ADVANCE SOCIAL SCIENCE ARCHIVE JOURNAL
Available Online: https://assajournal.com
Vol. 05 No. 01. Jan-March 2026.Page#.384-404
Print ISSN: 3006-2497 Online ISSN: 3006-2500
Platform & Workflow by: Open Journal Systems

Evaluation of Anticancer, Antioxidant and Anti-diabetic activity of Silver Nanoparticles using leaf extract of Grewia asiatica

Muhammad Ateeb*
University College of Conventional Medicine, Faculty of Medicine and Allied Health Sciences, The Islamia University of Bahawalpur, 63100, Pakistan
Research Associate, Office of Research, Innovation and Commercialization, The University of Faisalabad, Faisalabad 38000, Pakistan
Correspondence Email: muhammadateeb786@gmail.com
Abid Hussain
University College of Conventional Medicine, Faculty of Medicine and Allied Health Sciences, The Islamia University of Bahawalpur, 63100, Pakistan
Hafiz Muhammad Asif*
University College of Conventional Medicine, Faculty of Medicine and Allied Health Sciences, The Islamia University of Bahawalpur, 63100, Pakistan
Correspondence Email: muhammad.asif@iub.edu.pk
Rabia Zahid
University College of Conventional Medicine, Faculty of Medicine and Allied Health Sciences, The Islamia University of Bahawalpur, 63100, Pakistan
Ali Raza Ayub
A Key Laboratory of Cluster Science of Ministry of Education, School of Chemistry and Chemical Engineering, Beijing Institute of Technology, Beijing 100081, P. R

Abstract
Diabetes and cancer are two severe, multifaceted, and long-lasting diseases. Each year, diabetes mellitus and cancer claim the lives of about 11 million individuals. Recently, metal-based nanoparticles (MNPs), which were produced utilizing plant extracts in an ecologically benign way, have appeared as a superior choice for the management of these deadly illnesses. The current study investigated the antioxidant, anti-diabetic, and anticancer properties of greenly generated silver nanoparticles (AgNPs) and Grewia asiatica Linn (G. asiatica), a leaf extract from the Malvaceae family. The results indicate the color transition of the DPPH solution from purple to yellow, with the crude extracts exhibiting robust antioxidant activity (78.68% ± 0.02) and the nanoparticles demonstrating even higher activity (81.62% ± 0.02) against DPPH. Furthermore, with IC50 values of 177.3 µg/ml, 99.8 µg/ml, and 73 µg/ml, respectively, AgNPs efficiently prevent the development of human breast (MCF-7), cervical (HeLa), and liver (HepG2) cancer cells at exceptionally low doses. Additionally, the AgNPs exhibited superior anti-diabetic activity, as demonstrated by alpha-glucosidase and alpha-amylase inhibition assays (85.03% ± 0.02 and 75.15± 0.01 accordingly). AgNPs were therefore shown to be a potent α-glucosidase inhibitor, a free radical scavenger, and an efficient medicinal substance that targets human breast cancer cell lines (MCF-7). To determine the nanoparticles' anticancer and antidiabetic actions, further research is necessary.

Keywords: *Grewiaasiatica, alpha-amylase, alpha-glucosidase, MTT assay.*

Highlights

- GA-AgNPs were fabricated using G. asiatica leaf extracts.
- UV spectroscopy and a reaction-induced color shift were used to confirm GA-AgNPs.
- With strong in vitro anticancer, antioxidant, and anti-diabetic effects, GA-AgNPs can be used as a suitable source for environmentally friendly AgNP production.

Introduction

Oxidative stress, which results from an imbalance between the production of free radicals and reactive metabolites, is a feature of the majority of disorders (Phaniendra et al., 2015). This imbalance causes harm to critical macromolecules and cells, potentially affecting the whole body. DNA damage is the root cause of both the genesis of cancer and its progression (Alhmoud et al., 2020). In many chronic illnesses, pathophysiological factors such as cytotoxicity, inflammation, and oxidative stress serve as essential factors. The redox condition of cancer cells often varies compared to normal cells, and high reactive oxygen species (ROS) levels are harmful to cells. Cancer cells exhibit elevated levels of ROS because of signaling and metabolic abnormalities (Gorrini et al., 2013). Cancer is a potentially fatal illness that is described as the uncontrolled development of abnormal cells that penetrate and destroy surrounding tissues. The illness caused 9.6 million deaths and 18 million new cases in 2018 (Kaniyala Melanthota et al., 2022). The mortality and morbidity of cancer are rapidly increasing around the globe. Now it is the world's second leading cause of death (Chandrasekaran et al., 2022). There are different causes for different cancers, as it is difficult to evaluate which factor is responsible for initiating the cancer. However, some factors are in common, e.g., environmental factors, lifestyle, diet, social circumstances, and psychological factors (Anand et al., 2008).

ROS and oxidative stress are associated with ageing and associated dysfunctions, including cancer and diabetes (Bernatoniene & Kopustinskiene, 2018). An antioxidant defense system is the elimination of these free radicals via protective mechanisms (Ahmed, Hassan, et al., 2017; Ahmed, Soliman, et al., 2017; Perry et al., 2011). Diabetes mellitus (DM) is linked to significant physiologic abnormalities. It is mainly a metabolic (endocrine) disorder defined by persistent hyperglycemia that causes several biochemical abnormalities and oxidative stress in the body (Ghosh et al., 2015). The damaging levels of reactive free radicals in the body and the coordinated destruction of defense mechanisms against antioxidants may result in perilous deterioration of enzymes and cellular organelles, leading to peroxidation of lipids and the emergence of resistance to insulin. These effects of oxidative stress might result in complications of diabetic mellitus (Suganthy & Pandima Devi, 2016). In addition, glucose oxidation and nonenzymatic protein glycation both contribute significantly to the generation of free radicals in specifically diabetic patients (Azeem et al., 2022). Multiple environmental and genetic factors might be influencing. The metabolic abnormalities are mainly caused by inadequate insulin response of target tissues, as well as insulin resistance of the liver (to a lesser degree). The signal transduction system, insulin receptors, effector enzymes, or genes might induce metabolic disorders (Meyts, 2016). Diabetes-related macro- and microvascular complications are the leading causes of blindness, amputations, and renal failure, resulting in morbidity, high mortality, and a poor prognosis (Cade, 2008). Diabetes mellitus is among the oldest known diseases to humanity, representing a global health issue, with its incidence increasing from 4 percent to 5.4 percent since 1995. Diabetes is expected to affect 592 million people by 2035, or 642 million people will have the disease by 2040, according to the WHO. Notably, a recent analysis indicates that diabetes took the lives of 1.5 million people in 2019 (*Diabetes*, n.d.; Kharroubi & Darwish, 2015). In 2013, almost 382 million individuals globally had diabetes. Diabetes usually develops around 42.5 years of age because of a high-calorie diet, genetic

