

Green Synthesis of Ag₂O/Ag NPs via Ginkgo Biloba and Their Anticancer Effects

Ola abedallah manaty

University of Wasit

Nadia Naeema

University of Wasit

Ahmed Thamer

Ahmedkh224@uowasit.edu.iq

University of Wasit

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Abstract

Green nanomaterials have received much attention lately due to their potential in medicines. In the present research, we use a simple, cheap, and non-toxic method for synthesizing silver oxide/silver nanoparticles using plant extracts of the Ginkgo biloba. This plant has a variety of significant phytochemicals that may be utilized to treat various illnesses, including cancer. Cancer is the most important cause of mortality worldwide, and breast cancer is the second most common reason for death. Several therapeutic techniques have been used to treat cancer, including radiation, surgery, chemotherapy, immunotherapy, and hormone therapy. However, these approaches are expensive and have side effects, limiting their utilization. Ag₂O/Ag nanoparticles are synthesized using the green method by Ginkgo biloba plant extract, which acts as an oxidizing, reducing, and capping agent. Study the physicochemical properties of the Ag₂O/Ag nanoparticles using FTIR, XRD, TEM, and EDX spectroscopies. The result shows the formation of Ag₂O/Ag nanoparticles' spherical shape and in the nanoscale range. The Ag₂O/Ag nanoparticles showed an excellent anticancer effect against the human breast cancer type MCF-7 cell line; it was observed that the number of cancer cells decreased as the dilutions of the Ag₂O/Ag nanoparticles increased.

1. Introduction

Cancer is the most significant cause of mortality worldwide, and the breast cancer type MCF-7 cell line is the second most common reason for death [1]. Several therapeutic techniques have been used to treat cancer, including radiation, surgery, chemotherapy, immunotherapy, and hormone therapy [2]. However, these approaches are expensive and have side effects, limiting their utilization. As a result, there is a need for practical, non-toxic, low-cost treatments with minimal side effects. Nanotechnology is one of the most important new research areas in current material science, and nanoparticles are employed in every science phase, influencing all aspects of human life. Nanoparticles, which have a size range of (1–100nm), are the most straightforward structure [3, 4]. These nanoparticles have therapeutic promise for various ailments due to their physicochemical features and qualities [5]. Silver oxide nanoparticles (Ag₂ONPs) are a novel nanometal particle type widely applied in biology, medicine, and engineering [6]. Synthetic physiologically active Ag₂ONPs, in particular, have shown a wide range of therapeutic potential, including anticancer, anti-fungal, anti-bacterial, anti-inflammatory, anti-oxidative, anti-oxidative, anti-diabetic action, and wound healing [6, 7]. Various approaches are available for synthesizing metal oxide nanoparticles, such as; Biological, physical, and chemical. In the biological technique, nanoparticles are produced by the biosynthesis of plants, algae, yeast, bacteria, and fungi [8]. The physical and chemical processes have various advantages, such as a narrow size range of nanoparticles. Still, the main disadvantages are low efficiency, costs and time, and toxic byproducts [9].

On the other hand, biological approaches have several advantages over conventional techniques: low cost, eco-friendly, no downstream processing, and excellent yields.

Furthermore, natural processes are often known as green synthesis. As a result, using plant extracts to produce nanoparticles enables a straightforward one-step reduction approach with large-scale manufacturing [9]. The plant extract stabilized, capped, and reduced the agent [8]. Numerous studies have been published on the creation of nanoparticles from plant extracts with biologically significant properties; Fayyad et al. (2021) synthesized AgNPs using henna (Li) aqueous plant extract for antimicrobial activity [10], and Sarala et al. (2020) synthesized zinc ferrite NPs using aqueous extract of henna (Li) plant for anticancer properties against the human breast cancer type MCF-7 cell line [11]. Al-Sheddi et al. (2018) synthesized AgNPs using plant extract of *Nepeta deflersiana* for anticancer activity against human (HeLA) cancer cells [12]. Tamarindus indica aqueous fruit shell extract was used to synthesize Ag NPs by Gomathi et al. (2020) for the human breast cancer type (MCF-7) cell line [8]. This paper aims to synthesize Ag₂O/Ag NPs using the green approach via Ginkgo biloba plant extract as a capping and reducing agent. Also, study the structural and morphological analyses of Ag₂O/Ag NPs and their cytotoxic efficacy in breast cancer type (MCF-7) cell lines. Many of previous study in 2020 revealed that Ginkgo Biloba-AgNPs suppress cancer cell proliferation and induce apoptosis by upregulating intracellular ROS generation and mitochondrial pathway in cervical cancer cells[6] also in 2020 The green synthesis of silver nanoparticles using various plant extracts, including Ginkgo Biloba, has been found to be superior to chemical methods[13].

