Exp. Theo. NANOTECHNOLOGY 10 (2026) 1325-1335 https://doi.org/10.56053/10.3.1325

Eco-friendly synthesis and characterization of silver nanoparticles using turmeric extract and study anticancer activity

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Received 8/4/2026, *Received in revised form* 13/5/2026, *Accepted* 18/6/2026, *Published* 15/7/2026

Plant secondary metabolites have been identified as biologically active molecules for green synthesis and the unique biological applications of biosynthesized nanoparticles are also attracting interest in them. Focusing on the characterization of these nanoparticles and the assessment of their anticancer potential, this work presents a straightforward, environmentally friendly, dependable, and cost-effective method of synthesizing silver nanoparticles utilizing turmeric extract as natural reducing and capping agents. Various methods are used to analyze the biosynthesized silver nanoparticles, including ultraviolet-visible spectroscopy, scanning electron microscopy, energy-dispersive analysis, X-ray diffraction, and Fourier transform infrared. Characterization studies and a color shift verified the effective production of silver nanoparticles. The presence of a peak at 446 nm in the UV-Visible spectra suggests the development of nanoparticles. EDAX verified the existence of silver, whereas SEM showed uniformly shaped nanoparticles. XRD testing confirmed that the nanoparticles, which had a size of less than 100 nm, are in fact crystalline. Through research with Fourier transform infrared (FTIR) technology, it has been established that the extracts can contain bio-reducing compounds. The plant is able to reduce the viability of cancer cells in a dose-dependent manner particularly with the MCF-7 cell line (IC50 173.1) compared to the human normal cell line (WRL-68) (IC50 519.8). Scientists reported that the extracts of the turmeric are helpful in the eco-friendly synthesis of silver nanoparticles and the nanoparticles are found to have the potential to kill cancer cells. The results of this research bring hope in the quest for sustainable cancer treatment via plant-mediated synthesis. Silver nanoparticles reduced cell viability in a dose dependent manner for the MCF-7 human normal cell line (519.8) compared to the human normal cell line WRL-68 (173.1). The result indicates that it is possible to produce silver nanoparticles with turmeric extract in an efficient and environmentally friendly manner. These nanoparticles have anticancer properties. These results demonstrate the potential of plant-mediated synthesis in developing environmentally friendly anti-cancer drugs.

Keywords: Green synthesis; Silver nanoparticle; Characterization; Cytotoxic.

1. INTRODUCTION

NANOTECHNOLOGY emerged as a new science that brings about desired properties with novel material at the nanoscale. The optical, chemical, mechanical and therapeutic properties of nanoparticles are unique [1]. Environmentally friendly and non-toxic nanoparticle creation using biological processes, such as green synthesis, is now receiving a lot of attention. Biogenic AgNPs are synthesized from living organisms like plants, bacteria or fungus [2]. Biogenic AgNPs have several advantages over chemically produced AgNPs, including low cost, ease of production, and biocompatibility. Among the benefits of the use of turmeric extract is its known medicinal properties, and the diversity of biomolecules, which it has, favours the reduction of silver ions and the stabilization of nanoparticles [3]. One of the medicinal benefits of turmeric—a member of the Zingiberaceae family whose roots are in the Curcuma longa plant—is the presence of curcumin, the spice's primary colorant. Turmeric extract contains curcumin, a bioactive compound that has multiple biological activities such as anti-inflammatory, anti-oxidative and anti-cancer properties [4]. It has been shown that curcumin can reduce the toxicity of silver nanoparticles (Ag-NPs). This is because the efficacy, stability, bioavailability, and plasma half-life of turmeric are enhanced by its incorporation with metal nanoparticles [5]. The extracts prepared from these plants can be used for the synthesis of silver nanoparticles as they contain different types of bioactive compounds which have an inherent reducing property such as proteins, terpenoids, polyphenols and flavonoids [6]. Numerous phytochemicals including phenolic acids, tannins, alkaloids, saponins and flavonoids can be used to convert silver ions to silver nanoparticles through the reduction of Ag+ to Ag0 [7]. The biogenic reduction process is crucial to control the size, shape and stability of the silver nanoparticles produced, which are directly responsible for the antibacterial and anticancer effects [8]. This bottom-up green synthesis methodology involves using plant extracts to reduce silver ions (Ag+) to form the stable nanoparticles (AgNPs) is an eco-friendly and safe method to obtain such particles. This approach has several advantages over traditional chemical methods, such as being more cost-effective and scalable, which are crucial in various medicinal contexts [9]. This biogenic way of synthesis of nanoparticles leads to the formation of nanoparticles, which are capped with natural capping agents, thus minimizing agglomeration and increasing stability without the need for any other stabilizers. In addition, plant-based nanomaterials usually exhibit improved stability compared with other biological materials, making them particularly suitable for a variety of applications [10]. This plant derived nanoparticles are widely used in many nanomedicine applications particularly in the food and pharmaceutical industry because they are biocompatible and have broad therapeutic potential [3]. Although Numerous research has documented the green synthesis of silver nanoparticles utilizing turmeric extract; nevertheless, many predominantly concentrated on nanoparticle synthesis and physicochemical characterization. The present study integrates a comprehensive analysis of turmeric-mediated silver nanoparticles with a comparative assessment of cytotoxicity against MCF-7 breast cancer cells and WRL-68 normal cell lines. This approach provides additional insight into the potential biomedical applicability and selective cytotoxic effects of biosynthesized AgNPs.

