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Morus Alba Leaf Extract-Based Biogenic Production of Silver Nanoparticles: Characterization, Antibacterial, and Antiviral Evaluation

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Abstract

The current study used a green technique that involves an aqueous leaf extract of Morus alba (mulberry) to synthesize silver nanoparticles (AgNPs) and assess their antibacterial and antiviral capabilities. The optical studies revealed a color shift from colorless - light yellowish to dark yellowish-brown as an indication of the reduction of silver metal ions & creation of silver nanoparticles and Ultraviolet-visible spectroscopy (UV-Vis) examination revealed a surface plasmon resonance (SPR) peak at 430 nm, indicating nano-silver (AgNPs) production. In addition, Fourier-transform infrared spectroscopy (FTIR) studies indicated that proteins, carbohydrates, and secondary metabolites may act as reducing and capping agents. While the Transmission electron microscopy (TEM) examination, revealed the produced nanoparticles were spherical, oval, and triangular with particle sizes ranging from 20 to 100 nm. Moreover, X-ray diffraction (XRD) measurements, show the crystalline structure of green synthesized (AgNPs) that are preferentially orientated along (1 1 1), (2 0 0), (220), and (311) planes. Furthermore, Silver nanoparticles had antibacterial action against both Gram-positive and Gram-negative bacteria, with the best results against *Staphylococcus aureus* ATCC 6538 at the highest concentration (conc.) of biosynthesized silver nanoparticles (400 µg/ml) with a maximum diameter of inhibition activity (24.33 mm), whereas the Minimal Inhibitory Concentration (MIC) was recorded at conc. (50 µg/ml) against both gram-positive and gram-negative bacteria, with the highest action against gram-negative bacteria *Pseudomonas aeruginosa* with a maximum diameter of inhibition activity (13.56 mm). In addition, silver nanoparticles showed promising antiviral activity against both Hepatitis B virus (HBV) and Hepatitis D virus (HDV) with maximum inhibition percent of 94.64 and 100 at conc. of 15.63 µg/ml of AgNPs for each virus respectively, and with minimal cytotoxic concentration (conc.) up to 99.5 percent and 90.66 percent viability on Human derived hepatoma (HepG2) cell line treated with silver nanoparticles at conc. of 7.81 µg/ml and 15.6 µg/ml respectively.

Keywords

Silver nanoparticles; Morus alba; leaf extract; Characterization; XRD; Antimicrobial; Antiviral

1. Introduction

Biogenic methods of producing metal nanoparticles are becoming increasingly popular in today's world due to their simplicity, lack of toxicity, ease of usage, and environmental friendliness. Copper, zinc, and silver are the most often used metals for nanoparticle synthesis due to their biological properties [1], [2]. Due to properties such as non-toxicity, optical, catalytic, bio-sensing, drug transport, antioxidant, cytotoxic, and antibacterial, silver nanoparticles (AgNPs) are becoming increasingly popular, with a demand of 500 tons per year [3]–[5]. Ag and AgNP-based materials which have excellent form, stability, and biophysical characteristics, are currently a particularly active sector in this cutting-edge technology [6], [7]. Silver nanoparticles have been utilized extensively in the biomedical area as antibacterial, antifungal, antiviral, anti-inflammatory, and anti-cancer medications [8 .[9] ,[

Several physical and chemical techniques have been used for the formation of silver nanoparticles (AgNPs) including chemical reduction [10], thermal decomposition [11] photochemical reduction [12], and microwave irradiation [13]. Most