2. Material and Methods

2.1 Plant Extract Preparation Method

In Iraq/Wasit, the Ginkgo Biloba plants were purchased from neighborhood nurseries. It was washed with tap water, gently scraped to remove dirt and other dust, and then rinsed with distilled water. The Ginkgo Biloba leaves were minced into tiny pieces and distributed evenly for proper drying. Ginkgo Biloba leaves were air dried under shade for ten days in a dry, shady environment to prevent microbial fermentation and the subsequent loss of plant components. The dried leaf sections are mechanically ground into tiny particles to make the plant tissues into powder. 2g of Ginkgo Biloba powder was dissolved in 100 ml of distilled water with a pH of 3.9 using a hot plate stirrer, then heated and stirred for an hour at 45°C. With filter paper, a side-arm flask, and a Buchner funnel, the Ginkgo Biloba solution was vacuum filtered. The resultant aqueous solution should be kept at room temperature for future use

2.2 Ag₂O/Ag NPs green synthesis

The green-synthesis approach was used to manufacture silver oxide nanoparticles using silver nitrate (AgNO₃) from (Glenthams life sciences LTD, UK) and Ginkgo Biloba plant extract. One molar of aqueous silver nitrate (AgNO₃) solution was produced by dissolving (AgNO₃) in 100 ml of distilled water. The dissolving process was placed at 75°C for 1 hour and vigorously mixed at 700 rpm. Then, 100 ml of silver nitrate aqueous solution was gently mixed with 100 ml of henna plant extract before being heated for an hour at 70°C. The green synthesized Ginkgo Biloba forms a dark brown, water-based colloidal mixture. To study and analyze the crystal structure, elemental composition, and surface morphology of Ginkgo

Biloba, XRD, EDX, and TEM techniques were used. The final Ginkgo Biloba colloidal solution was sprayed on a glass substrate using a micropipette and a hot plate stirrer to produce a film layer. A temperature lower than 50°C was used for the drop-casting process

2.3 Anticancer Activity assay

The anticancer activity of colloidal Ag₂O/Ag NPs was investigated using the MTT assay. The antibiotic-antimitotic solution was utilized in DMEM with 10% fetal bovine serum to cultivate the MCF-7 breast cancer cells. The MCF-7 cells were then grown in 96-well plates at a density of 1x10⁴ cells/well and treated for 24 hours with various Ag₂O/Ag NP dilutions; (0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.015625 per well) and grew in 5 percent CO₂ at 37°C. Following the incubation period, the MTT solution was applied to each well. For 4 hours, the plate was put in incubation under dark circumstances. The formazan crystals generated during the reaction were decomposed in 100µl of DMSO. A microplate reader was used to assess the spectrophotometric absorbance of the purple-blue formazan dye at 570 nm. Eq. (1) calculates the relative cell viability of breast cancer cells.

$$RelativeCellviability = \frac{AverageODvaluefortest}{AverageODvalueforcontrol} \times 100\% \dots\dots (1)$$

The anticancer activity of the Ag₂O/Ag NPs of the MCF-7 live cancer cells was determined using Hoechst blue-fluorescent staining.