predisposition, not enough exercise, and a poor lifestyle (Tao et al., 2015; Zheng et al., 2017).
The incidence and fatality rates of diabetes and cancer remain high, despite significant advancements in treatment and medication. Current diabetes therapies attempt to normalize blood glucose levels in the vasculature. However, most modern drugs have side effects that might lead to serious medical issues (Tran et al., 2020). The most efficient method to treat tumor cells is with chemotherapy, while surgery and radiation therapy are also effective options. All of these therapy options are costly and have a number of undesirable side effects. No adequate treatment for cancer is still accessible in modern medicine (Chandrasekaran et al., 2022). As a consequence, for a long time, traditional medications have used and continue to be used as an alternative treatment. Also, in recent years, emerging plant-derived bioactive substances have shown stronger anti-diabetic potential than oral hypoglycemic medicines used in clinical therapy (Tran et al., 2020). Consequently, researchers are engaged in developing diabetes treatment options that are both effective and safe. Biomimetic plant-based nanocomposites (nanomedicine) are one of the most effective active components, with anticancer, antibacterial, anti-inflammatory, antidiabetic, and antioxidant effects (Hu et al., 2022).
The application of nanotechnology in medicine is to monitor, diagnose, treat, and control illness, which is known as "nanomedicine" (Satalkar et al., 2015). Nanoparticles (NPs) are better at delivering drugs and imaging than other types of particles, so researchers are becoming more interested in using them to treat and manage diabetes mellitus and cancer. Inorganic NPs, polymer-based NPs, and liposomes are the main types of systems for drug delivery (Mauricio et al., 2018). Nanotechnology-based techniques may significantly improve the treatment of diabetic patients. Insulin sensitivity may now be better mimicked by glucose-responsive nanoparticles, which are based on glucose nanosensors (Veiseh et al., 2014). Because of this, the majority of researchers are focusing their attention on nanotechnology in the hopes of developing reliable nanomaterials with which to combat these ever-increasing challenges. In previous studies, the fabrication of nanomaterials via chemical and physical methods was the primary area of study. However, the majority of these methods are rather expensive, require significant and difficult laboratory processes, and make use of toxic substances (Badawi et al., 2021; Dawadi et al., 2021). So, biological method/ green synthesis or biosynthesis, are ways for synthesizing and fabricating nanoparticles that are low-toxic, economical, efficient, and less harmful to the environment than traditional techniques (Barani et al., 2021; Es-Haghi et al., 2021; Hassanisaadi et al., 2021, 2022; Mahmoud et al., 2021). For the manufacture of metal oxide nanoparticles, biological systems such as fungi, bacteria, viruses, yeast, and plant extracts are used (Jamkhande et al., 2019; Shah et al., 2015). Greater solubility, better pharmacological potential, increased bioavailability, and sustained release are the advantages of using Nanotechnology in the extraction of plant extracts. Furthermore, the medicine is protected against chemical and physical depletion using this method (Servat-Medina et al., 2015).
Metal nanoparticles have found widespread application in various fields, including medicine as well. Their unique physicochemical and biological features make MNPs ideal for biomedical applications, i.e., antibacterial, optical polarizability, electrical conductivity, biocompatibility, antioxidant, antidiabetic, photocatalytic, anticancer applications, and more. Silver nanoparticles, on the other hand, are better and safer (less toxic) than copper, gold, and other nanoparticles (Xu et al., 2020). Additionally, as we all know Medicinal plant extracts are far safer than synthetic medications. Because of this, nanoparticles made from these plant extracts will be both safe and efficient in the management of diabetes, cancer, and several other fatal diseases. Thus, green silver nanoparticle synthesis may be an efficient and safe method of eradicating multidrug-resistant diseases and their complications. Green synthesis of metal ions, such as silver, displayed

both in vivo and in-vitro antidiabetic and anticancer action, prompting efforts to develop alternative anticancer and antidiabetic medicine (Ahmad et al., 2023; lakshmi, 2022; Lan Chi et al., 2022; Nagaraja et al., 2022; Oves et al., 2022; Perumalsamy & Krishnadhas, 2022; Veeragoni et al., 2022; Zubair et al., 2022)

The *Grewia asiatica* plant has been thoroughly investigated for its many biological properties, including antidiabetic, anticancer, and potent antioxidant (Zia-Ul-Haq et al., 2013). The previous work (Ateeb et al., 2023) looked at the synthesis and characterization of *Grewia asiatica* silver nanoparticles as well as some of their biological activities. However, no findings on this plant AgNPs to demonstrate anti-diabetic, antioxidant, and anticanceractivity have been published so far, and this needs to be addressed. So, this study was designed to evaluate anticancer, anti-diabetic, and antioxidant activity of AgNP susing leaf extract of G.asiatica.

Material and Methods

Material

Chemicals used are Silver nitrate, Sodium nitrate, Potassium hydroxide, Sodium hydroxide, Disodium phosphate, Potassium dihydrogen phosphate, DPPH, 4-Nitrophenyl-β-D-glucopyranoside, Alpha-glucosidase, from Sigma Aldrich, Merck, Germany, Ascorbic and gallic acid (Sinopharm Chemical Co. Ltd), Methanol (HPLC), DMSO (Merck Darmstadt, Germany). P-nitrophenyl butyrate (Sigma Chemical, Germany), Alpha-amylase (from porcine pancreas, China EC 3.2.1.1), Dinitro salicylic acid (Sigma–Aldrich), Acarbose (Carbo synth United Kingdom), Foetal Bovine Serum (Sigma, Aldrich, USA), Dulbecco's Modified Eagels medium (Caisson's Lab, USA) and Tetrazolium salt (MTT Roche applied sciences). The University of Lahore's cell and tissue culture facility provided the cell lines: HepG2, MCF-7, and HeLa cells. *Grewia asiatica* leaves that were gathered in the Bahawalpur region were identified and verified by a botanist.

Preparation of Grewia asiatica leaves extract

In the initial phase of the crude extract preparation, 500g of the *Grewia asiatica* plant was thoroughly washed twice and allowed to dry entirely at room temperature beneath a shade cover. Using a grinder, the dried leaves of *Grewia asiatica* were crushed into powder form. I prepared a large amount of plant extract so that I may do further activities in the future to demonstrate its additional pharmacological effects. The pulverized plant material was now immersed in an aqueous-ethanolic solvent (30/70 V/V) for 3 days with periodic stirring throughout the day (Sultana et al., 2013). It was then filtered using muslin fabric and finally through filter paper. The extract was evaporated using a rotary evaporator at a temperature between 30 and 40 degrees and at a vacuum pressure. The result was a thick semisolid (gummy) extract that was assessed for percentage yield and then stored at 4°C in the freezer to be used in the anti-diabetic, anticancerous, and antioxidant analysis. The process of collecting, identifying, making the crude extract, and getting the percentage yield is seen in Figure 1.

Figure 1 The process of going from collection to % yield step by step.

Experimental

Synthesis of GA.AgNPs utilizing *Grewia asiatica* leaves extract solution

Grewia asiatica extract was employed as an agent for reduction. to make Ag-NPs of different sizes from silver nitrate. It also helped to keep the Ag-NPs stable by capping them. At 0.01M, it was made by solubilizing 0.0339 g of AgNO3 (silver nitrate) in 20 mL of deionized water. Three conical flasks labeled 1-3, each containing 20 mL of 0.01 M AgNO3 solution, were placed on a magnetic stirrer, and Grewia asiatica extract dispersed in 20 mL of water was added drop-by-drop into each flask 1-3 with burette in different concentrations- 20 mg, 30 mg, and 40 mg respectively, with continuous stirring to obtain three separate samples of varying sizes of AgNPs labeled as 1, 2, and 3. At room temperature for 3 hours, these three samples were stirred until the colour changed from light yellowish-brown to darkish brown, indicating that Ag-NPs had been synthesized. All three samples (1-3) were exposed to complete darkness for a whole night to verify that almost all Ag+ ions had transformed to Ag0. For the validation of the findings, a UV-spectrophotometer was used. Samples were then centrifuged at 6000 RPM for 30 minutes, rinsed with ethanol, and dried. They were then measured and stored at 4°C for future use and characterization.

