Nanomed Res J 8(3):259-267, Summer 2023

RESEARCH ARTICLE

Synthesis of Silver Oxide Nanoparticles using Different Precursor and Study Cytotoxicity Against MCF-7 Breast Cancer Cell line

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ARTICLE INFO

Article History:
Received 22 Jan 2023
Accepted 03 Apr 2023
Published 01 May 2023

Keywords:
Green synthesis
Silver oxide nanoparticles
Orange leaves extract
Anti-cancer activity

ABSTRACT

In this work, the eco-friendly synthesis of silveroxidenanoparticles (Ag_2O-NPs) was successfully performed using orange leaf extract with silver nitrate and silver sulfate as precursors. Several techniques were used to characterize the surfaces of the silver oxide nanoparticles (Ag_2O-NPs), including FT-IR spectra, which showed the functional groups present in the synthesis of silver oxide nanoparticles, FE-SEM images showed that the green synthesis of Ag_2O-NO_3-NPs and Ag_2O-SO_4-NPs has a spherical shape. EDX analysis spectrum confirmed the presence of elemental silver signals in the Ag_2O-NPs, proving that the Ag_2O-NPs were prepared within the nanoscale range and that there is a clear and tangible effect of negative ions bonded (NO_3^- and SO_4^{-2}) with Ag metal on the size, shape, and distribution of Ag_2O-NPs. Also, the cytoxicity of the established Ag NPs at 0-200 µg/ml was assessed in breast cancer MCF-7 cell lines in vitro using MTT assay, and verified these strong cytotoxicity. As a result, the use of orange leaf extract is considered an eco-friendly and cost-effective method.

How to cite this article
Names N. Synthesis of Silver Oxide Nanoparticles using Different Precursor and Study Cytotoxicity Against MCF-7 Breast Cancer Cell line. Nanomed Res J, 2023; 8(3): 259-267. DOI: 10.22034/nmrj.2023.03.004

INTRODUCTION

Nanomaterials behave differently than macroscale materials and have highly desirable properties due to size confinement, the predominance of interfacial phenomena as a result of increased surface area, and quantum effects [1]. These unique properties make nanomaterials highly sought after in various industries, including electronics, medicine, energy, Food, pharmacy, cosmetics, paints, plastics, paper, and textiles [3]. In electronics, nanomaterials are used to create smaller and more efficient devices such as transistors and memory chips. In medicine, nanomaterials are being developed for targeted drug delivery and imaging. Additionally, nanomaterials have the potential to revolutionize the energy industry by improving the efficiency of solar cells and creating new materials for energy storage [2]. There are many nano-oxides of importance such as iron oxide, zinc oxide, magnesium oxide, aluminum oxide, silver oxide particles, especially silver oxide nanoparticles (Ag_2O-NPs) these wonderful materials have drawn a lot of attention because of their distinct chemical and physical properties. They have enormous applications in the fields of oxidation catalysis, sensors, photovoltaic, and data storage devices and have great potential in environmental remediation as they can be used in many biomedical applications desired in cancer treatment and wound healing.[3-6]. There are several different forms of silver oxides, including AgO, Ag_2O, Ag_2O_3, and Ag_3O_4. Ag_2O semiconductors come in a variety of shapes, with a reported bandgap values between 1.2 eV to 3.4 eV[7]. Ag_2O-NPs can be prepared either by combining aqueous $AgNO_3$ solutions with alkaline hydroxide [8]. Or by using other synthesis methods based on various chemical reagents such as photo-adhesion, wet deposition, electrochemical synthesis, chemical reduction [9], electro, chemistry [10, 11], electron