2.4 Ag₂O/Ag NPs Characterization

Using the PHILIPS X-Ray Diffractometer (XRD) (type PW 1730), the physicochemical characterization of the green synthesis Ag₂O/Ag NPs was investigated. This method, which measures intensity as a function of Bragg's angle, was used to investigate the crystalline structure of the Ag₂O/Ag NPs layer film. The target is copper (Cu) with a wavelength of 1.54060 Å, current and voltage of 30 mA, and a voltage of 40 kV, respectively. Five degrees per minute of angle change is scanned from 10 to 80 degrees at an angle of two degrees. The elemental composition in the Ag₂O/Ag NPs layer film was analyzed using Energy Dispersive Spectroscopy (EDX) model TESCAN Mira3. The surface topography and particle size measurements were done using the Transmission electron microscopy (TEM) model PHILIPS CM30 /V = 200KV. While the Fourier transforms infrared spectroscopy (FTIR) indicated the (4000–400 cm⁻¹) wavenumber range of the plant extract and the green generated Ag₂O/Ag NPs colloidal solution using FTIR device model IRAffinity-1 from SHIMADZU

3. Results and Discussions

3.1 The Crystal Structure of Ag₂O/Ag NPs Analysis

The x-ray diffraction at grazing incidence angle (GIXRD) technique was utilized to investigate the crystal structure of an Ag₂O/Ag NPs layer film. This technique studied the sample's surface layer rather than the substrate material (i.e., glass). A surface-sensitive diffraction method limits penetration into bulk

materials while maximizing intensity using a small incident angle X-ray beam. The polycrystalline Ag2O/Ag NPs layer film's X-ray diffraction patterns are represented in Fig. 1

The most muscular three X-ray diffraction patterns 2-theta at 32.889°, 38.103°, and 55.011° can be well-matched with miller indices (111), (020), and (202) crystal planes of silver oxide in cubic phase can be well-matched with Crystallography Open Database (COD) card number 96-101-0487 (cubic) Ag2O. On the other side, 2-theta at 44.54°, 64.59°, and 65.69° can be well-matched with miller indices (200), (202), and (131) crystal planes of silver in the cubic phase can be well-matched with (COD) card number 96-500-0219 (cubic) Ag. Table 1. gives the crystal structure characterization of the green synthesized Ag2O/Ag NPs layer film. The crystallite size was calculated via Eq. (2) (Debye-Scherer), and the crystallite size of the average most substantial three peaks was ~ 20nm.

$$D = \frac{K\lambda}{\beta\cos\theta} \dots\dots\dots (2)$$

D refers to the crystallite size in nanometers, λ refers to the wavelength of the X-ray Cu-K radiation, which is 1.54060 °A, β refers to the full width at half maximum in radians, θ refers to the Bragg's diffraction angle in radians. K refers to the shape factor of 0.94 [10]. The spacing (d) between the crystalline layers was calculated using Eq. (3).

$$n\lambda = 2d_{hkl}\sin\theta \dots\dots\dots (3)$$

Here, n represents the order of the diffraction peak [10].

Table 1
crystal structure characterization of Ag2O/Ag NPs layer film.

<i>2 θ (Deg.)</i>	<i>FWHM (Deg.)</i>	<i>hkl</i>	<i>d_{hkl}.(Å)</i>	<i>Crystallite Size (nm)</i>	<i>COD No.</i>
32.889	0.554	111	2.7176	15.62	96-101-0487 (cubic) Ag ₂ O 96-500-0219 (cubic) Ag
38.103	0.515	020	2.3547	17.05	
55.011	0.341	202	1.6665	27.43	

3.2 Elemental Composition Analysis

The Ag2O/Ag NPs layer film's components are identified using an x-ray method known as energy dispersive x-ray analysis (EDX). Ag2O/Ag NPs contain various elements, each with a distinct atomic structure, causing it to emit a spectrum with a particular collection of peaks. The energy-dispersive X-ray spectrum of the greenly synthesized Ag2O/Ag NPs is shown in Fig. 2, along with a quantitative result corresponding to oxygen (O) and silver (Ag) elements and the element's normalized concentration

expressed as an atomic weight (A%) and a proportion of weight (W%). Owing to surface plasmon resonance (SPR), strong and medium AgL α and AgL β absorption peaks of silver (Ag) nanocrystals were seen at (2.970 and 3.170 KeV). Also, a weak absorption peak of (Ok α) of the oxygen element was observed at (0.50 KeV). This result agrees with the references [14].