2. EXPERIMENTAL

2.1 Plant extract preparation

One gram of turmeric powder is dissolved in 100 mL distilled water to form a solution. The mixture is blended for uniform dispersion, and after that heated for 15–20 minutes at the heating mantle at 60 °C. This heating method enhanced the extraction of powder active ingredients and water solubility. We removed insoluble particles by filtering the mixture through Whatman No. 1 paper after heating. The filtrate is collected in a sterile container. Depending on their application, extracts are dried or left in solution. The isolated chemicals are refrigerated at 4 °C until use [3].

2.2 Green synthesis of silver nanoparticles

Green synthesis produced silver nanoparticles. We added 1 mL of turmeric extract to 100 mL of a 1 mM aqueous AgNO3 solution and heated it to 60–80°C for one hour with a magnetic stirrer to decrease Ag+ ions. The dark brown to reddish-brown hue change suggested silver nanoparticle production. In the dark, this reaction prevented static silver nitrate photoactivation. After collecting the nanoparticles, the supernatant is discarded after 25 minutes of centrifugation at 15,000 rpm. Storing isolated nanoparticles in opaque glass containers [11].

2.3 Characterization of Silver Nanoparticles

Characterize silver nanoparticles using UV–vis spectrophotometry by scanning from 300 to 600 nm, with distilled water for baseline correction. Examine their shape and dimensions using SEM; validate the structure through EDX and XRD. Employ FTIR to identify functional groups.

2.4 Cytotoxic Activity

2.4.1 Cell line

The University of Malaya Faculty of Medicine, Centre for Natural Product Research and Drug Discovery, Malaysia, contributed the human breast cancer MCF-7 and normal human WRL-68 cell lines. Cells are cultured in RPMI-1640 media at 37°C for 24 hours in a humidified 5% CO_2 environment.

2.4.2 MTT Assay

The cytotoxic effects of various concentrations (6.2, 12.5, 25, 50, 100, 200, and 400 μg/mL) of green synthetic nanoparticles loaded with turmeric are evaluated in vitro on MCF-7 cells using an MTT assay (Bio-Rad, USA) in 96-well microtiter plates. Following the cell line maintenance guidelines outlined by Freshney in 2010, the cells are allowed to form a confluent monolayer in the culture vessel and are subsequently ished with PBS. We added three milliliters of Trypsin/versine solution and incubated the cells at 37 °C for 1–2 minutes. Add 15 milliliters of RPMI media and incubate the cells at 37 °C in 5% CO2. Counting cells using a hemocytometer measured cell concentration. Inoculated 200 µL of MCF-7 cells (1 × 104–1 × 106 cells/mL) and incubated at 37 °C for 24 hours. Add plant concentrations (6.2-400 µg/mL) to each well and incubate for 24 hours. Incubate 10 µL of MTT solution at 37 °C with 5% CO2 for 4 hours after the first incubation. A five-minute incubation following the addition of 100 milliliters of the kit is solubilization solution to the medium. A microplate reader for ELISA measured formazan production at 570 nm.

3. RESULTS AND DISCUSSION

3.1 Biosynthesis and detection of silver nanoparticles

The first step in synthesizing nanoparticles is to combine turmeric extract with a 1 mM AgNO3 solution. Following the effective creation of silver nanoparticles, the color changes from yellow to reddish-brown, as seen in Figure 1. Surface plasmon resonance, an optical characteristic exclusive to noble metals, is responsible for this color shift. The use of UV-visible spectroscopy, a commonly used method for analyzing nanoparticle structure, further verified the existence of nanoparticles in the water-based solution. In this study, a peak at 446 nm, shown in the matching absorbance curve (Fig. 2), is indicative of the creation of Ag NPs as a result of surface plasmon resonance (SPR) of electrons on the surface of the nanoparticle demonstrating the effective creation of nanoparticles using the green synthesis method with turmeric extract serving as capping and reducing agents [3,11]. This peak is typical of silver nanoparticles. Synthesized silver nanoparticles' size, shape, morphology, and content greatly affect the SPR bands [12].