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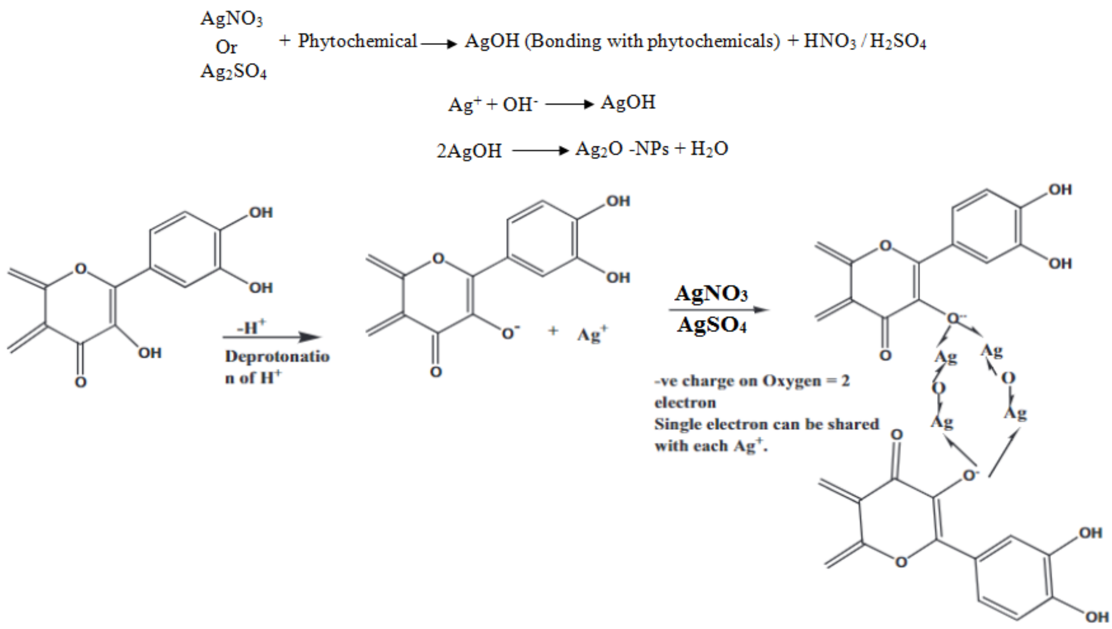

Scheme 1. The reaction mechanism during the synthesis of Ag_2O-NPs.[17].

irradiation [12], gamma-irradiation [13], photo chemical methods [14], and Langmuir–Blodgett [15]. However, these techniques typically make use of expensive or harmful substances (e.g. reducing agents and stabilizers) [5, 6]. In addition, the remaining by products may make Ag and Ag_2O-NPs unsuitable for biomedical application. Therefore, recently, many researchers have resorted to green biosynthesis methods based on microorganisms, and plant extracts [9-12]. Plant extracts are frequently used in the environmentally friendly, non-toxic, and biologically safe green synthesis of metals and metal oxides NPs [13-15]. The green synthesis method has gained popularity in recent years due to its ability to produce NPs with specific sizes and shapes, which can be tailored for various applications in fields such as medicine, catalysis, and electronics [27]. Furthermore, the use of plant extracts as a source of reducing agents for NPs synthesis is cost-effective and sustainable compared to traditional chemical methods that rely on toxic chemicals and high-energy inputs [28]. The process involves simple steps such as mixing the plant extract with a metal salt solution followed by heating or exposure to light. The resulting NPs are stable and exhibit unique properties that make them suitable for various applications. Overall, the green synthesis of NPs using plant extracts is a promising approach that offers numerous advantages over conventional methods and has the potential to revolutionize the field of nanotechnology [16-18]. The reaction between Ag^+ from $AgNO_3$ and the hydroxyl groups (-OH) of the phytochemicals initially form AgOH, which is then changed into Ag_2O[32] As shown in scheme 1. Therefore, many researchers have conducted scientific studies using various plant extracts, such as Camellia Sinensis (green tea) [19-21], pine[22], persimmon[23], ginkgo[24], magnolia[25], and Platanus[26], are being used for Ag and Ag_2O-NPs synthesis. The goal of this work is to synthesize Ag_2O-NPs from different precursors ($AgNO_3$ and Ag_2SO_4) using orange leaf extract, then study the effect of carbonate (NO_3^-) and sulfate (SO_4^{-2}) ions on the size, shape, and distribution of the silver oxide nanoparticles that were synthesized.