Table 1 Reaction parameters (AgNO3/plant extract ratios, Ag-NP weights)

Sample codes (Ag-NPs)	AgNO (0.01M) Solution (mL)	Plant extract (mg)	Quantity (mg)
1	20	20	26.5
2	20	30	39.1
3	20	40	54.6

Characterization techniques

Initially, silver nanoparticles were identified by their reaction-induced colour change from vivid yellow to darkish brown and then confirmed via UV spectroscopy, as shown in Figure 2. The solution changed colour due to the formation of Ag-NPs. The techniques, including XRD, EDS, FTIR, and SEM, were used to characterize Ag-NPs (Ateeb et al., 2023).

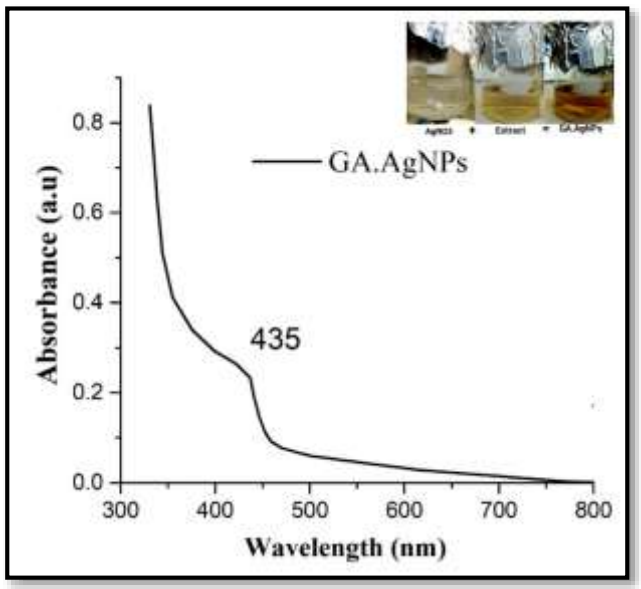

Figure 2 The UV absorption spectrum of AgNPs with their reaction-induced colour change

Percentage yield of AgNPs

The actual silver nanoparticle yield was determined using the following equation:

% yield = actual nanoparticle weight / theoretical weight × 100

Anticancer activity: MTT assay

Cell viability and cytotoxicity were evaluated in breast cancer, cervical cancer, and liver cancer cells through MTT assay in accordance with the previously established procedure with certain modifications (Ahmad et al., 2023; Al-Khedhairy & Wahab, 2022). Suspension cells were harvested through centrifugation. The adherent cells were released from their substrate by scraping or trypsinization. The cells were resuspended by spinning the 96-well plates at 1000 RPM at 4°C for 5 minutes in a microplate-compatible centrifuge, then gently aspirating the media. Each well received 50 µL of serum-free medium and 50 µL of MTT solution. The plate was then incubated at 37 °C for about 3 hours. After adding 150 µL of MTT solvent to each well, the plate was sealed in foil and agitated with an orbital shaker for about 15 minutes. After an hour, the absorbance was measured at 590 nm.

The measurement of growth inhibition was calculated using the formula below:

Percentage cell viability = 100-(Ae - Ab) ÷ (Ac - Ab) x100

Where,

Ae (the sample's absorbance value)

Ab (the blank's absorbance value)

Ac (control absorbance value)

Preparation of Phosphate Buffer

To prepare a 0.2 M potassium dihydrogen phosphate solution, 27.3g of KH_2PO_4 was weighed and then dissolved in 1000ml of deionized water. After this, 8g of NaOH was dissolved in another conical flask with deionized water to make a 1000 ml solution of sodium hydroxide (0.2 M). Now, 250ml of KH_2PO_4 and about 110ml of NaOH solution were combined into a 1000ml beaker from

the above-mentioned prepared solution, and the required volume (1000ml) was created by adding deionized water. The pH measuring equipment was used to determine and modify the pH (6.9) accordingly (Prietzel et al., 2016)

Anti-oxidant activity by DPPH assay

Using the scavenging (inhibition) activity of the stable DPPH free radical as a measure to assess the antioxidant capacity of plant extracts. To prepare a 100 µL solution of DPPH, 1 milligram in 25 mL of methanol was dissolved (Parveen & Akhtar, 2013). To determine the IC_{50} value, multiple dilutions were made. 10 µL of the sample solution (Hydro-ethanol extract 500 µg/ml, AgNPs 500 µg/ml) and 90 µL of the DPPH solution were added to a 96 well microplate. The 96 microplates were then incubated at 37^{o}C for half an hour. After incubation, these samples were evaluated at a wavelength of 517nm with the use of a microplate reader. There were three measurements of absorbance that were made and compared to the reference standard (Jain et al., 2008).

The following formula was used to calculate the actual % inhibition:

DPPH (%) = [(A 0 -A s)/A 0]100%

Inhibitory percentage (%) = (Absorbance of Control - Absorbance of Test) / Absorbance of Control × 100

Absorbance of the test = Radical effect of the test compound

Absence of control = Total radical effect without the presence of any inhibitor

Alpha-amylase Enzyme Inhibition

In this investigation, a modified version of the approach described by Nickavar and Yousefian (2009) is employed, which was initially suggested by Bernfeld (1955). One milligram of alpha-amylase was dissolved in 100 milliliters of 20 mM phosphate buffer at pH 6.9 to create the enzyme solution. Concentrations ranging from 500µg/ml to 31.3µg/ml were used to generate the DMSO sample solutions for the AgNPs and plant extract fractions. As a substrate, a starch solution (0.5 percent w/v) was made by boiling the potato for 15 minutes in distilled water. The coloring reagent was a DNS solution containing 20 mL of 96mM dinitro salicylic acid, sodium potassium tartrate (12 g), as well as 8 mL of 2M sodium hydroxide, with 12 mL of deionized water. 0.5 mL of plant sample (AgNPs/Extract) and 0.5 mL of enzyme solution were incubated for 30 minutes. One milliliter of starch solution was added, and the mixture was then incubated for three more minutes at 25°C. It was then heated for 15 minutes at 85°C. with 1 mL of DNS solution included in the blend. The liquid was then diluted with distilled water (9 mL) after it had been allowed to cool in the test tube. After thoroughly mixing the mixture, the absorbance at 540 nm was measured. The procedure was the same for making the blank, with the exception that a basic DNS solution was added before the starch solution was added. Acarbose 250 µg/ml was taken as a standard (positive control), while DMSO was employed as a control. The actual enzyme inhibition percentage was determined using the following equation: % Inhibition = [(Ac – As) /Ac] x 100

The sample and absorbances of the control are represented by As and Ac in the above formula. An exponential regression curve was obtained by plotting the percent inhibition against the concentration of each sample to compute the IC50 (the sample concentration necessary to inhibit an enzyme by 50 %) value (All et al., 2014).