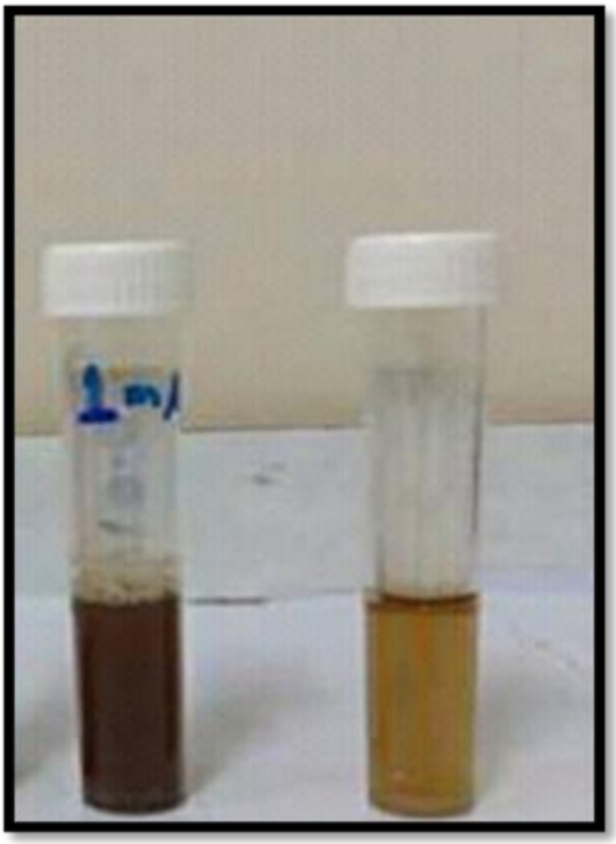

Figure 1 Silver nanoparticle (AgNP) solution (a) before and (b) after Ag NPs synthesis.

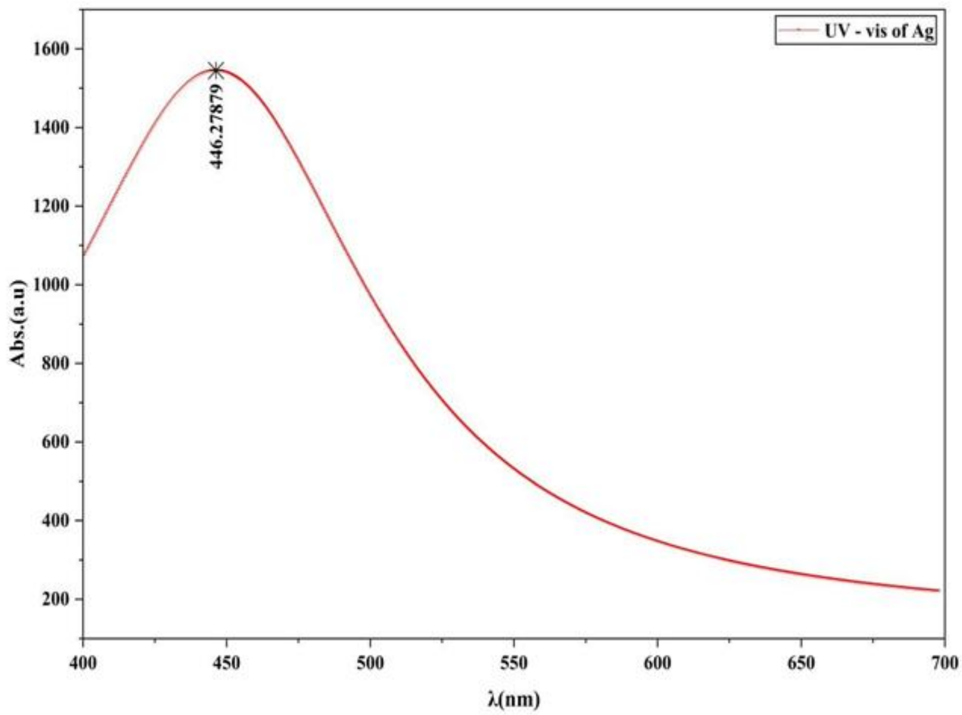

Figure2 UV- vis spectrophotometry of Ag NPs synthesis.