3.3 Surface Topography and Particle Size Analysis

The surface topography and particle size of Ag₂O/Ag NP are investigated using the transmission electron microscopy (TEM) method. The distribution of particle sizes of Ag₂O/Ag NPs is shown in Fig. 3-d; the nanoparticles range from 12.64 to 115.9 nm, averaging particle size of (20.71 nm). In contrast, Fig. 3 (a-c) TEM images show the surface topography of Ag₂O/Ag NPs at three different magnifications. According to TEM images, the shape of these nanoparticles is semi-spherical, and the morphology will be termed spherical if aggregations, as seen in some locations, are ignored. TEM images further reveal that a capping layer of Ginkgo Biloba plant extract encased the Ag NPs, indicating that the plant extract works as a capping layer, molding the nanoparticles during their growth.

3.4 Fourier Transform Infrared Spectroscopy (FTIR)

The 400–4000 cm⁻¹ wavenumber range of the FTIR spectrum was utilized to identify the main functional groups and chemical bonds of Ginkgo Biloba plant extract and green synthesized Ag₂O/Ag NPs colloidal solution. FTIR analysis confirms that the phytochemical substance of Ginkgo Biloba plant extract is capping off the surface of Ag₂O/Ag NPs.

Figure 4 shows the Ginkgo Biloba plant extract and Ag₂O/Ag NPs colloidal solution FTIR spectrum. Ginkgo Biloba shows a medium and broad peak at 3503.2 cm⁻¹ and 3331.3 cm⁻¹ due to stretching vibration of the hydroxyl group H-bonded (O-H stretch) and amine stretching vibration (N-H stretch), respectively. A broad and robust peak at 2897.4 cm⁻¹ is due to sp² alkenes (C-H stretch). Two minor peaks at 1599.39 cm⁻¹ and 1525.02 cm⁻¹ are due to carbonyl and aromatic groups of (C = O and C = C stretch), respectively.

Figure 4 Ginkgo Biloba plant extract and Ag₂O/Ag NPs colloidal solution FTIR spectrum.

A sharp and medium peak at 1028.38 cm⁻¹ is attributed to carbohydrates (C-O bending). In the fingerprint region, minor peaks appeared between (783.4-725.3) cm⁻¹, representing carbohydrates, aromatic, and alkyne of C-O and C-H bending. On the other hand, Ag₂O/Ag NPs showed shifting in the FTIR spectra, with medium and broad peaks appearing at 3521.4 cm⁻¹, 3339.7 cm⁻¹, and 3017.65 cm⁻¹ due to the hydroxyl group (O-H stretch) H-bonded and stretching vibration of amine (N-H stretch), and sp² alkenes (C-H stretch), respectively. Minor peaks at 1642.25 cm⁻¹ of C = O stretching were attributed to the carbonyl group. Strong and little peaks appearing at 1361.5 cm⁻¹ and 1060.2 cm⁻¹ are due to

carbohydrates and aldehyde groups of C-O and C-H binding, respectively. In the fingerprint region, Ag₂O/Ag NPs peaks obtained at 555.9–515.1 cm⁻¹ represent the Ag-O mode of vibration.

3.5 Anticancer Activity

The anticancer activity of the green synthetic form Ag₂O/Ag NPs was evaluated using the MTT assay against human breast cancer (MCF7) cells within the dilutions range (from 0.5 to 0.015625). The association between the relative cell viability of the cancer (MCF7) cell line and different dilutions of the green synthesized Ag₂O/Ag NPs is shown in Fig. 5-a.

According to the chart findings, colloidal Ag₂O/Ag NPs demonstrate considerable toxicity at greater dilutions (doses), steadily dropping from 0.5 to 0.015625. On the other hand, the viability of breast cancer (MCF7) cells increased steadily from 37.08 percent to 96.88 percent, which means that the amount of surviving breast cancer cells is inversely proportional to the Ag₂O/Ag NPs dilutions. In addition, prior studies have revealed that many nanoparticles have dose-dependent cytotoxicity [1–3].