Alpha-glucosidase Enzyme Inhibition

In this investigation, the inhibitory activity of alpha-glucosidase was evaluated using the proven methodology (Nair et al., 2013). Ethanol extract (500 µg/ml) and AgNPs (500 µg/ml) were dissolved in DMSO/Methanol, centrifuged or sonicated for 10 min, and filtered through 0.2 µm filters. Then it was put in an Eppendorf tube for the analysis. For the purposes of this study, 10 µL of the testing sample was incubated for 15 minutes at 30°C in a 96-well microplate containing

70 µL of 0.1 M phosphate buffer (100 mM), pH 6.8, and 10 µL of alpha-glucosidase (0.5 unit/mL). Following pre-read, 10µL of PNPG (p-Nitrophenyl—D- glucopyranoside) substrate solution was then added and incubated for another 30 mins. Now measure the absorbance at 410 nm after adding 3 ml of 50 mM sodium hydroxide to this mixture. An HT BioTek microplate reader equipped with an ELISA spectrophotometer was used to measure the samples' absorbance. To serve as a control, the reaction system was employed with no sample (just methanol or DMSO), whereas the acarbose solution (250 µg/ml) served as a positive control/standard. The blank was made the same way, except using a 40ul buffer solution rather than an extract solution. Each procedure was carried out in triplicate to ensure accuracy. The following equation was used to compute the enzyme inhibitory values of the samples: % inhibition= (Ac−As)/(Ac) × 100, where Ac = Absorbance of control and As = Absorbance of sample.

Results

Percentage yield (%)

The yield of AgNPs (in percent) was established using the known weight (mass) of AgNO3 and the crude extract.

Table 2 Yield (%) of different formulations of silver nanoparticles

Ag-NPs Formulations	AgNO3 (0.01M)	Plant extract (mg)	%Age yield
1	20	20	49%
2	20	30	61%
3	20	40	74%

Formulation 3 had the highest percentage yield. A higher concentration of *Grewia asiatica* leaf extract results in an increased presence of functional groups, thereby enhancing the reduction of silver. This large quantity of reducing agents causes silver nanoparticle aggregation. As a result, bigger nanoparticles are synthesized, increasing the yield.

Anticancer activity

Antineoplastic study on Breast Cancer Cells (MCF-7)

The table no 2 below illustrates the anti-cancer effects of silver nanoparticles on MCF-7 cancer cells. Taxol, a chemotherapeutic drug used as a positive control, demonstrated a suppression of tumor growth of 89%. The cytotoxicity of 200 µg/ml GA.AgNPs was 67.9%, close to the positive control. GA.AgNPs at 10 µg /ml, 25 µg /ml, 50 µg /ml, and 100 µg /ml had 13.2%, 39.6%, 45.5%, and 57.1% cytotoxic activity, as shown in Figure 3. 73 µg/ml was the IC50 for GA.Ag nanoparticles. This study shows that silver nanoparticles have an anticancer effect against this cancer cell line.

Table 3 *Cytotoxic activity of Grewia asiatica silver nanoparticles on MCF-7 cell line*

Sr.no	Concentration µg/ml	Percentage cell viability	Inhibition percentage
1.	Positive control	11%	89%
2.	200µg	32.1%	67.9%
3.	100µg	42.8%	57.1%
4.	50µg	54.4%	45.5%
5.	25µg	60.4%	39.6%
6.	10µg	86.7%	13.2%

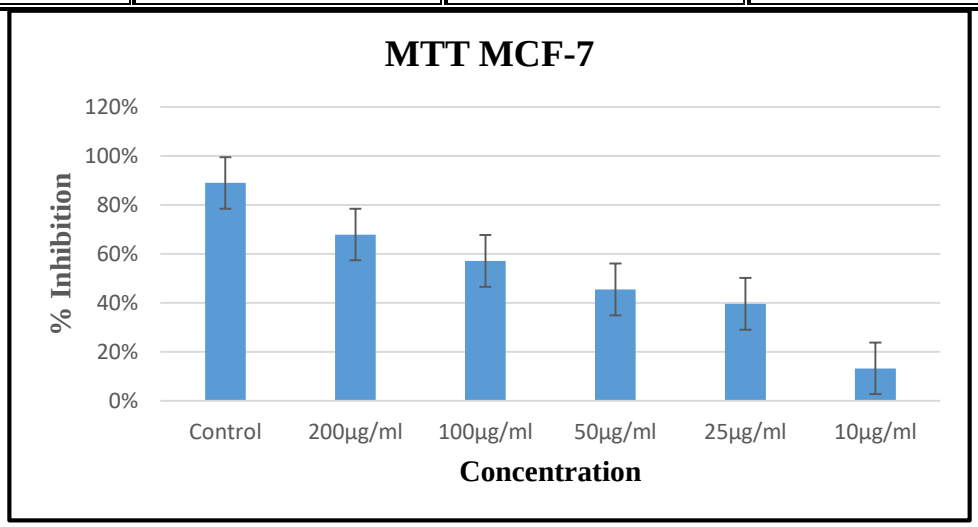

Figure 3 Evaluations of cytotoxicity in MCF-7 using the MTT assay

Antineoplastic study on cervical cancer cells (HeLa)

The anti-cancer effects of silver nanoparticles on HeLa cancer cells are seen in Table 3. The drug Taxol (positive control) has demonstrated a tumor inhibition rate of 93.6%. *Grewia asiatica* silver nanoparticles showed cytotoxicity of 62.3 percent at a 200 µg/ml concentration, which was slightly comparable to the positive control. Further GA.AgNPs demonstrated cytotoxic action at concentrations of 10 µg /ml, 25 µg /ml, 50 µg /ml, and 100 µg /ml, with respective percentages of 9.4 percent, 21.5 percent, 35.8 percent, and 50.1 percent as shown in Figure 4. IC50 value for GA.Ag nanoparticles have been calculated as 99.8 µg/ml. These findings demonstrate that being exposed to silver nanoparticles leads to a dose-dependent enhancement of anticancer effects, as well as a change in the morphology of several cell types due to cell death.

Table 4 *Cytotoxic activity of Grewia asiatica silver nanoparticles on HeLa cell line*

Sr.no	Concentration µg/ml	Percentage cell viability	Inhibition percentage
1.	Positive control	6.4%	93.6%
2.	200µg	37.6%	62.3%
3.	100µg	49.9%	50.1%
4.	50µg	64.2%	35.8%
5.	25µg	78.4%	21.5%
6.	10µg	90.6%	9.4%