MATERIALS AND METHODS

Materials

Silver nitrate ($AgNO_3$) was purchased from BDH, Sigma Aldrich, Sodium hydroxide (NaOH) (THOMAS BAKER, 99%), silver sulphate (Ag_2SO_4) (BDH, Sigma Aldrich, 98%), Ethanol (scharlau, 99%), Deoxygenated distilled water and fresh leaves of orange was collected from orange trees in Baquba / Diyala government. In this study, all glass tool was washed using distilled water and dried in the oven till used in the experiments, and all solutions were prepared using distilled water.

Preparation of plant leaf extract

Orange leaves were washed in tap water to remove dust, washed in distilled water, dried for 12 days at room temperature in a shaded area, and then crushed to create a fine powder. About 30 g of the powder from these leaves were added to a glass flask with 100 mL of distilled water to create the extract. To change the color of the solution, the mixture was boiled for two hours at 80°C. The extract was then chilled at room temperature, filtered with Whatman No. 42 filter paper, and kept in a glass container at 4°C for later use [27].

Biosynthesis of silver oxide nanoparticles

Silver nitrate ($AgNO_3$) and silver sulphate (Ag_2SO_4) were used to the synthesis of silver oxide nanoparticles (Ag_2O-NPs). Add10 ml of plant leaf extract solution was gradually added to the 20ml of (1 mM) precursor solution while stirring at (40°C) in A 250-ml capacity flask was used in this study, and the solution temperature was raised to (90°C). The production of Ag_2O-NPs through biological synthesis was evident through a noticeable color change and the formation of a brown-black solid following a 5-minute duration, achieved by adjusting the pH to approximately 12 using a 1M sodium hydroxide solution. Subsequently, the solid was subjected to washing with methanol and multiple rinses with deionized water. It was then dried in an oven at 80 °C for 2 hours and subjected to calcination at 550 °C for 6 hours to yield silver oxide nanoparticles. These nanoparticles were subsequently stored in a glass container for subsequent analysis[28].

CHARACTERIZATION OF SILVER OXIDE NANOPARTICLES (Ag_2O-NPs)

The vibrational modes of the functional groups present in the nanoparticles were analyzed using a Perkin Elmer FTIR spectrophotometer (Jasco 6100, Japan) with the KBr pellet technique. The size, shape, and purity of the Ag_2O-NPs were characterized using Field Electron Scanning Electron Microscopy (FESEM) with an EV018 instrument (CARL ZEISS) and Energy Dispersive X-ray Spectrometer (EDX) with an X Flash R Quantax 200 instrument. The structural properties of the Ag_2O-NPs were investigated through X-ray diffractometry (XRD) analysis using a Philips PW1730 instrument.

MTT assay

According to the MTT assay's instructions, the effects of Ag2O-NO3-NPs and Ag2O-SO4-NPs on the viability of MCF-7 cells were evaluated. Briefly, cells were seeded in 96-well plates at a density of 1× 10^5 cells per well for 24 hours before being exposed to concentrations of Ag2O-NO3-NPs and Ag2O-SO4-NPs ranging from 0-200 g/ml for 24, 48, and 72 hours. After that, 10 L of the 5 mg/ml MTT medium was added to the wells. After 4 hours of incubation at 37°C, the optical density (OD) of the wells was calculated using an ELISA reader at 570 nm wavelengths. The following formula was used to calculate the optical density (OD) value and the percentage of cell viability:[29].

$$\boldsymbol{Precentage\ of\ cell\ viability = \frac{OD\ cell\ with\ AgNPs}{OD\ cell\ with\ out\ AgNPs} \times 100}$$

Statistical analyses

The results obtained in this study are shown in the form of average± SD, Student's t-test was conducted to determine the statistical differences between groups. Finally, a P value < 0.05 was considered statistically significant.