Figure 6 (b-h) displays fluorescence microscopy pictures demonstrating the vitality of cancer (MCF7) cells using Hoechst blue-fluorescent staining. Images (c-f) give the cancer cell viability treated with Ag₂O/Ag NPs at different dilutions: 0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.015625, respectively, in comparison to image (b) of control cancer cell viability (no treatment). The rise in the dilutions of the Ag₂O/Ag NPs was associated with a steady decrease in cancer cell viability. As a result, the initial finding in Fig. 5-a is affirmed. The reference [1] agrees with this result. The anticancer action of Ag₂O/Ag NPs is related to producing reactive oxygen species and silver ions. Ag₂O/Ag NPs directly communicate with cell membranes, causing cell membrane collapse, rupture, and death. Reactive oxygen species can affect mitochondrial respiration, disequilibrium, and cellular redox damage in mitochondria.[15]

Conclusion

The green method successfully prepared Ag₂O/Ag NPs using Ginkgo biloba plant extract. The polycrystalline cubic Ag₂O and Ag phases were produced with a 20 nm-sized crystallite from the XRD analysis. EDX analysis shows strong and medium absorption peaks of AgL α and AgL β of silver (Ag) nanocrystals were identified at 2.97 and 3.17 KeV, respectively. Also, it shows a weak absorption peak of OK α at 0.5 KeV, indicating the presence of oxygen (O) element. TEM technique showed the surface topography of Ag₂O/Ag nanoparticles is semi-spherical with an average particle size of 20nm, and the capping layer of Ginkgo Biloba plant extract encased the Ag NPs, indicating that the plant extract works as a capping layer, molding the nanoparticles during their growth. The principal functional group peaks of the Ginkgo Biloba plant extract were shifted in the spectrum of the FTIR compared to Ag₂O/Ag NPs. In the fingerprint region, Ag₂O/Ag NPs peaks obtained at 555.9–515.1 cm⁻¹ represent the Ag-O mode of vibration. The results demonstrated excellent anticancer efficacy using the green synthesized Ag₂O/Ag NPs against human breast cancer-type MCF-7 cells.

Declarations

ETHICAL STATEMENT:

- Funding: (N/A)
- Conflict of Interest: (N/A)
- Ethical approval: (N/A)
- Informed consent: (N/A)
- Author contribution: Ola abedallah manaty (main idea for reserch), Nadia Naeema (data analysis), and Ahmed A. Thamer (interpretation data and analysis)
- Data Availability Statement : N/A