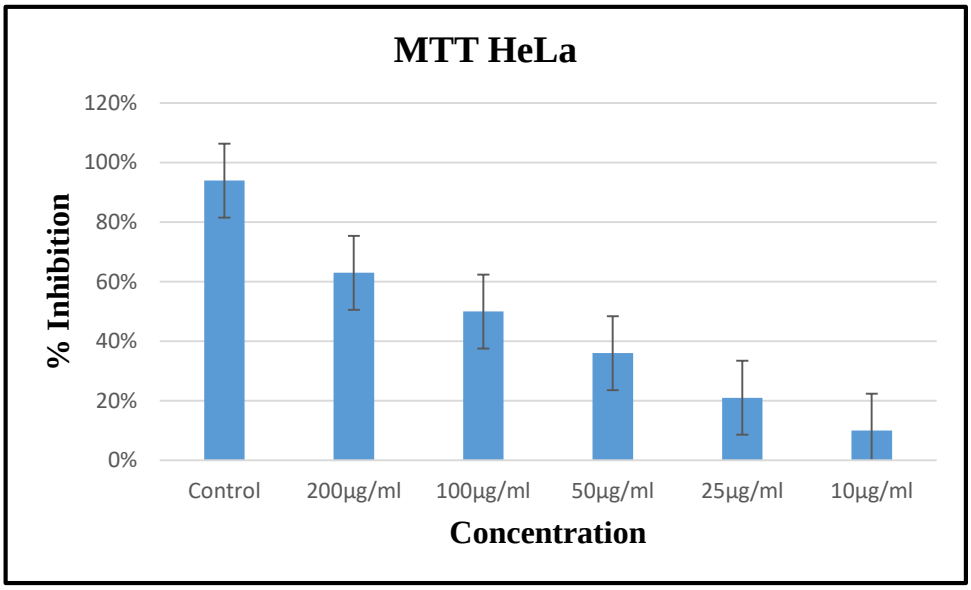

Figure 4 Evaluations of cytotoxicity in HeLa cell line using the MTT assay

Antineoplastic study on liver cancer cells (HepG-2)

The anti-cancer effects of silver nanoparticles on MCF-7 cancer cells are illustrated in the table below. Taxol, a chemotherapeutic drug used as a positive control, demonstrated a suppression of tumor growth of 93.6%. The cytotoxicity of 200 µg/ml GA.AgNPs was 53.4%. GA.AgNPs at 10 µg /ml, 25 µg /ml, 50 µg /ml, and 100 µg /ml had 2.3%, 14.9%, 25.5%, and 37.1% cytotoxic activity as shown in Figure 5. IC50 value for GA.Ag nanoparticles have been calculated as 177.3 µg/ml.

Table 5 *Cytotoxic activity of Grewia asiatica silver nanoparticles on HepG2 cell line*

Sr.no	Concentration µg/ml	Percentage cell viability	Inhibition percentage
1.	Positive control	6.4%	93.6%
2.	200µg	46.6%	53.4%
3.	100µg	62.9%	37.1%
4.	50µg	74.4%	25.5%
5.	25µg	85.1%	14.9%
6.	10µg	97.6%	2.3%

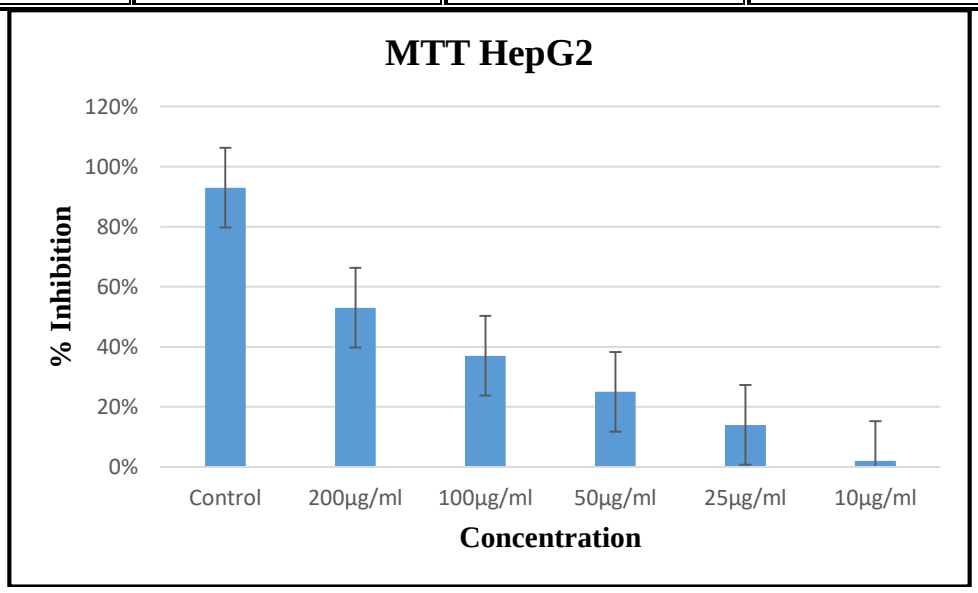

Figure 5 Evaluations of cytotoxicity in HepG2 cell line using the MTT assay

Antioxidant activity as a DPPH scavenger

During the process of the investigation, the crude ethanolic extract's antioxidant capacity, GA.AgNPs and four additional concentrations of both samples (250, 125, 62.5 and 31.3µg/ml) were examined. Standard ascorbic acid, *Grewia asiatica* hydro-ethanolic extract and GA.AgNPs of these compounds were shown to be effective in changing the DPPH colour from purple to yellow (shown in Figure 6). Quantitative measurement of this colour shift was made using an ELISA kit. The findings demonstrate the colour change of the DPPH solution to yellow, showing a high amount of antioxidant activity shown by the crude extracts as well as nanoparticles (GA.AgNPs) against DPPH. The extract inhibits DPPH 78.68 percent at a dose of 500 µg/ml and GA.AgNPs 81.62% at a dose of 500µg/ml. Table 5 contains the outcomes of the study and Figure 7 depicts a comparison of standard ascorbic acid (250 µg/ml) with *Grewia asiatica* extract and nanoparticles.

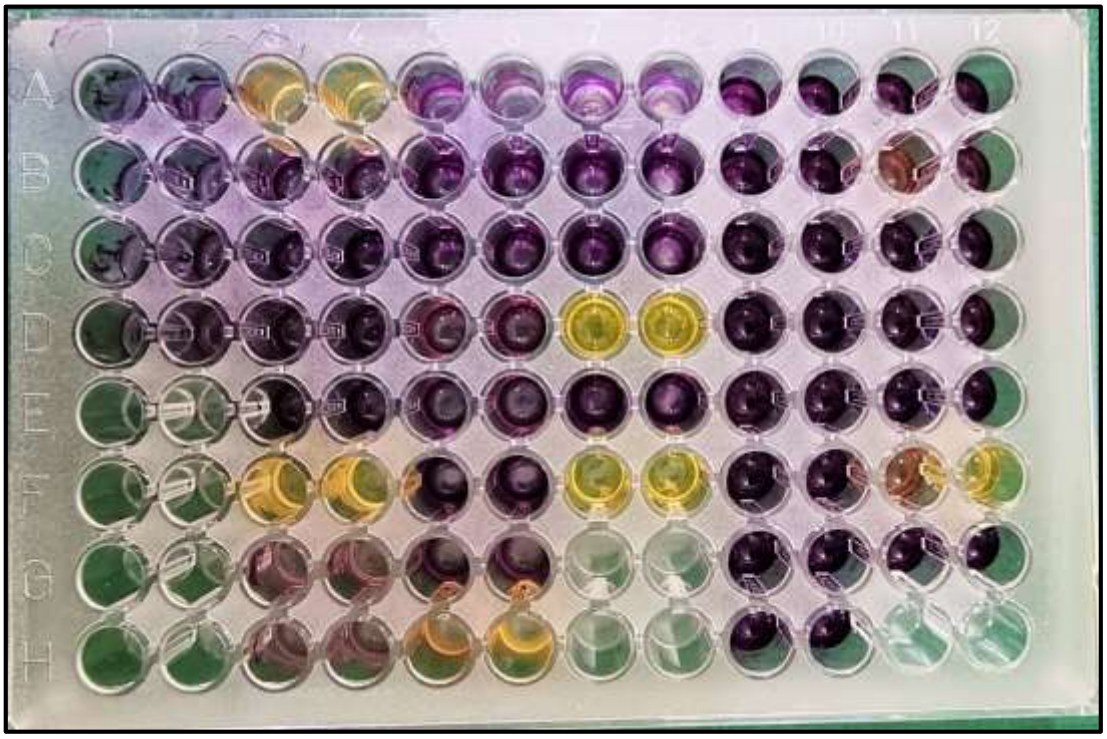

Figure 6 Color shift in DPPH assay using 96 microwell plates

Table 6 Antioxidant activity and IC50 value of Grewia asiatica and its synthesized nanoparticles