3.2 Characterization of green synthetic silver nanoparticles

SEM, EDX, XRD, and FTIR verify the production of silver nanoparticles. Scientists used the scanning electron microscope to measure the size and shape of produced silver nanoparticles (Ag NPs). Figure 3 shows that scanning electron microscopy (SEM) showed that the nanoparticles are very tiny and spherical, with particle size ranges from 37 to 39 nm. Few individual nanoparticles are found, but most are aggregated. SEM images show the nanoparticles' size, shape, and homogeneity, supporting their spherical shape. The spherical form of these Ag NPs influences their properties and usage. The high surface area-to-volume ratio of spherical nanoparticles has several applications in electronics, medicine, and catalysis [13]. Many scientific fields are interested in nanoparticles because their optical and antibacterial capabilities rely on size and form [14]. Similar findings demonstrated the synthesis of silver nanoparticles with turmeric extract, exhibiting uniform shape and spherical morphology [13].

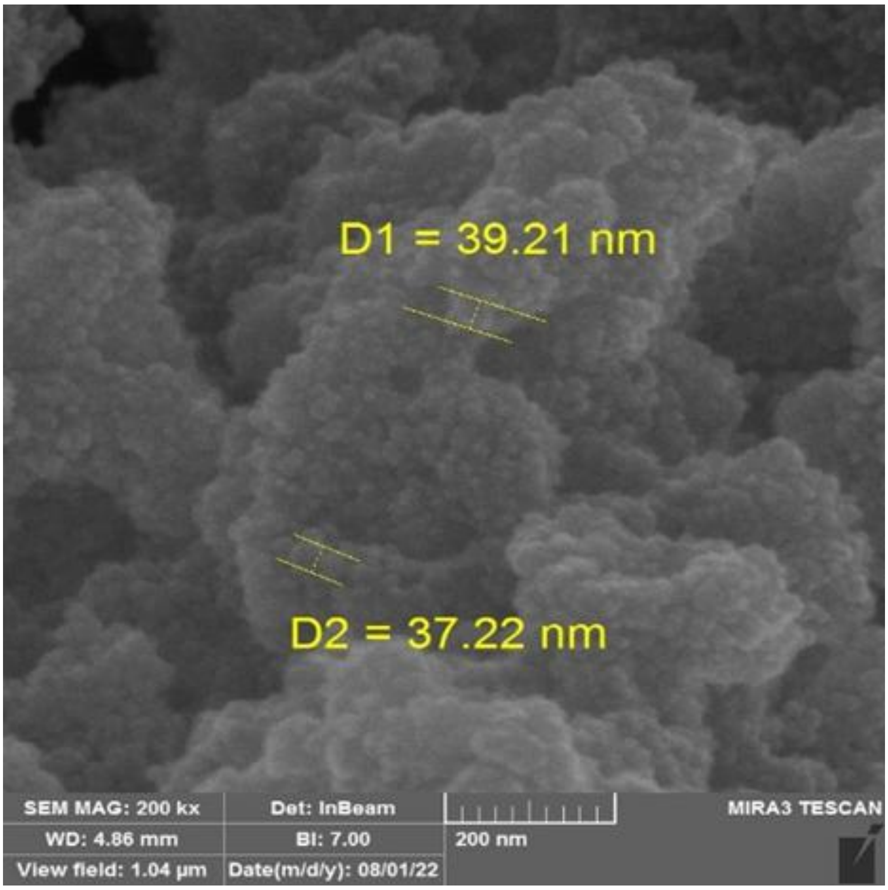

Figure 3 SEM image of Ag NPs synthesis.

The elemental composition of the synthesized nanoparticles is confirmed by the results of the Energy Dispersive X-ray Spectroscopy (EDX) investigation, the results of which are shown in Figure 4. The identification of a clear signal at 3 keV suggests that there is a metallic silver nanoparticle present and confirms it has an absorption feature. At this energy level, the surface plasmon resonance is most pronounced with metallic silver nanoparticles [15]. You may get quantitative information on the biosynthesized silver nanoparticles (AgNPs) in Figure 8. The results are consistent with the targeted production of silver nanoparticles with the turmeric extracts and show the main elemental components of the nanoparticles produced, with silver (Ag) being a major element. Besides this, the presence of carbon (C) and oxygen (O) also indicates that plant extracts might contain organic compounds which will stabilize and reduce silver ions in green synthesis. Negligible levels of silicon (Si) and chlorine (Cl) may be due to trace contamination or environmental sampling contamination during sample preparation and analysis [16]. Overall, the EDAX analysis confirms the effective synthesis of silver nanoparticles with a majority silver content, along with organic components, providing insight into the elemental composition of the turmeric-mediated silver nanoparticles.