RESULT AND DISCUSSION

FTIR ANALYSIS

FT-IR measurements provide valuable insights into the chemical composition and structure of the nanoparticles. The FT-IR spectrum was recorded at wave number ranging from 4000 – 400 cm^{-1}. The spectrum of Ag_2O-NO_3-NPs and Ag_2O-SO_4-NPs powder were shown in the (Fig 1, a and b), shows various bending and stretching bands viz, 3309, 1650, 1623, 1457, 1354, 544 and 516 cm^{-1}. The broad band at 3309 and 3335 cm^{-1} was related to the presence of O-H group [45]. The bands at 1623 and 1620 cm^{-1} related to the stretching vibrations of C=C or the O–H bending. The absorption at 1457, 1440 and 1354 cm^{-1} can be related to $-CH_3$ group. The band at 515 and 544 cm^{-1} is related to Ag–O bond, thus confirming the presence of Ag_2O-NPs [32]. From FTIR measurements, it can be concluded that some of the phytochemicals which are found in the orange leaves extract such as flavonoids, alkaloids, and phenolic may be involved in reducing the silver salt to (Ag^0), and are responsible for the on the Ag_2O-NPs. In addition, it is possible to observe a clear effect of the carbonate and sulfate ions on the locations of the silver oxide nanoparticles peaks prepared from both salts.

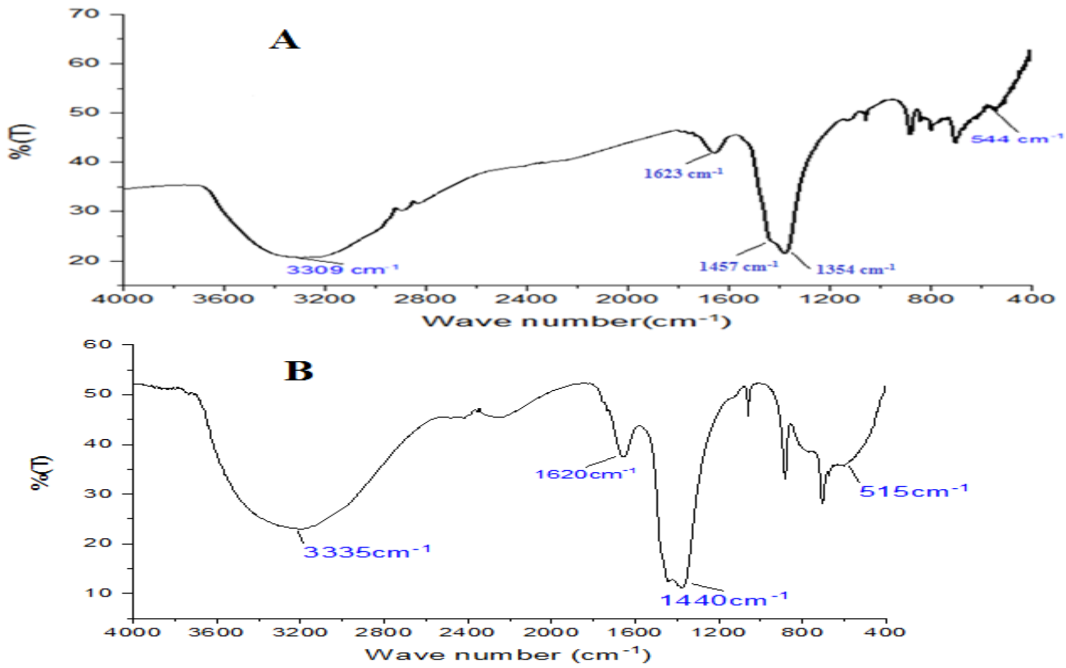

Fig. 1. FT-IR analysis of Ag_2O-NPs (**a**) from $AgNO_3$, and (**b**) from Ag_2SO_4.