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of these procedures are expensive, hazardous, and involve chemical compounds that harm living systems, which necessitates the development of a new technique that uses benign natural substances for nano-silver production [14], [15]. However, the biogenic technique used bacteria [1], [15], fungus [16], [17], actinomycetes [2], [18], leaf extract [14], root extract [19], fruit extract [20], and many other things to make bio-friendly silver nanoparticles (AgNPs). Plant extracts have an advantage over microorganisms in metal nanoparticle synthesis since isolating and maintaining microbe cultures in aseptic conditions is expensive [21]. Many substances can reduce and stabilize metal nanoparticles in plant extracts, including polysaccharides, proteins, amino acids, organic acids, and phytochemicals [22]. a variety of other plant extracts have lately been used to make silver nanoparticles including *Lavandula coronopifolia* [23], *Moringa oleifera [*24], *Datura stramonium* [25], *Cassia roxburghii* [26], *Bergenia ciliata* [27], *Carica papaya* [28], and *Morus alba* [29]. *Morus alba* (mulberry) leaf extract is a plant that is both economically and medicinally important, mulberry species, both wild and cultivated, can be found all over the world [30]. Mulberry is presently exploited industrially since every part of the mulberry is used in the creation of numerous goods in the pharmaceutical, food, cosmetic, and health care industries [30], [31]. Previous studies have used mulberry leaf extract to biosynthesize silver nanoparticles with antibacterial and antioxidant potential [29]. Moreover, silver nanoparticles (AgNPs) derived from plant extracts are a worthy material for new antiviral medications due to their many targets for action. These silver nanoparticles are antiviral in the presence of viruses including HIV [33], Hepatitis B virus [34], Herpes simplex virus [35], and Chikungunya virus (CHIKV) [36]. So, Current work aimed to synthesize and characterize silver nanoparticles using a green, sustainable, and affordable method based on mulberry leaf extract, as well as assess their effectiveness against some pathogenic Gram-positive and Gram-negative bacteria and their antiviral properties against both Hepatitis B virus (HBV) and Hepatitis D virus (HDV) and their cytotoxicity on a human-derived hepatoma (HepG2) cell line.

Materials

Roswell Park Memorial Institute (RPMI-I640) medium (Hi media, India), Silver nitrate (AgNO3), Whatman No. 1 filter paper (CytivaTM - Sigma-Aldrich), Fetal bovine serum (10% v/v) (FBS), L-glutamine, penicillin G and streptomycin, amphotericin B and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide are all available from Sigma-Aldrich (Sigma-Aldrich, USA). human-derived hepatoma (HepG2) cell line, Hepatitis B and D viruses (Vacsera, Agouza, Giza, Egypt, gram-positive *Staphylococcus aureus* ATCC 6538, and gram-negative *Clostridium difficile* ATCC 9689 *Escherichia coli* ATCC. 8739 and *Pseudomonas aeruginosa* ATCC. 9022 (culture collection of the Medical Microbiology and Immunology faculty of Ain Shams University. Deionized water was used for each experiment. Analytical-grade chemicals, reagents, and microbiological media were all used without the need for prior purification.

Plant extract preparation

Fresh, mature, shoot tissues (mulberry leaves) were collected from Kafr Abaza, Zagazig, Sharkiia Governorate Egypt at site coordinate (30°29'30.4"N 31°34'39.4"E) Fig. (1). To remove dirt and organic impurities, the leaves were surface washed multiple times with double distilled water, then air-dried for 45 minutes at room temperature to eliminate the water content. About 20 g of leaves were finely chopped and boiled in 100 ml of double-distilled water for 60 minutes. The yellowish aqueous extract was filtered with Whatman No. 1 filter paper (Cytiva™ - Sigma-Aldrich) and centrifuged at 2000 revolutions per minute (rpm) for 5 minutes to remove suspended impurities. The supernatant was used to make biogenic silver nanoparticles.

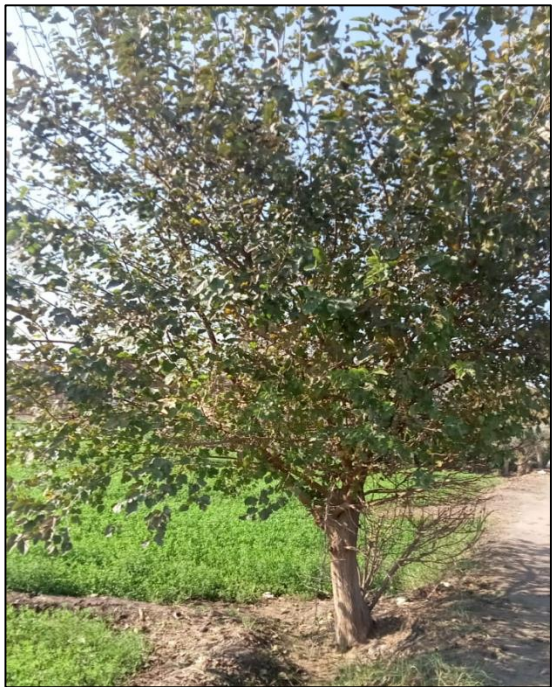


Fig. (1) *Morus alba* tree field location.