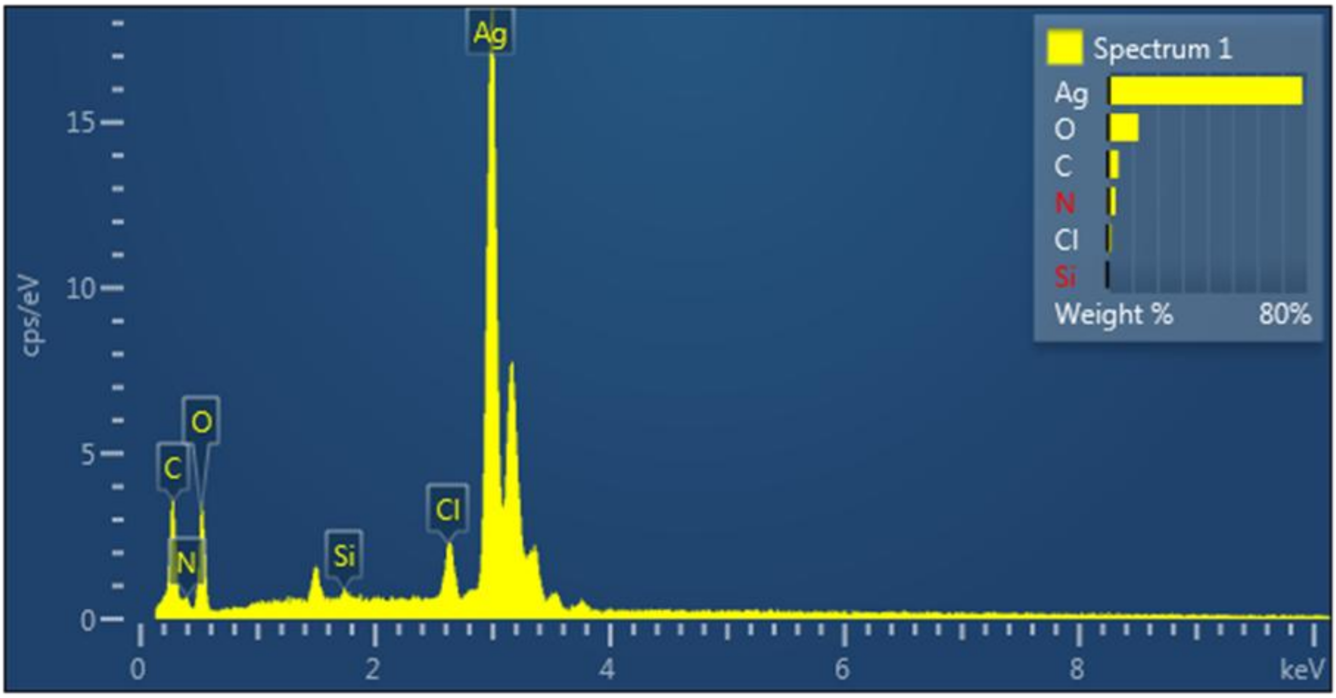

Figure 4 EDX image of Turmeric mediated silver nanoparticles Ag NPs.

We studied the crystalline structure and lattice characteristics "of the produced silver nanoparticles (AgNPs) using the X-ray diffraction (XRD) technique. Silver nanoparticles' XRD pattern verifies their crystalline structure, as seen in Figure 5. The (2θ) values of 38.09°, 44.30°, 64.49°, and 77.71° show four separate diffraction peaks, which correspond to the (111), (200), (220), and (311) reflection planes of the face-centered cubic structure of silver, respectively". The AgNP structure is determined to be face-centered cubic based on a comparison with standard data (ICSD file No. 01-074-2076) and the observed diffraction peaks. By using the Debye-Scherrer formula, we are able to determine the typical crystalline size of the silver nanoparticles.