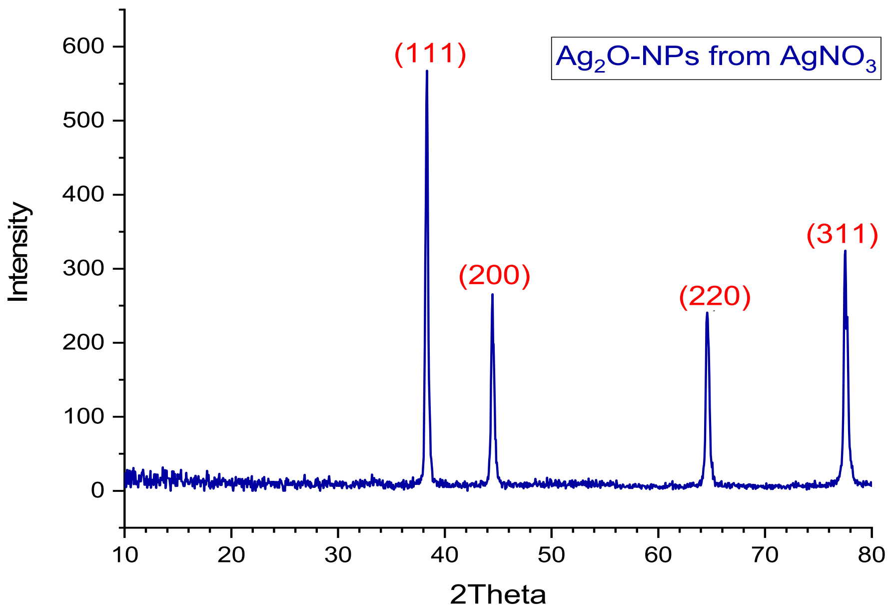

Fig. 2. XRD of Ag_2O- NPs from $AgNO_3$

XRD ANALYSIS

The crystal structure and phase identification of the Ag_2O-NPs are identified from the X-ray diffraction pattern. The XRD spectra of the synthesized Ag_2O-NO_3-NPs and Ag_2O-SO_4-NPs are show in (Fig. 2 and Fig. 3) respectively. Bragg's diffraction peaks for Ag_2O-NO_3-NPs and Ag_2O-SO_4-NPs are observed at 38.32°, 44.475°, 64.575°,and 77.525° in Ag_2O-NO_3-NPs and 38.32°, 44.446°, 64.59°, and 77.49° in Ag_2O-SO_4-NPs equivalent to 111, 200, 220, and 311, respectively, representative face centered cubic

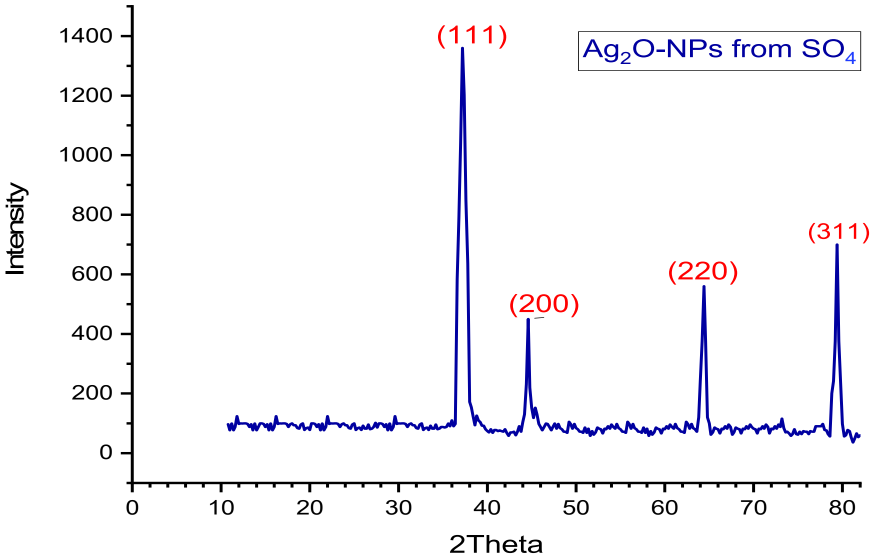

Fig. 3. XRD of Ag_2O- NPs from Ag_2SO_4

Table 1. Show XRD data of Ag_2O-NO_3-NPs

Pos. [°2Th.]	FWHM Left [°2Th.]	d-spacing [A°]	Height [cts]	Rel. Int. [%]	hkl	D,nm	δ	ε
38.3163	0.246	2.34915	561.43	100	111	35.72	0.000784	0.002146
44.4621	0.246	2.03767	260.33	46.37	200	36.45	0.000753	0.002145
64.5933	0.3444	1.44288	232.63	41.44	220	28.52	0.001229	0.003001
77.4962	0.1968	1.23173	318.44	56.72	311	54.09	0.000342	0.001713