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Figures

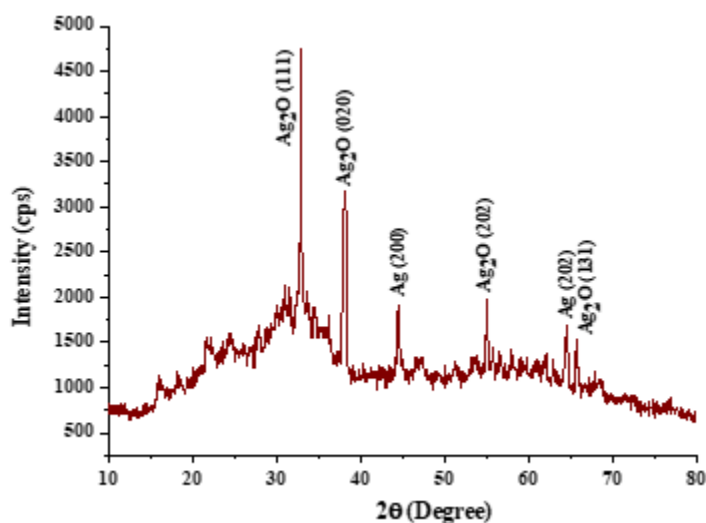


Figure 1

X-ray diffraction (XRD) patterns of Ag₂O/Ag NPs layer film

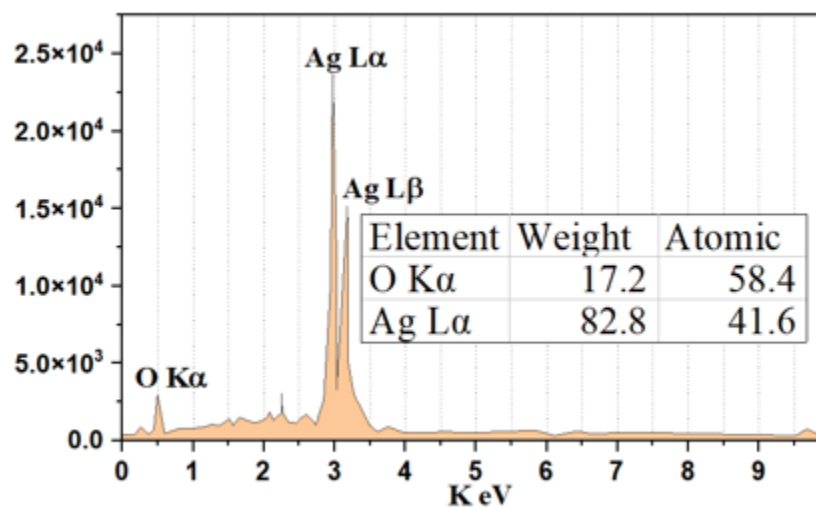


Figure 2

EDX spectrum and quantitative results of Ag₂O/Ag NPs.