D ¼Kλ=β cos θ (1)

In this context, β stands for the angular width at half maximum of the diffraction peak, λ is the Bragg angle in degrees, D is the estimated crystal size from XRD patterns, and K is the constant, usually taken to be 0.9. With the corresponding measured angles and the Debye-Scherrer equation, we get 69.67, 36.56, 31.10, and 35.06 nm. X-ray diffraction (XRD) spectroscopy is an essential technique for accurately determining the dimensions and crystalline structure of nanoparticles [17]. Recent studies have thoroughly examined the green production of silver nanoparticles (AgNPs) and their subsequent XRD spectroscopy characterization. AgNPs have been produced in an environmentally friendly manner using a variety of plant extracts. According Singh et al. [18], the use of turmeric extract as a reducing agent for the environmentally friendly production of AgNPs. In this research, the findings unequivocally confirmed the crystalline nature and face-centered-cubic (FCC) crystal structure of the produced AgNPs. The biosynthesized silver nanoparticles exhibit a crystalline size of less than 100 nm. Based on the required size range and qualities, the size distribution of green-synthesized silver nanoparticles may provide light on their structural traits and their uses.

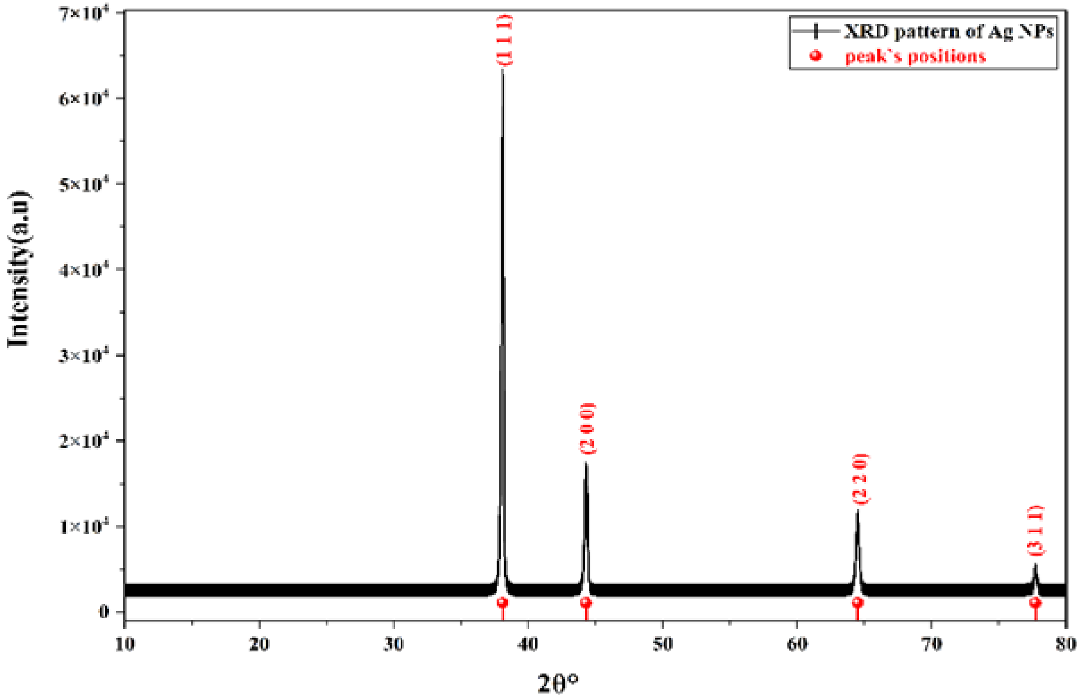

Figure 5 Green-synthesized silver nanoparticle XRD spectrum.

The main "functional groups present in the turmeric extract and their probable role in the production and stability of AgNPs are identified through Fourier transform infrared (FTIR) investigations [3]. The FTIR spectra of the produced AgNPs (Fig. 6) displays significant absorption peaks at 3057.88, 2439.76, 2127.25, 1718.25 and 1467.77 cm^{-1} indicating the presence of capping agents, which includes phytoconstituents. The peak of 3075 cm^{-1} is due to the O-H stretching vibration of phenolic chemicals in turmeric extract that plays a vital role in the reduction of Ag^+ ions to silver nanoparticles". According Salman et al. [11], highlighted the important role that plant-derived polyphenols play in stabilizing and reducing nanoparticles. The 2439 cm^{-1} band implies stretching vibrations of the O=C=O bond, or the presence of organic functional groups from absorbed CO_2, which suggests interactions between the organic components and the surfaces of the nanoparticles. Similar findings have been documented in biosynthesized nanoparticles made using extracts from medicinal plants. The peak at 2127 cm-1 is assigned to the C≡C/N≡N stretching modes, indicating bioactive organic molecules that caps and stabilizes the silver nanoparticles. Similar shifts in FTIR spectra following AgNP synthesis are noted by Suo et al. [19], who proposed that these changes are caused by organic molecules coordinating with the nanoparticle surface. The signal located at 1718 cm^{-1} corresponds to the carbonyl C=O stretching vibration, which is characteristic of the curcumin and other phenolic groups of turmeric, suggesting the interaction between the silver nanoparticles and turmeric phytochemicals. Lastly, the signal at 1467 cm^{-1} is representative of aromatic C=C stretching and C-H bending vibration of phenolic compound which suggests the presence of aromatic rings in the phytochemicals of turmeric. According to these results, phytochemicals of turmeric extract cap and reduce the size of silver nanoparticles. The functional groups involved in AgNPs formation are indicated by the comparison of the peaks shift of the generated AgNPs.