Table 2. Show XRD data of Ag_2O-SO_4-NPs

Pos. [°2Th.]	FWHM Left [°2Th.]	d-spacing [A°]	Height [cts]	Rel. Int. [%]	hkl	D,nm	δ	ε
29.6974	0.1968	3.00833	170.11	3.42	111	43.64	0.000525	0.001717
32.8408	0.1968	2.72722	405.66	8.14	200	43.97	0.000517	0.001716
35.8334	0.2952	2.50602	4981.19	100	220	29.55	0.001145	0.002573
39.069	0.3936	2.30561	4661.16	93.58	311	22.38	0.001997	0.003426

(FCC) structure of silver. The average crystallite size of Ag_2O-NO_3-NPs and Ag_2O-SO_4-NPs were calculated to be about 38.69 nm and 34.89 nm respectively. The results of the XRD analysis concur with earlier studies that showed silver to have a cubic structure.

In Tables 1 and 2. It can be observed that the lattice constant increases (1.150962581 - 1.891936997) ^{0}A due to the use of a different salt as a precursor. The small increase in lattice constant for the Ag_2O-SO_4 NPs indicates that the particles are strained, which may be due to the ion (SO_4) changing.

FE-SEM IMAGES

The image of FE-SEM showed the surface morphology of Ag_2O-NPs, the results in (Fig .4-A and B) refer to Ag_2O-NPs derived from silver nitrate and silver sulfate, respectively. In Fig. (4-A), the scanning electron microscopy (SEM) image displays the synthesized silver oxide nanoparticles (Ag_2O-NO_3-NPs). These nanoparticles are spherical in shape and have an average size of approximately 77.75 nm. On the other hand, Figure (4-B) depicts the SEM image of the synthesized silver oxide nanoparticles (Ag_2O-SO_4-NPs) derived from silver sulfate (Ag_2SO_4).

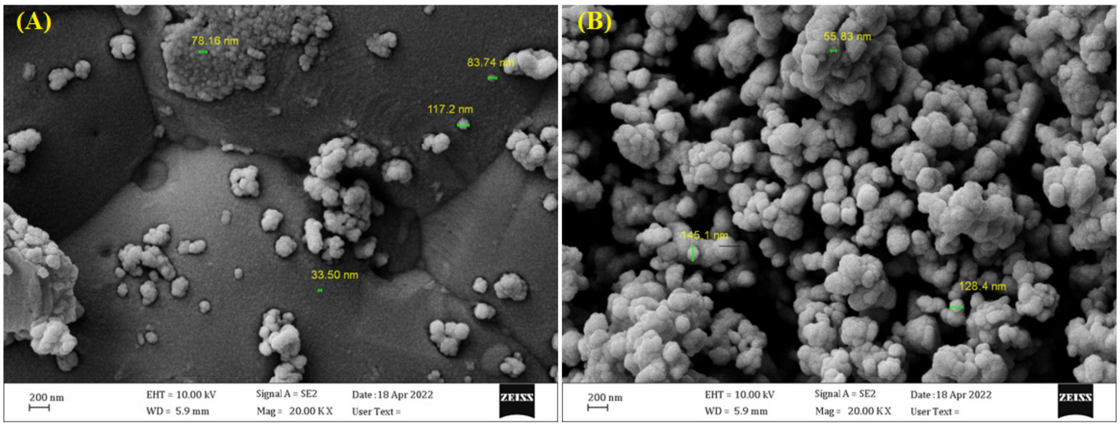

Fig. 4. FE-SEM image of silver oxide nanoparticles (A) Ag_2O-NO_3-NPs, and (B) Ag_2O-SO_4-NPs