Biogenic Synthesis of silver nanoparticles

To make silver nanoparticles (AgNPs), 10 ml of *Morus alba* leaves extract was dropped into a 90 ml aqueous solution of silver nitrate (Sigma-Aldrich) and stirred continuously for 10 minutes with a magnetic

stirrer. The extract's reducing and capping agents cause the solution's color to shift from light yellow to dark brown, indicating the creation of silver nanoparticles (AgNPs).

Characterization of silver nanoparticles

Ultraviolet-visible spectroscopy (UV–Vis) analysis

UV-Vis spectroscopy (JANEWAY 6305 Spectrophotometer) of biogenic synthesized silver nanoparticles (AgNPs) at a range of 300–500 nm, shows specific surface plasmon resonance peaks of silver nanoparticles (AgNPs).

Transmission Electron Microscopy

The size and shape of silver nanoparticles (AgNPs) are often determined through Transmission Electron Microscopy (TEM- JEOL 1010 Japan).

Fourier-transformed infrared spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy FTIR analysis was used to examine the binding characteristics of biosynthesized silver nanoparticles (AgNPs) from aqueous extract of *Morus alba* leaves. The characterization includes (FTIR) analysis of the dry powder of the biosynthesized AgNPs (Agilent system Cary 630 FTIR model).

X-ray diffraction analysis (XRD)

X-Ray Diffraction patterns for silver nanoparticles (AgNPs) were obtained with the XRD-6000 series (Shimadzu Scientific Instruments (SSI), Kyoto, Japan) then the result gotten was compared with the standard JCPDS library for deciding the crystalline structure of biosynthesized silver nanoparticles (AgNPs).

Anti-microbial activity and minimum inhibitory concentration (MIC)

The antibacterial activity and minimum inhibitory concentration (MIC) of synthesized silver nanoparticles using mulberry leaf extract were tested using the agar well diffusion method. Five distinct conc. (25, 50, 100, 200, and 400 µg/ml) were used to investigate the antimicrobial activity of diluted silver nanoparticles by double-fold serial dilution. Gram-positive *Staphylococcus aureus* ATCC 6538, *Clostridium difficile* ATCC 9689 gram-negative *Pseudomonas aeruginosa* ATCC. 9022 and *Escherichia coli* ATCC. 8739 test organisms were cultivated overnight on nutritional broth before their application. 100 µl nutrition broth test organism was uniformly mixed with nutrient agar plate and allowed to harden. After 30 minutes, one hindered microliter (100 µl) of manufactured nanosilver was added to the nutrient agar plate at an adequate concentration. After 24 hours of incubation at 37°C, the diameter of the inhibitory zone was measured in millimeters.

Cell Culture

The human-derived hepatoma (HepG2) cells line and Hepatitis B and D viruses (Vacsera, Agouza, Giza, Egypt) were maintained in Roswell Park Memorial Institute (RPMI-I640) media HI media, India) supplemented with fetal bovine serum (10% v/v) (FBS, Sigma-Aldrich, USA), 2 mM L-glutamine (Sigma-Aldrich, USA), 100 units/ml penicillin G with 100 µg/mL streptomycin (Sigma-Aldrich, USA) and, amphotericin B 50 µg/ml (Sigma-Aldrich, USA). Cells were incubated at 37˚C in a 5% CO_2 humidified atmosphere.

Cytotoxicity and Anti-viral activity assays

The cytotoxicity of silver nanoparticles was evaluated by cell viability using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on the HepG2 cells line at 96 well tissue culture plate. The color intensity was measured at 560 nm using an enzyme-linked immunosorbent assay (ELISA) reader [37], the experiments were performed in triplicates, and the percentage cell viability was then calculated concerning control (cells incubated without silver nanoparticles) as follows:

Cell viability %= (sample absorbance / control absorbance) x100 [38] .