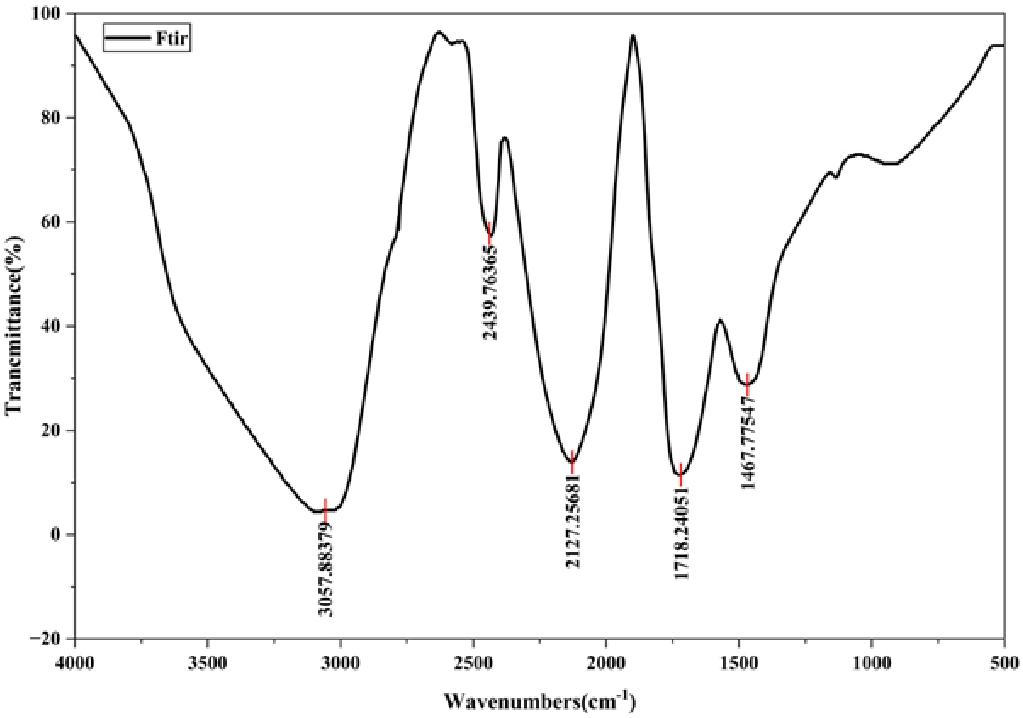

Figure 6 FTIR spectra of the synthesized AgNPs.

3.3 Cytotoxicity of biosynthesized AgNPs

The MTT assay evaluated the cytotoxic effects of green synthetic turmeric nanoparticles on MCF-7 breast cancer cells. This investigation assessed tumour cell viability and inhibition rates at varying nanoparticle dosages. At a concentration of 400 µg/ml, MCF-7 cell viability diminished to 40.86±2.766, whereas at 6.2 µg/ml, it reached its peak at 94.79±2.034. In comparison to WPL-68, treated cells exhibited a dose-dependent reduction in cell viability (Table 1). Figure 7 indicates that the lethal concentration of produced silver nanoparticles (AgNPs) for cancer cells is 519.8 µg/ml, while the IC50 for the normal cell line WRI-68 is 173.1 µg/ml. The synergistic interaction between curcumin's bioactive chemicals and silver nanoparticles may explain their increased anticancer activity [20]. Silver nanoparticles may produce reactive oxygen species (ROS), mitochondrial malfunction, DNA damage, and cancer cell death, whereas curcumin includes antioxidant and anticancer phenolic and flavonoid chemicals [21]. Nanoparticles boost treatment effectiveness by increasing surface area in touch with tumor cells and improving cellular absorption [22]. Similar to current result, biosynthesized AgNPs using Solanum surattens leaf extract induced 50% MCF-7 cell death at 75 µg/ml [23-24]. The current findings suggest that turmeric-loaded silver nanoparticles constitute a promising nanobiotechnological system with potential uses in cancer treatment. However, further research is necessary to clarify the properties of cytotoxicity and the death or proliferation of cells generated by turmeric silver nanoparticles extract.