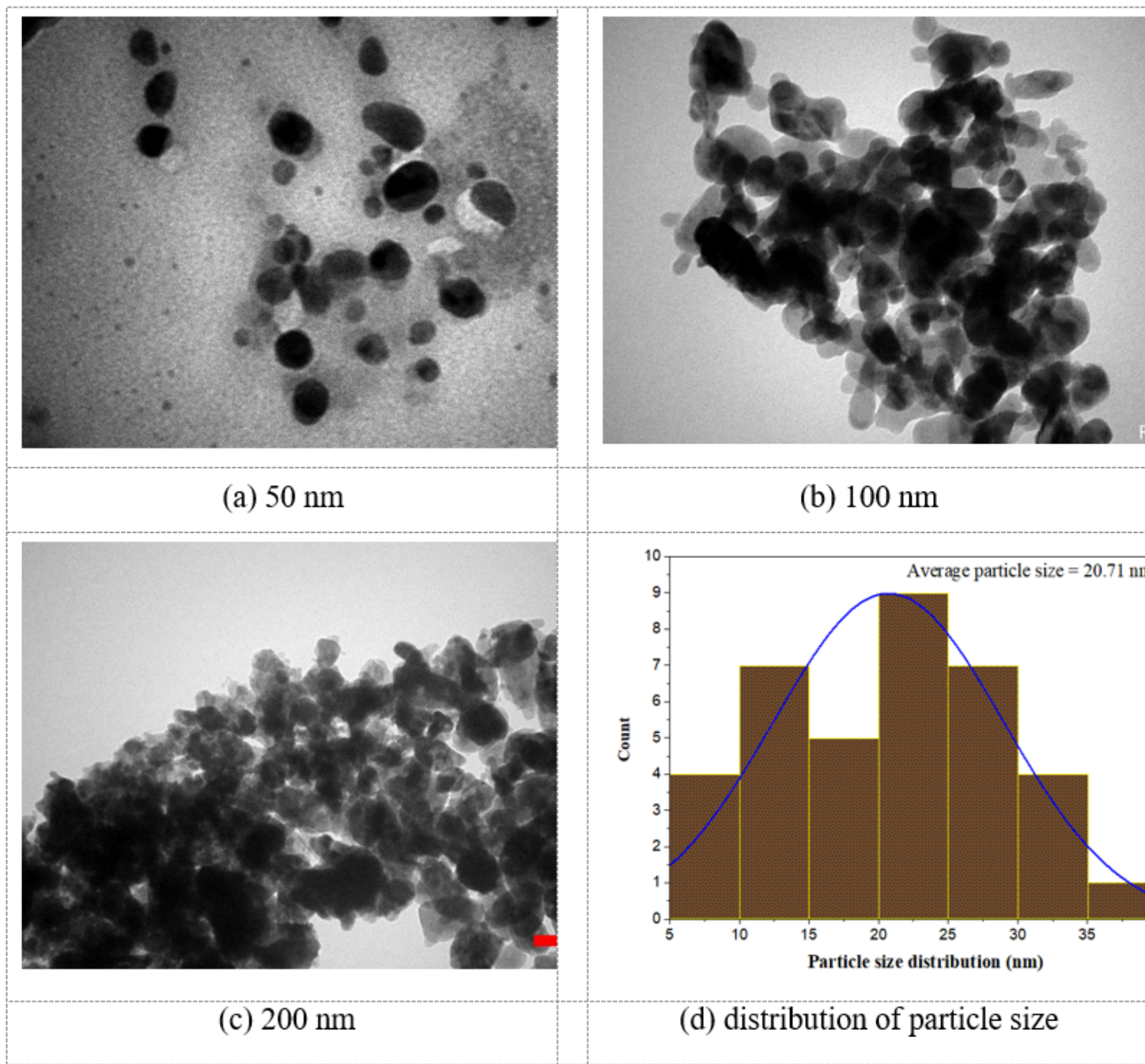


Figure 3

Surface topography with three various magnifications: (a) 50 nm, (b) 100 nm, (c) 200 nm, (d) particle size distribution of Ag₂O/Ag NPs.

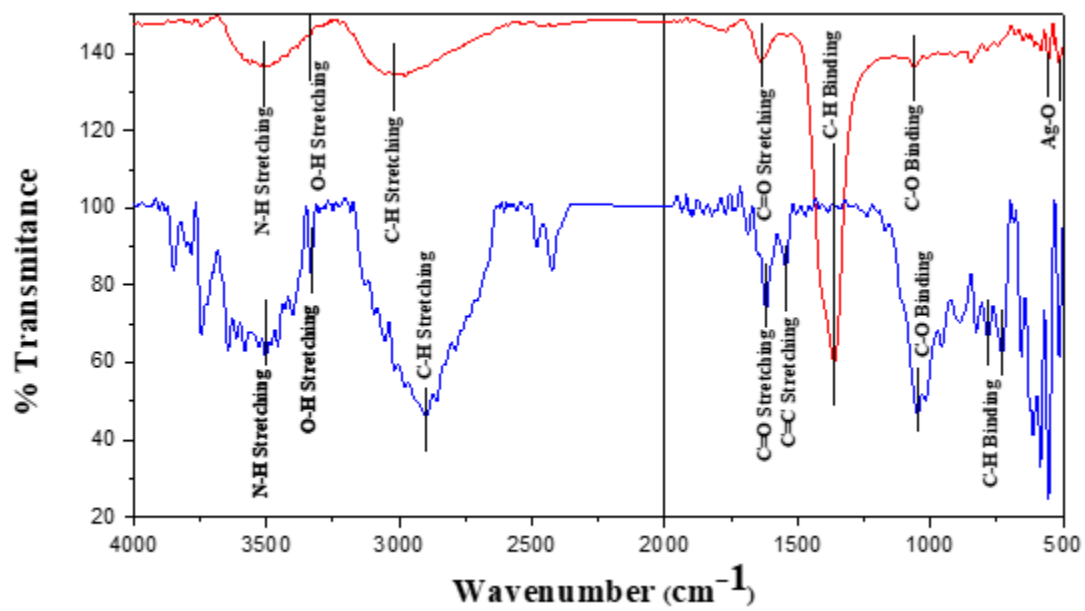


Figure 4

Ginkgo Biloba plant extract and Ag₂O/Ag NPs colloidal solution FTIR spectrum.