MTT assay was used also to evaluate antiviral activity using 10,000 viral cell plates cultured in 200 µL media in a 96-well plate [39]. Three wells are left blank for blank controls, and the cells (2×10^5 cells/well) were cultured in a growth medium incubated overnight at (37°C, 5% CO_2) to allow the cells to attach to the wells, then incubated for 1 hour with an equal volume (1:1 v/v) of nonlethal dilution of the tested sample and exposed to a non-toxic concentration of silver nanoparticles (AgNPs), Hepatitis B and D viruses suspension, 100 µL of viral/ sample suspension added and placed on a shaking table at 150 rpm for 5 minutes. The viral/sample suspension is incubated for one day at (37 °C, 5% CO_2) to allow the virus to take effect. Formazan (MTT metabolic product) is resuspended in 200 µL DMSO and shaken for 5 minutes at 150 rpm on a shaking table to fully mix the formazan into the solvent. At 560 nm, the optical density is determined, and at 620 nm, the background is eliminated. The optical density should be proportional to the number of cells. The 50% antiviral effective concentration (IC50) was expressed as the concentration that achieved the 50% protection of virus-infected cells from the Hepatitis Viruses induced destruction. The percent protection was calculated by the following formula:

(ODt)v - (ODc)v / (ODc)m - (ODc)v X 100 (%)

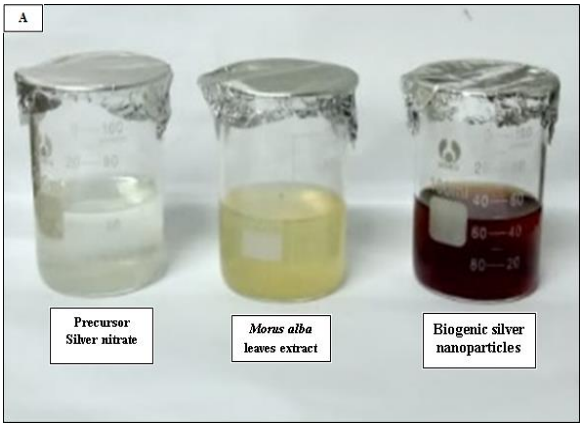
The abbreviations (ODt)v, (ODc)v, and (ODc)m correspond to absorbances in virus-infected cells with test compounds, without test compounds (control), and in the very-infected control, respectively [40], [41].

6. Results and discussion

Visual observation and Spectral study of UV–visible light

The use of biological approaches to synthesize nanoparticles (NPs) using plants and microorganisms has various advantages over physical and chemical methods. Physical methods of NPs creation consume a lot of energy, whereas chemical methods produce hazardous and poisonous chemicals that prevent the biological usage of the nanoparticles produced [42]. The biogenic synthesis of silver nanoparticles by *Morus alba* leaves extract was mediated when supernatant cell-free filtrate was incubated with an aqueous solution of 1 mM of $AgNO_3$ under optimized conditions & observed by the color shift from colorless - light yellowish to dark yellowish-brown as an indication of the reduction of silver metal ions & creation of silver nanoparticles as shown in Fig. (2-A). The color change is caused by the excitation of surface plasmon properties in nanoparticles and is unique to silver nanoparticles [43], [44]. Color change during nano formation is influenced by numerous factors, including the medium and the organic content of the plant extract. A UV-Vis spectrophotometer was used to confirm the production of silver nanoparticles after 24 hours of incubation, revealing a typical absorption peak at 430 nm for biosynthesized silver nanoparticles, as shown in Fig (2-B). The long-term stability of the AgNPs generated was also discovered.

Our findings are consistent with those of Balavijayalakshmi and Ramalakshmi, 2017 [45], who found that the greatest wavelength peak of Nanosilver generated with 10^{-2} M and 10^{-3} M silver nitrate was at 450 nm and 435 nm, respectively, with a redshift as concentration increased. The existence of a long-wavelength SPR signal indicates a large particle size. [46].



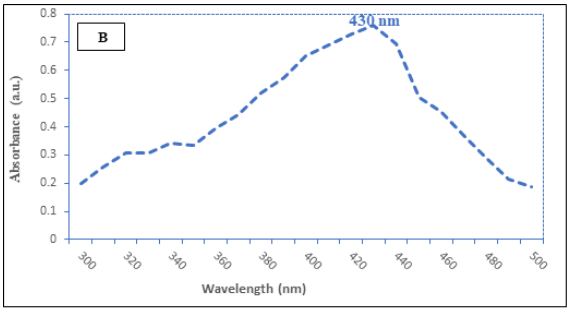


Fig. (2) A- Color change observation Phyto-synthesis of silver nanoparticles from *Morus alba* leaves extract.
B- UV-visible spectroscopy of Phyto-synthesis of silver nanoparticles from *Morus alba* leaves extract.

