

A specific mechanism of synthesis of gold NPs using different biological agents is still unknown. But we can assume that different chemical compounds that are already mentioned as a part of biomatrices may reduce agents reacting with metal ions leading to their reduction and thereby the synthesis of metal nanoparticles [20].

Pay attention that using *Camellia* extracts is preferred among other phytoextracts and has some advantages in the case of nanoparticle production. Namely, tea catechin in green tea or theaflavins in black tea is known as stronger antioxidant compounds. Moreover, tea extracts are more readily available. Tea-derived Au NPs in the present research are characterized by

narrow size distribution, high stability without aggregation, and high luminescence intensity, which are key parameters for their biomedical applications [25]. Today, there are many studies for green synthesis of Au NPs by *C. sinensis* leaf aqueous extract. Namely, gold nanoparticles produced by *Camellia* extract are reported to have a dietary therapeutic potential compared to Daunorubicin in an animal model of acute myeloid leukemia [17]. *C. sinensis* from *Camellia* genus is one of the important medicinal plants. In recent years, researchers have tried to survey the therapeutic activities of gold nanoparticles using plants. In this regard, it has been revealed that gold nanoparticles had excellent antibacterial effects against Gram-negative and Gram-positive bacteria. TEM investigated the size and morphology of gold nanoparticles. According to the TEM data, it is evident that gold nanoparticles have a nearly spherical and triangular morphology with a distribution of particles with a size between 20-30 nm [17]. The Au NPs showed a characteristic peak centered at 536 nm, indicating the surface plasmon absorption of nanosized Au particles and nanoparticles' formation. About the antioxidant activity of *C. sinensis*, it is believed to be a source of beneficial antioxidants, like that found in fruits. It is particularly rich in polyphenols, including thearubigins, theaflavins, and catechins. Polyphenols present in *C. sinensis*, being powerful antioxidants, may play an important role in preventing cancer by decreasing DNA damage in the cell and activation of cancer, leading to malignancy [17].

Another study has found that gold NPs produced by an aqueous extract of *C. sinensis* L had a diameter around 23.33±6.69 nm. In the high magnification of TEM it can be observed clearly that the C. sinensis L. extract surrounds Au-NPs. This result approved that the size of the synthesized Au-NPs depended on the stirring reaction time, reaction temperature, volume of *C. sinensis* L. extract, and volume of $AuCl_4$. The proposed model can be used as an alternative tool for predicting the size of the synthesized Au-NPs [26].

It is important that comparing similar studies of biosynthesis of nano Au by tea extracts is agreed well with our data. For example, in the paper [26], gold NPs were also synthesized using phytochemicals presented in tea leaves. These nanoparticles had UV-Vis maximum at the range of 530-563 nm, had spherical morphology, which corresponds to our results. Another study also suggests a gold nanoparticles production using a single step by reducing $HAuCl_4$ with polyphenols from black tea extracts [27]. The authors advise on the following properties of nanoparticles: absorption bands strongly depend on tea concentration as using 1% tea solution UV-Vis maximum is at 538 nm confirms the smaller size of these NPs (8–24 nm diameter), relative to the absorption band at 566 nm observed for the Au NPs 5% tea solution or that at 602 nm for the larger-sized Au NPs 10% tea (57–113 nm) [27]. Such dependence on the concentration of the initial extract was observed in our study as well.

Our results are also comparable to [25]. Three different shapes of chitosan-capped gold nanoparticles (nanospheres, nanostars, and nanorods) were synthesized and investigated using green tea extract. All three types of gold nanoparticles showed their characteristic surface plasmon resonance bands upon UV-visible spectrophotometry. The optimization of size and shape, together with surface modification and functionalization, will lead to the development of nanoparticles for future use in nanomedicine [25].

It is important to emphasize that in contrast to the existing studies, gold nanoparticles obtained in our research have significantly smaller dimensions (average diameter of 5 nm), and they are more stable, maintaining their physical properties for up to 3 months without degradation. So, small size, stability, intense luminescence, and surface uniformity together with the environmentally friendly method of their synthesis make the resulting Au

nanoparticles promising probes in medical and biotechnological applications, such as DNA and drug carriers, tools for biosensors, cancer treatment, etc.

Summing up, the biological synthesis of gold NPs is a novel, cost-effective, and biocompatible method. A shorter time is required for biosynthesizes of gold NPs in comparison to chemical synthesis. We have established that the extract of green tea leaves allows obtaining stable luminescent NPs with a diameter that does not exceed 10 nm, offering biological or medical applications, such as tissue engineering, drug and gene delivery, or fluorescent labeling.

4. Conclusions

This study found that phytoextracts derived from the mixture of *Camellia sinensis* var. *sinensis* and *Camellia. sinensis* var. *assamica* (green tea) can be effective biomatrices for the synthesis of gold NPs. Synthesized gold NPs were investigated by UV-Vis and Raman spectrometry as well as by AFM microscopy. It was revealed that an absorption peak at a wavelength of 530-550 nm corresponds to gold nanoparticles' absorption. The synthesized gold NPs had an average size of 5 nm. Obtained gold NPs were stable in time due to their stabilizing by organic compounds presented in phytoextracts. Thus obtained Au NPs can be a new generation of diagnostic probes, promising tools for pharmaceutical or cell biology applications.

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Conflicts of Interest

The authors declare no conflict of interest.

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