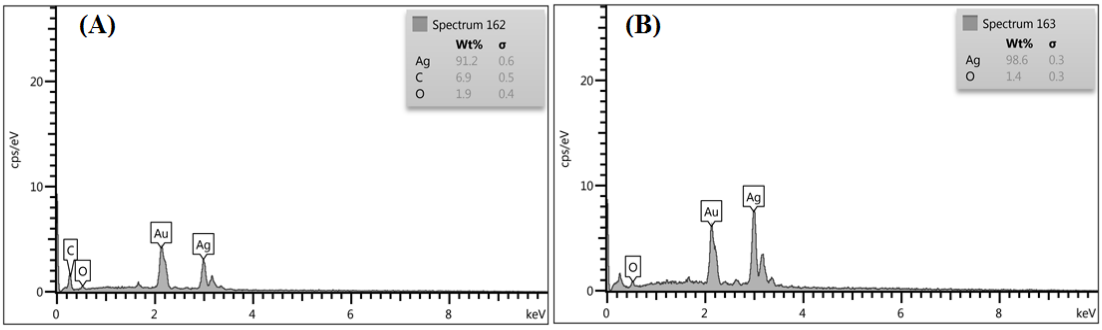

Fig. 5. EDX spectra of silver oxide nanoparticles (**A**) Ag_2O-NO_3-NPs , and (**B**) Ag_2O-SO_4-NPs.

These nanoparticles also exhibit a spherical shape and tend to agglomerate. The average size of these nanoparticles is approximately 109 nm [30]. In previous studies, a comparable outcome was reported [31-33].

EDX ANALYSIS

The elements that may play a role in the formation of Ag2O-NPs can be assessed quantitatively and qualitatively using EDX techniques. The peak spectrum shown in (Fig 5 A and B) confirmed the found of Ag in nanoparticles in both samples. The silver region at 3 kV displayed a broad and distinct signal from the elemental composition analysis, while the oxygen O and carbon C atoms produced weaker signals. This study's findings were consistent with those of other studies [34, 35].

Ag NPs induced cytotoxicity against MCF-7 cells

The effects of Ag_2O-NO_3-NPs (6A) and Ag_2O-SO_4-NPs (6B) on the viability of MCF-7 cells at 0-200 µg/ml concentrations were evaluated with 24, 48 and 72 hours of treatment. Irrespective of the Ag_2O-NO_3-NPs 10µg/ml within 24 hours, other concentrations at 24, 48 and 72 hours of treatment showed great cytotoxicity versus MCF-7 cells (6A). Besides, regardless of Ag_2O-SO_4-NPs at 10 and 25 µg/ml within 24 hours of treatment, other concentrations at all periods resulted in a substantial cytotoxicity versus MCF-7 cells (6B). These effects were time- and dose-dependents.

Due to the distinct physical and chemical characteristics, such as small size, high specific surface area, high reactivity, etc. AgNPs, also known as silver nanoparticles, are being used more and more in a variety of industries, including food, health care, industry, and pharmaceuticals [36]. Ag or AgNPs have recently been mentioned as potential anticancer medications or adjuvants, though the molecular mechanism is unclear. AgNPs may activate p53 to cause cancer cells to undergo apoptosis in order to have an antitumor effect, according to the literature [37, 38]. The most recent research, however, demonstrates that AgNPs can kill cancer cells that are both p53-positive and