FTIR (Fourier transformed infrared) spectroscopy

Infrared spectroscopy was used to see if functional groups were entangled in the reduction, stability, and capping of silver nanoparticles, which were linked to chemical bonds in the mulberry extract filtrate, indicating that many plant extract components are involved in the bioreduction of metallic silver into nanoparticles. Absorption bands were found in the FTIR spectra of biosynthesized silver nanoparticles from dried aqueous plant extract were found at 3441.2, 1636.5, 1329.5, 1269.8, 1113.6, 624.6, 563.2, and 425.6 cm^{-1} as indicated in Fig (3). Hence, the peak at 3441.2, was corresponding to the N–H vibration mode which overlapped with –OH vibration stretching of alcoholic and phenolic compounds [47]. While the strong peak at 1636.5, 1329.5, and 1269.8, cm^{-1} relates to primary, and secondary amine vibrations, and the C–N vibration stretch represent the amide I band of proteins identified in leaf extracts [48]. Strong C–O– and C–OH stretching vibrations of carboxylic acid, alcohol, ester, and ether bonds of protein and carbohydrate present in the extract are represented by the intense band at 1113.6 cm^{-1} of plant extract [49]. In produced nano silver, the peak at 624.6 cm^{-1} indicates N–H vibration of primary aliphatic amines [50]. C–Cl stretching vibration and C–C skeletal vibration of branch alkenes are indicated by absorption bands at 563.2, and 425.6 cm^{-1} respectively [51]. In a similar investigation of mulberry leaf extract, Liang et al., 2018 [52] found protein, glucose, glycoprotein, phenols, flavonoids, amino acids, carotene, and anthocyanins. Thus, the presence of carbohydrates, proteins, and various secondary metabolites in the plant extract is defined by the functional groups observed in IR spectra, which are engaged in silver ion reduction and act as stabilizing agents.

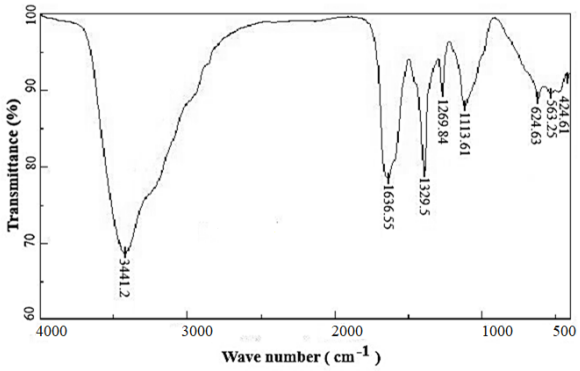


Fig. (3) FTIR spectra of Phyto-synthesized (AgNPs) by *Morus alba* leaf extract.

X-ray diffraction analysis (XRD)

Examining the diffraction peaks of the AgNPs generated from *Morus Alba* Leaf Extract using XRD can be utilized to detect the existence of silver nanoparticles in plant tissues because XRD is frequently used to determine the chemical composition and crystal structure of a material. So that the crystalline character of biologically produced silver nanoparticles was demonstrated using the XRD pattern of dried nanosilver, as seen in Fig. (4). The values of (111), (200), (220), and (311) Bragg's reflections plane of face-centered cubic silver was found to correspond to four significant diffraction peaks which were indexed for standard JCPDS library file no. 04-0783, which validated the face-centered cubic structure of biosynthesized silver nanoparticles. Peaks (200), (220), and (311) have modest and broad intensities, however (111) has a high and powerful peak, indicating that nanocrystals are oriented in the direction of (111). There are also other smaller peaks visible those peaks are the consequence of bio organics in plant leaf extract crystallizing and this peak is noticeably weaker than those of Ag showing that Ag is the primary constituent of the composite [53].

[54] previously found similar XRD orientation while producing silver nanoparticles using aqueous Chlorella Vulgaris extracts, respectively. Almost matches exactly the reported standard value of silver which is 4.086 Å (JCPDS file no: 04-0783). A similar finding regarding the biogenic synthesis of AgNPs was made regarding the organic components of extracts that result in the reduction of the silver ion into silver nanoparticles [55], [56].