Sr. No.	Samples	Concentration	% Inhibition	IC_{50}(µg/ml)
1	*Grewia asiatica*	500µg/ml	78.68 ± 0.02[b]	68.24 ± 0.3
2	GA. AgNPs	500µg/ml	81.62 ± 0.02[c]	61.57 ± 0.4
3	Ascorbic acid	250µg/ml	93.74 ± 0.12[a]	44.78 ± 0.9

Superscript a, b, and c showed that mean± standard deviations in the same column with different superscript are significantly different ($p < 0.05$). Values are mean (n=3) ± standard deviation

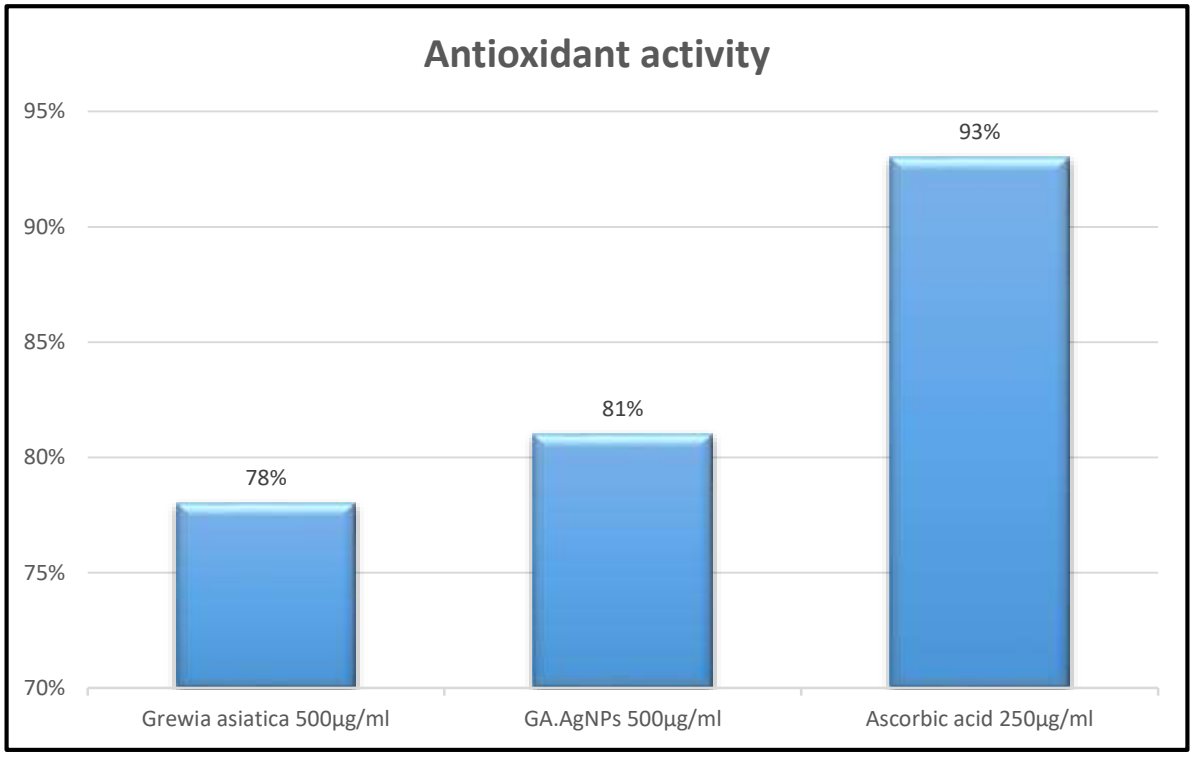

Figure 7 Graph of the anti-oxidant property of extract and nanoparticle as compared to control.

4.4. Alpha-amylase enzyme inhibition

Alpha-amylase inhibitory activity of hydro-ethanolic extract and nanoparticles at various concentrations is investigated in this study. At 500µg/ml plant extract showed 71 percent inhibition, while at 500µg/ml, GA.AgNPs exhibit 75 percent inhibition. This activity demonstrates that at various concentrations, different samples exhibit varying percentage inhibition of alpha-amylase, with both extract and nanoparticles exhibiting a comparable percentage of inhibition of alpha-amylase compared with the standard drug acarbose (250µg/ml).

Table 7 Alpha amylase inhibitory activity and IC50 value of Grewia asiatica and its synthesized nanoparticles

Sr. No.	Samples	Concentration	% Inhibition	IC_{50}(µg/ml)
1	*Grewia asiatica*	500µg/ml	71.11± 0.05^{a}	74.9 ± 0.1
2	GA. AgNPs	500µg/ml	75.15± 0.01^{b}	69.5 ± 0.4
3	Acarbose	250µg//ml	79.18 ± 0.07^{c}	42.8 ± 0.2

Superscript a, b, and c showed that mean± standard deviations in the same column with different superscript are significantly different ($p < 0.05$). Values are mean (n=3) ± standard deviation