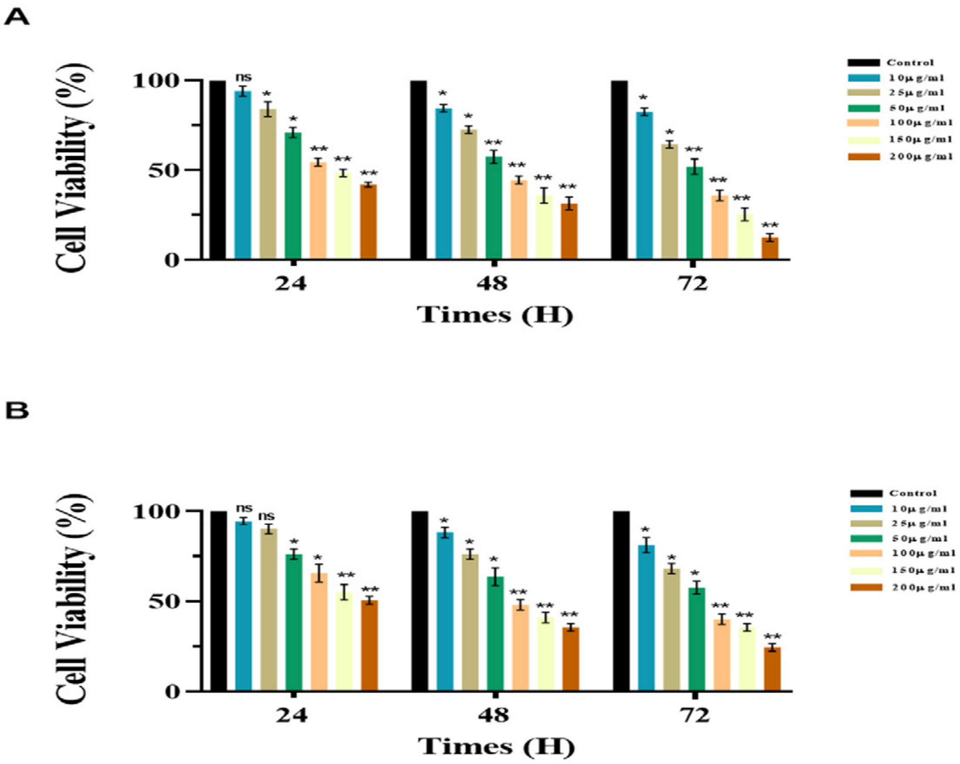

Fig. 6. The effects of Ag_2O-NO_3-NPs (6A) and Ag_2O-SO_4-NPs (6B) on the viability of MCF-7 cells at 0-200 µg/ml concentrations.

p53-negative [39]. When tested in vitro and in vivo, it has been found that AgNPs also have cytotoxic effects on normal cells [40], which makes it difficult to use as a standalone antitumor medication. Since AgNPs can be used in conjunction with other anticancer medications, some studies have suggested that they may develop into an assistant agent.

CONCLUSION

Green synthesis using plant extracts is an environmentally friendly technology that is easy, fast, and available, and its by-products are non-toxic. In this work, the green synthesis of Ag_2O-NPs was successfully carried out using orange leaf extract with silver nitrate and silver sulfate as precursors. Results of this study illustrated that orange leaf extract with various groups of phytochemicals such as terpenoids, phenols and flavonoids had suitable property in green synthesis of Ag_2O- NPs. The green synthesis was confirmed using several techniques (FTIR, XRD, FE-SEM, and EDX). The FTIR spectra demonstrated that the presence of phytochemicals in orange leaf extract played an important role in the synthesis of Ag_2O-NPs. FCC crystals of Ag_2O-NO_3-NPs and Ag_2O-SO_4-NPs, with average crystallite sizes of 38.69 nm and 34.89 nm, obtained from XRD measurements, the spherical shape belonging to Ag_2O-NO_3-NPs with average diameter 77 nm, and agglomerations spherical for Ag_2O-SO_4-NPs with average diameter nanoparticles 109 nm proved by FE-SEM analysis. EDX spectra confirmed the found of silver in the prepared NPs. Clearly from results show effect of nitrate and sulfate ions on the size and shape of Ag_2O nanoparticles prepared, the results indicate that Ag_2O-NO_3-NPs and Ag_2O-SO_4-NPs had anticancer activities against human breast cancer cell lines in a dose-dependent manner. These green methods of Ag_2O-NPs formation open a new window for treating many infectious diseases and cancers.

CONFLICT OF INTEREST

Regarding the current manuscript, the researchers affirm that there are no conflicts of interest.

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