According to data from observed TEM image analysis, particle shapes, and sizes that directly transformed into cumulative number-based distributions, the average particle size was found to be 20–100 nm for produced silver nanoparticles. These particles have spherical, oval, and triangle shapes as well as varying size distributions (ISO/TC 229 Nanotechnologies) [61] (Fig. 5 A and B). In some regions, the variation in size distribution was predominantly due to nanoparticle clumping [57].

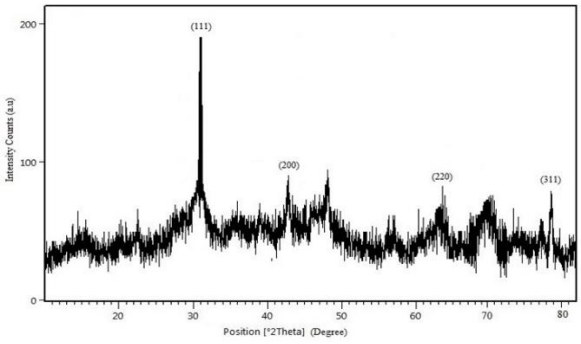


Fig. (4) XRD Pattern of Phyto-synthesized (AgNPs) by *Morus alba* leaf extract.

Transmission electron microscopy (TEM)

The nanoparticles' periphery is thinner than the center, implying that protein molecules serve as a capping agent [58]. The polydispersity was found to be 11 percent, indicating that most of the particles are monodispersed. [59] observed similar effects using banana peel and papaya leaf extracts. The size, shape, and polydispersity index of nanoparticles all play a factor in their use [60]. According to [60], Silver nanoparticles' bioactivity, notably antibacterial activity, is inversely proportional to their size. The goal of this research is to determine the bioactivity of nanoparticles of a smaller size.

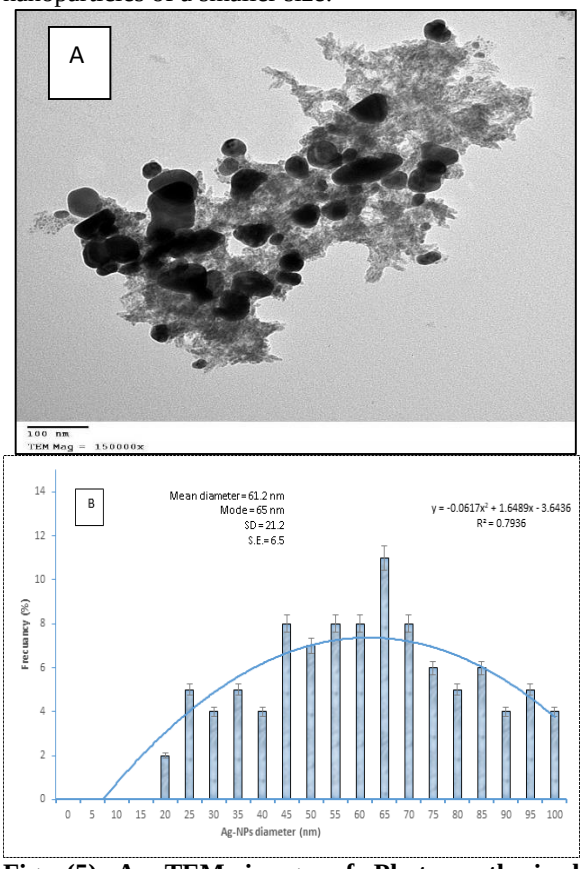


Fig. (5) A- TEM image of Phyto-synthesized (AgNPs) by *Morus alba* leaf extract.
B- Particle size distribution of Phyto-synthesized (AgNPs) by TEM observation [61] .

Antimicrobial activity

The diameter of the inhibition zone of biosynthesized silver nanoparticles made with mulberry leaf extract showed significant antibacterial activity against both gram-positive and gram-negative bacteria, with the highest activity against gram-positive bacteria *Staphylococcus aureus* at the highest concentration of biosynthesized silver nanoparticles (400 µg/ml) with a maximum diameter of inhibition activity (24.33 mm), whereas the Minimal Inhibitory Concentration was recorded at conc. (50 µg/ml) against both gram-positive and gram-negative bacteria, with the highest action against gram-negative bacteria *Pseudomonas aeruginosa* with a maximum diameter of inhibition activity (13.56 mm) as shown in (Table 1- Fig. 6).