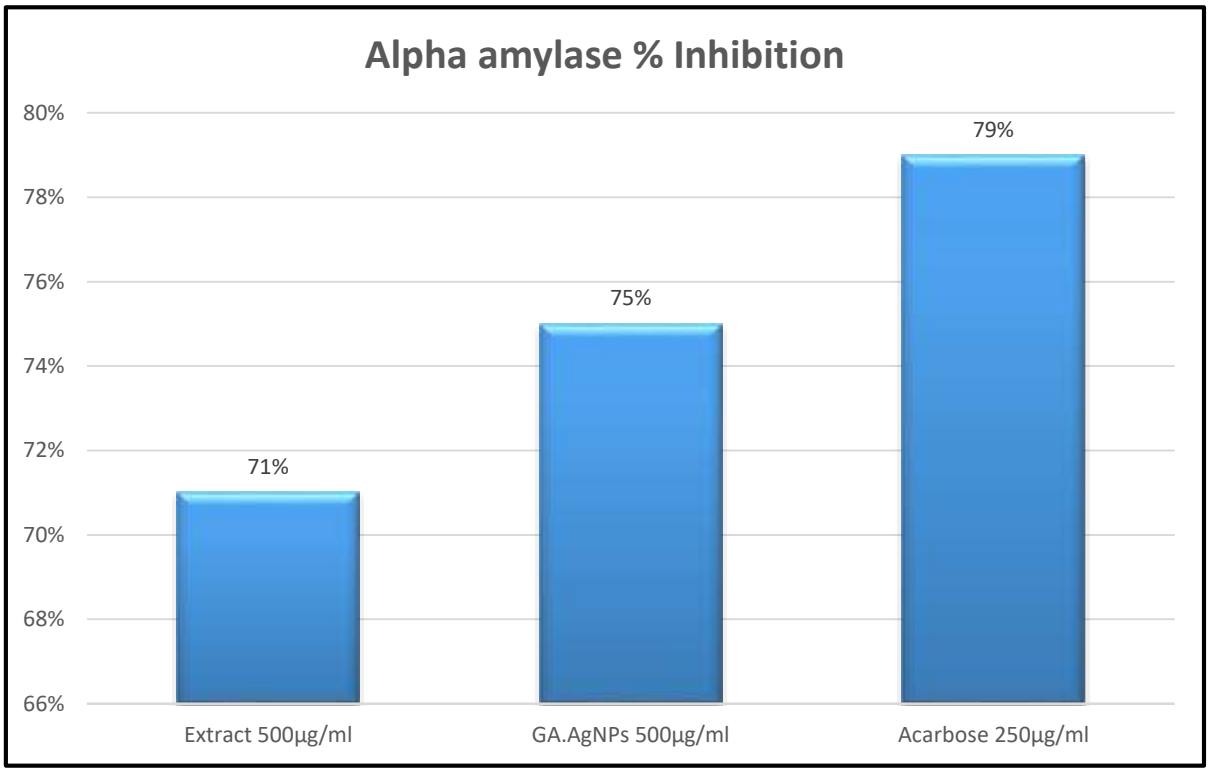

Figure 8 The graph shows the extract and nanoparticle inhibition of the Alpha amylase enzyme compared to the control.

Alpha-glucosidase enzyme inhibition

The alpha-glucosidase inhibitory assay was performed following literature protocols. Various concentrations of hydro-ethanolic extract and nanoparticles were assessed for their capacity to hinder the α-glucosidase enzyme in this investigation. At 500µg/ml, there is an 82.14 percent inhibition by crude extract. Whereas GA.AgNPs sample at 500µg/ml showed better results than those obtained with the extract. A total of 85.03 percent inhibition is shown at this concentration. According to the results of this activity, different samples display diverse percentage inhibition of alpha-glucosidase at different doses, with both extract and nanoparticles demonstrating a high percentage suppression of alpha-glucosidase when compared to the standard medicine acarbose.

Table 8 Alpha-glucosidase inhibitory activity and IC50 value of Grewia asiatica and its synthesized nanoparticles

Sr. No.	Samples	Concentration	% Inhibition	IC_{50}(µg/ml)
1	*Grewia asiatica*	500µg/ml	82.14 ± 0.01[a]	54.8 ± 0.13
2	GA. AgNPs	500µg/ml	85.03 ± 0.02[b]	48.5 ± 0.5
3	Acarbose	250µg/ml	89.16 ± 0.05[c]	38.2 ± 1.7

Superscript a, b, and c showed that mean± standard deviations in the same column with different superscripts are significantly different ($p < 0.05$). Values are mean (n=3) ± standard deviation

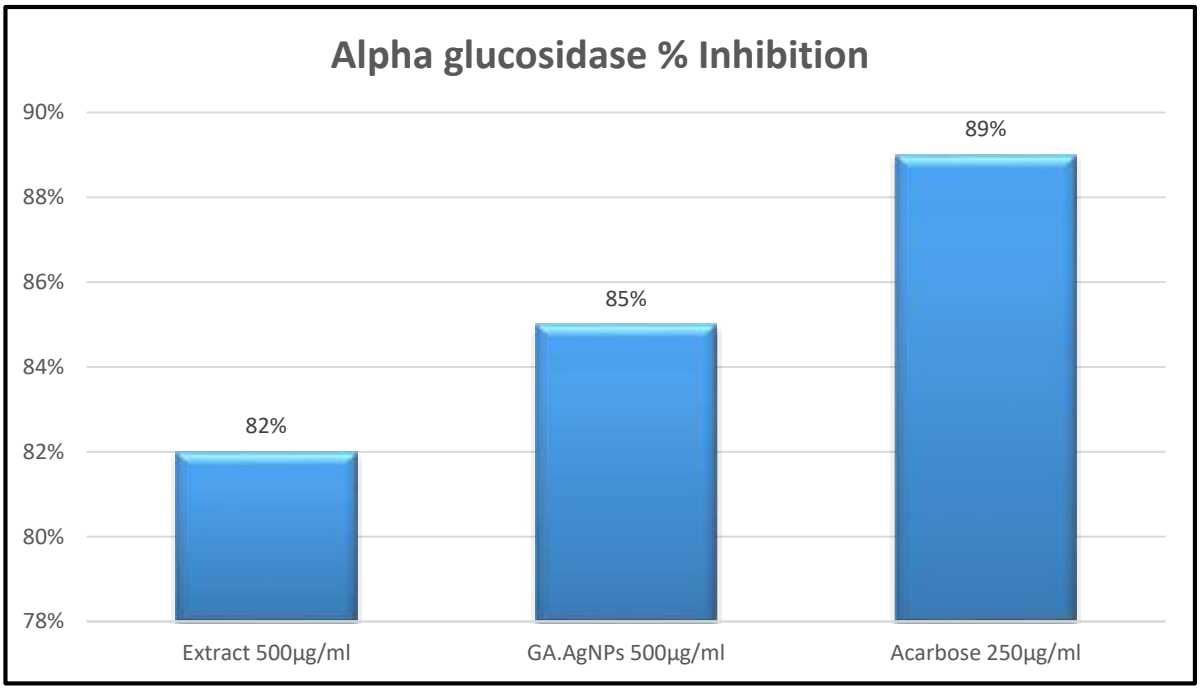

Figure 9: The graph shows the extract and nanoparticle inhibition of the alpha-glucosidase enzyme compared to the control.

Discussion

In the current study, anticancer, antioxidant, and antidiabetic potential of *G.aciatica* leaves extract and GA.AgNPs were evaluated. The findings suggest that GA.AgNPs are more potent in a dose-specific manner due to the presence of naturally occurring functional chains that inhibit MCF-7, HeLa and HepG2 cells from growing. Consequently, Ag-NPs' anticancer effects are linked to their interactions with intracellular functioning proteins, resulting in cellular modifications. According to our results the cytotoxicity of 200 µg/ml GA.AgNPs against MCF-7 cell line was 67.9 %, close to the positive control (Taxol), which was 89 %. In an earlier investigation carried out by Ali Sharif et al have inhibitory concentrations (IC50) of the *Kalanchoepinnata* n-hexane extract against hormone-dependent breast cancer (MCF-7) carcinoma cell lines were 75.7 g/mL (Sharif et al., 2017). *G. asiatica* silver nanoparticles showed cytotoxicity at a 200 µg/ml concentration against HeLa cancer cell line 62.3 %, which was comparable to the positive control (Taxol 93%). Another research found that at a dosage of 5 mg/mL, the MTT test demonstrated 100% activity of *Avicennia alba* methanol extract in HeLa cells (Eswaraiah et al., 2020). Similarly GA.AgNPs at a 200 µg/ml concentration exhibited a cytotoxicity against the HeLa cancer cell line 62.3 %, which was comparable to the positive control (Taxol 93 %) in previously study, which was carried out by Azeem MNA (Azeem et al., 2022). The studied biogenic GA.AgNPs showed an improvement in the DPPH scavenging. According to the FTIR and phytochemical analysis reported in our previous study (Ateeb et al., 2023), flavonoids and phenols derived from *G. asiatica*crude extract may be attributed to the antioxidant capacity of GA.AgNPs. Hence, the radical scavenging by the electron supply was the source of the decline in DPPH radical absorbance. According to the current study's findings, all treatments, including *G. asiatica* extract, GA.AgNPs and standard ascorbic acid significantly block DPPH radical recording, with respective values of 78 %, 81 %, and 93 %. Similar results were also observed in a study conducted by Manal N. Abdel Azeem et al (Azeem et al.,