Table 1 After 24 hours at 37ºC, green synthetic nanoparticles showed cytotoxicity on MCF-7 and WRI-68 cells.

"Green synthetic NPs Concentrations (µg/mL)	Viable cell counts of MCF-7 cell line Mean± S.D.	Viable cell counts of WRL-68 cell line Mean± S.D".
400	40.86±2.766	75.62±3.740
200	58.64±2.894	86.69±2.539
100	88.97±1.037	93.63±1.748
50	92.28±3.679	93.90±1.011
25	95.25±1.029	95.76±0.837
12.5	93.29±1.819	96.73±1.772
6.2	94.79±2.034	96.41±0.926

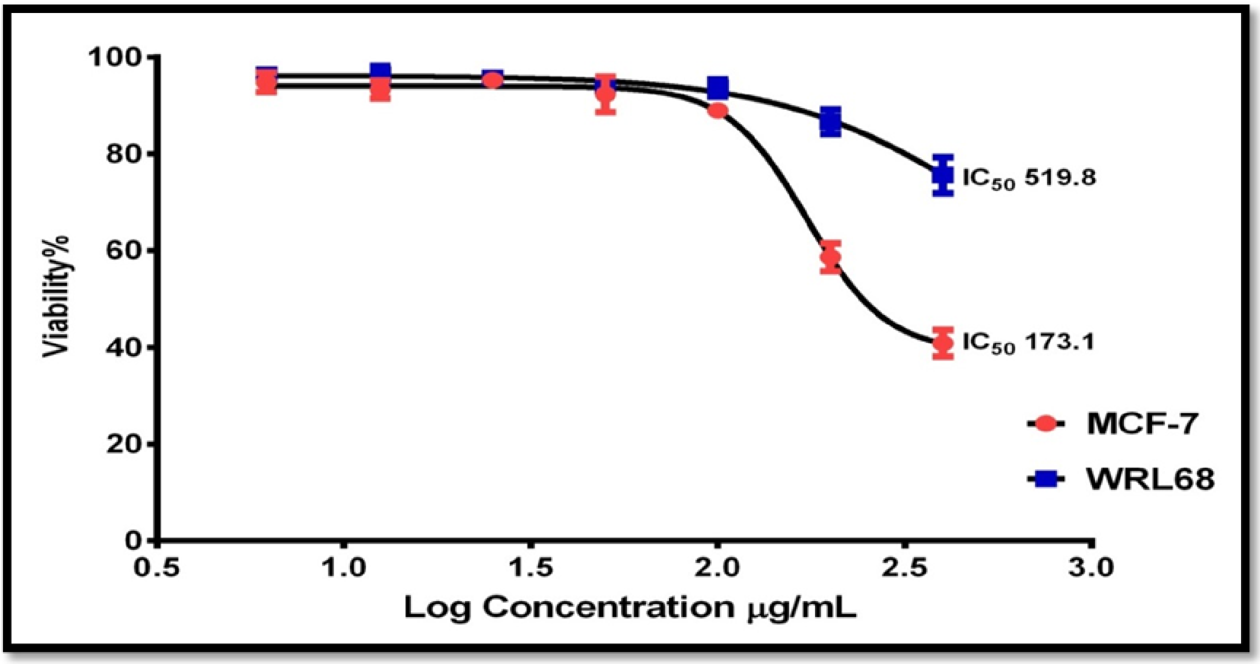

Figure 7 Cytotoxic effect for cell viability of AgNPs loaded turmeric extract.

4. CONCLUSIONS

Turmeric extracts show promise for environmentally friendly silver nanoparticle (AgNP) manufacturing, according to the studies. The impressive anticancer activity of these plant-mediated AgNPs proves that they might be a viable alternative to traditional antibiotics. The green synthesis technique offers an easy, inexpensive, and ecologically responsible way to synthesize AgNPs, in line with the principles of sustainable and environmentally friendly chemistry. In addition, the used turmeric extracts, which are renowned for their therapeutic qualities, improve the generated AgNPs, increasing their efficacy against cancer. The generated AgNPs have fantastic stability and anticancer capabilities, making them useful in many medicinal contexts. In addition to adding significantly to what is already known about green nanoparticle manufacturing, the results of this study have important consequences for the creation of new cancer treatments.

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