This could be explained by the fact that metals adhere to the surface of gram-positive bacteria more easily and in greater amounts [62]. Gram-positive bacteria's cell wall has a thick peptidoglycan layer, consisting of linear polysaccharide chains cross-linked by short peptides, making it difficult for silver nanoparticles to penetrate; however, Gram-negative bacteria's cell wall has a thinner peptidoglycan layer, allowing silver nanoparticles to easily release silver ions, causing membrane disruption and bactericidal action. [63], [64]. Aloe vera extracts were used to make silver nanoparticles [65], *Eriobotrya japonica* [66], *Sida acuta* [67], *Lycopersicon esculentum* [67], and *Artocarpus heterophyllus* [68], previously demonstrated high antibacterial action against several microbes.

Furthermore, by altering cellular architecture and releasing silver cations, silver nanoparticles inhibit bacterial development. [68]. According to the researchers, the electrostatic attraction between positively charged nanoparticles and the negatively charged microbial membrane was the driving force behind bactericidal activity [69].

Table (1) Antagonistic activity & Minimum Inhibitory Conc. (MIC) of biosynthesized AgNPs against different pathogenic test organisms.

Conc. (µg/ml)	Mean Diameter of inhibition zone(mm)			
	Staphylococcus aureus	*Clostridium difficile*	*Pseudomonas aeruginosa*	*Escherichia Coli*
25	0	0	0	0
50	12.66±0.58	11.56±0.88	13.56±0.58	11.51±0.58
100	16.67±0.33	14.33±0.58	15.67±0.33	16.87±0.88
200	19.67±0.33	16.67±0.33	18.27±0.88	17.67±0.33
400	24.33±0.33	19.27±0.58	22.67±0.58	20.33±0.58

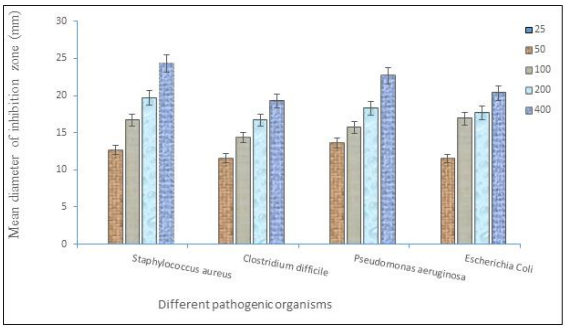


Fig. (6) Antagonistic activity & Minimum Inhibitory Conc. (MIC) of biosynthesized AgNPs against different pathogenic test organisms.

Cytotoxicity activity

Based on cell viability by MTT assay [70], the in-vitro cytotoxic effects of Phyto-synthesized spherical, oval, and triangle shapes silver nanoparticles (AgNPs) from *Morus alba* leaf extract with average particle size between 20 - 100 nm were assessed against the human hepatoma (HepG2) cells line. Since AgNP's size and shape affect their antiproliferative activity [71]. AgNPs' shape-dependent characteristics make them suitable for therapeutic use. Certain non-regularly shaped AgNPs can readily bind to different proteins and DNA, boosting biological activity and apoptosis through DNA fragmentation [72]. Additionally, AgNPs have the potential to destroy cell membranes by attaching to proteins in the membrane that contain sulfur. Furthermore, the tumorigenic wall may be fatally affected by silver nanoparticles, inducing a synergistic pharmacological activity, such as an anti-proliferative effect in several cancer cell lines, the water-soluble organic moieties on the surface of the nanoparticles make them beneficial in a variety of cancer treatments [73].

HepG2 cells treated with AgNPs at concentrations of 7.81 µg/ml and 15.6 µg/ml showed 99.5 percent and 90.66 percent viability for biosynthesized AgNPs after the incubation period, respectively; the viability of HepG2 cells decreased with increasing AgNPs Conc.

Meanwhile, viability dropped to almost 50% of the initial level, which showed 54.81 percent, at

conc. 31.2 µg/ml. Therefore, these values were selected as the IC50 conc.