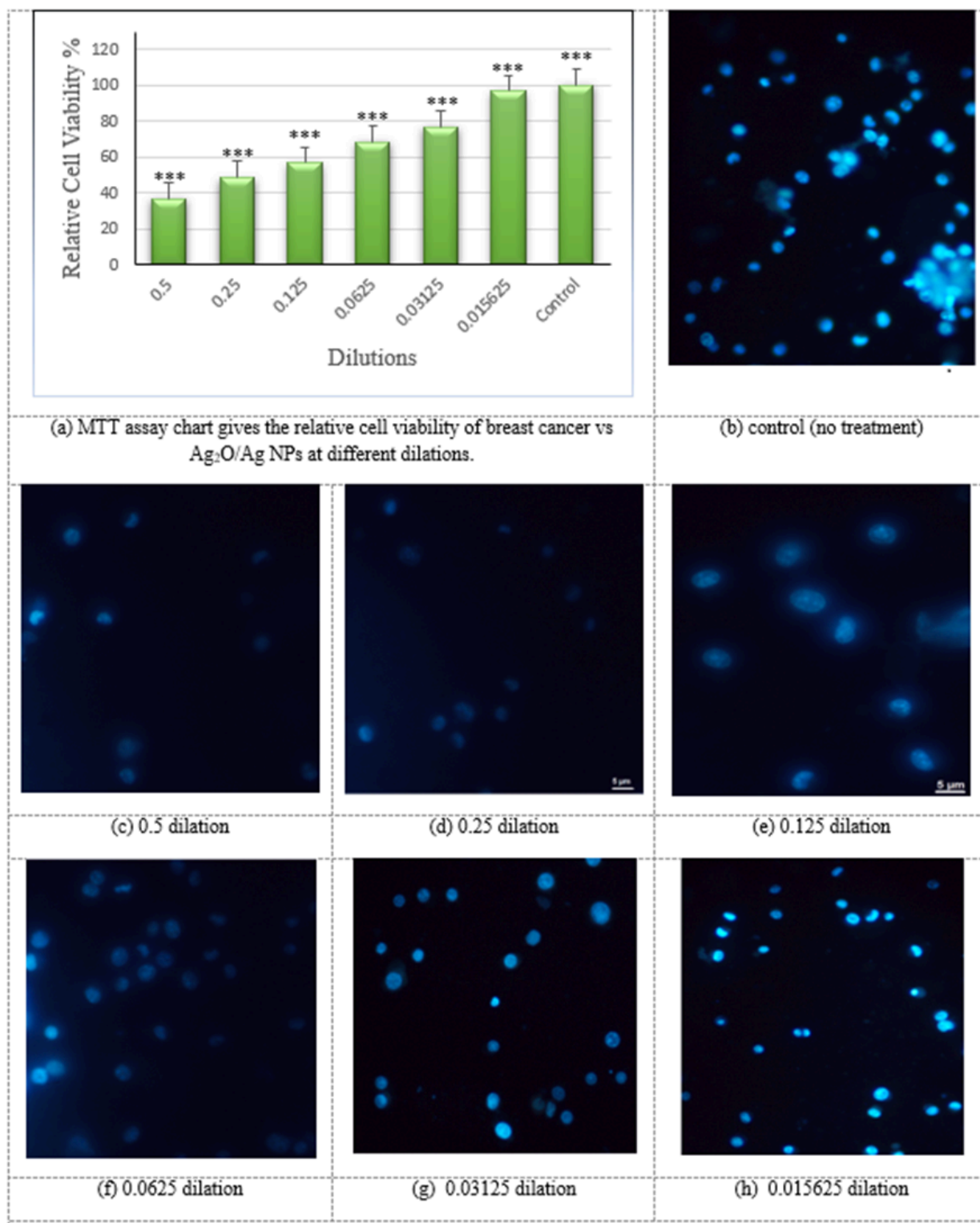


Figure 5

(a) MTT assay chart gives the relative cell viability of breast cancer vs $\text{Ag}_2\text{O}/\text{Ag}$ NPs at different dilutions, (b-h) Blue-fluorescent staining of cancer (MCF-7) cell viability.