Furthermore, the lowest viability percentages of 4.79 and 3.81 percent, respectively, were reported at maximal concentrations of 500 and 1000 µg/ml. as indicated in Fig. (7)

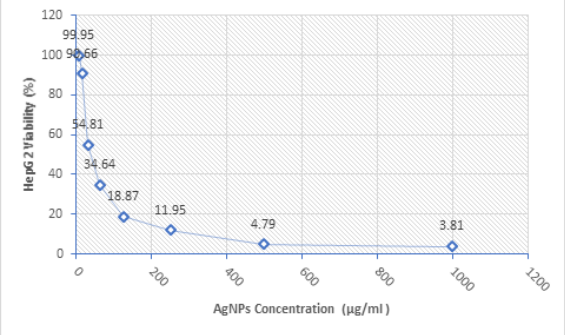


Fig. (7) Cytotoxicity by MTT assay of Phyto-synthesized silver nanoparticles treated HepG2 cells, at different concentrations of 7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000 µg/ml, and overnight incubation.

Antiviral activity

AgNPs have potential antiviral activity, due to their huge surface area, which enables interaction with virus particles. Antiviral medications typically interfere with structural interactions or activities of viral particles by binding to viral coat proteins. Here, viral-nanoparticle interactions are critically influenced by the size of the AgNPs [74].

The potential antiviral action of tested AgNPs concentrations was assayed against HBV and HDV within HepG2 cells using MTT assay Fig. (8). HepG2 cells treated with HBV, HDV, and AgNPs at concentrations of 15.63 µg/ml of AgNPs showed almost 100 percent and 94.64 percent inhibition for HDV and HBV replication after the incubation period, respectively.

Meanwhile, viral activity dropped to almost 50% of the initial level at AgNPs conc. of 1.95 µg/ml for HDV and 0.977 µg/ml for HBV Therefore, these values were selected as the IC50 conc.

The action of AgNPs occurs due to AgNPs interacting with the viral surface, either causing the viral genomic material to be destroyed or preventing it from entering the cell membrane. To prevent the virus from interacting with the cell membrane, AgNPs additionally adhere to the viral entity. The viral entity's nucleocapsids inside the cell are also inhibited by AgNPs. AgNPs also engage with the viral genomic material, preventing the host cell's genome from replicating. Finally, they stop cellular processes like protein synthesis to prevent the viral entity from replicating. Although other metals have antiviral capabilities, AgNPs are the most potent; regardless of the virus family, they have demonstrated strong antiviral activity [74],[75].

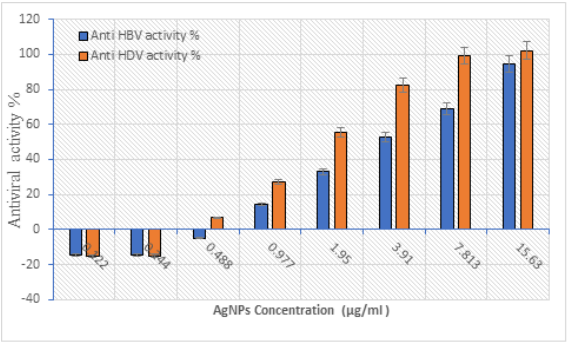


Fig. (8) Antiviral activity by MTT assay of Phyto-synthesized silver nanoparticles treated HepG2 cells against HBV and HDV, at different concentrations and 24 hrs. incubation.

7. Conclusion

In this study, mulberry leaf aqueous extract was used for the environmentally friendly production of silver nanoparticles (AgNPs). Ag-NPs synthesis was confirmed by UV-Vis spectroscopy in the 430 nm range and color shift from colorless to yellowish brown. Using XRD and FT-IR, respectively, the crystalline structure of Ag-NPs and the functional groups in charge of biosynthesis were assessed. According to the TEM investigation, nanoparticles with spherical, oval, and triangle forms and sizes between 20 and 100 nm were produced. Ag-NPs were also found to have biocontrol abilities against several harmful microbes as well as antiviral activity against the Hepatitis B and D viruses with little cytotoxic effects on the HepG2 cell line.

8. Conflicts of interest

The authors affirm that they have no known financial or interpersonal conflicts that would have appeared to have an impact on the research presented in this study.

9. Formatting of Funding sources

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