2022). *G. asiatica* 500 µg/ml extract showed 71 % and 82 % inhibition of alpha-glucosidase and amylase, respectively, while GA.AgNPs exhibited 75 %, 85% inhibition at the same concentration; the results are significant compared with the standard drug acarbose (250µg/ml), which showed inhibition 79 % and 89%, respectively. In a previous study conducted by Manal N. Abdel Azeem et al in 2022, similar results were obtained for macroalgae-based silver nanoparticles at a dose of 200 µg/mL for *Galaxauraelongata, Turbinariaornata, and Enteromorphafexuosa* respectively (Azeem et al., 2022). The results indicated that these silver nanoparticles had powerful anti-diabetic, antioxidant, and anti-cancerousproperties. Hence, it has been determined that the crude extract of *G.asiatica* plant and its AgNPs may be utilized to treat NCDs and multidrug-resistant illnesses.

Conclusion

Silver nanoparticles were synthesized in this work using a crude extract of the Leaves of the Grewia asiatica plant. These AgNPs were synthesized as antioxidant, anticancer, and anti-diabetic medicines for oral administration. We tested the synthesized nanoparticles for antioxidant activity, anti-cancer activity and enzyme inhibition for antidiabetic activity. The generation of silver nanoparticles in the green synthesis method was verified by colour change and UV-Visible spectroscopy. The greatest absorbance peak was seen at 435 nm, indicating the production of AgNPs. Different concentrations of crude extract were utilized to adjust the formulation based on the particle size. The improved formulation had a yield of 61 percent. The results indicated that these silver nanoparticles had powerful anti-diabetic, antioxidant, and anti-cancerous properties. Hence, it has been determined that the crude extract of Grewia asiatica leaves and its AgNPs may be utilized to treat NCDs and multidrug-resistant illnesses.

Future prospects

The potential of Grewia asiatica and its synthesized nanoparticles to inhibit enzymes, cancer cell lines and free radicals highlighted the plant's potential as a new therapeutic substance. Today, cancer, diabetes, and other dangerous bacterial infections are prevalent around the globe. While a wide range of synthetic medications is available on the market, there is still a need to identify novel chemical compounds with the highest benefit and least harm via exploration of natural sources, particularly plants. Additionally, neurological illnesses, cancer, cardiovascular disease, diabetes, and inflammation are the major problems induced by the generation of reactive oxygen species, which may be mitigated by polyphenolic compounds and flavonoids found in many plants, such as Grewia asiatica. Considering these facts, it is advised that more research be conducted on green nanoparticle synthesis in order to enhance bioavailability and promote natural and cost-effective therapies for a variety of vital ailments. Green nanoparticle synthesis utilizing plant extracts is an easy, one-step biosynthesis approach with tremendous promise for the advancement of biomedicine as a replacement for standard cancer, antibiotic, and antidiabetic therapies. However, we are currently unable to determine the chemical makeup of the plant metabolites engaged in the reduction process because of the vast number of bioactive chemicals found in plant extracts. It is advised that pure chemical components taken from plants be used in the biosynthesis of silver nanoparticles. We also advise using mouse models for in vivo assessment of these nanoparticles.

Acknowledgment

We would like to express our sincere gratitude to the University College of Conventional Medicine, Faculty of Medicine and Allied Health Sciences, The Islamia University of Bahawalpur, Pakistan, for providing lab facilities and moral support to execute this research work.

Conflict of Interest

The authors declared having no conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding: N/A

Author's Contributions

Author HMA designed & supervised the study while MA executed the experiments. Authors ARA and MA analyzed the results and revised the final draft. Authors AH & RZ helped in analyzing FTIR and SEM studies. Authors ARA, RZ, and AH helped in investigations, performed statistical analysis, and wrote the manuscript draft. All authors read and approved the final manuscript**.**

References

Ahmad, H. S., Ateeb, M., Noreen, S., Farooq, M. I., Baig, M. M. F. A., Nazar, M. S., Akhtar, M. F., Ahmad, K., Ayub, A. R., Shoukat, H., Hadi, F., & Madni, A. (2023). Biomimetic synthesis and characterization of silver nanoparticles from Dipterygium glaucum extract and its anti-cancerous activities. *Journal of Molecular Structure*, *1282*, 135196. https://doi.org/10.1016/J.MOLSTRUC.2023.135196

Ahmed, O. M., Hassan, M. A., Abdel-Twab, S. M., & Abdel Azeem, M. N. (2017). Navel orange peel hydroethanolic extract, naringin and naringenin have anti-diabetic potentials in type 2 diabetic rats. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*, *94*, 197–205. https://doi.org/10.1016/J.BIOPHA.2017.07.094

Ahmed, O. M., Soliman, H. A., Mahmoud, B., & Gheryany, R. R. (2017). Ulva lactuca hydroethanolic extract suppresses experimental arthritis via its anti-inflammatory and antioxidant activities. *Beni-Suef University Journal of Basic and Applied Sciences*, *4*(6), 394–408. https://doi.org/10.1016/J.BJBAS.2017.04.013

Al-Khedhairy, A. A., & Wahab, R. (2022). Silver Nanoparticles: An Instantaneous Solution for Anticancer Activity against Human Liver (HepG2) and Breast (MCF-7) Cancer Cells. *Metals 2022, Vol. 12, Page 148*, *12*(1), 148. https://doi.org/10.3390/MET12010148

Alhmoud, J. F., Woolley, J. F., Al Moustafa, A. E., & Malki, M. I. (2020). DNA Damage/Repair Management in Cancers. *Cancers*, *12*(4). https://doi.org/10.3390/CANCERS12041050

All, L., All, L., Ahmed, D., Younas, S., Mumtaz, Q., & Mughal, A. (2014). *Study of alpha-amylase and urease inhibitory activities of Melilotus indicus Study of alpha-amylase and urease inhibitory activities of Melilotus*. *January*.

Anand, P., Kunnumakara, A. B., Sundaram, C., Harikumar, K. B., Tharakan, S. T., Lai, O. S., Sung, B., & Aggarwal, B. B. (2008). Cancer is a Preventable Disease that Requires Major Lifestyle Changes. *Pharmaceutical Research*, *25*(9), 2097. https://doi.org/10.1007/S11095-008-9661-9

Ateeb, M., Asif, H. M., Ali, T., & Mahmood, M. (2023). Photocatalytic and Antibacterial activities of bio- synthesised silver nanoparticles (AgNPs) using Grewia asiatica leaves extract. *International Journal of Environmental Analytical Chemistry*, *00*(00), 1–19. https://doi.org/10.1080/03067319.2022.2158328

Azeem, M. N. A., Ahmed, O. M., Shaban, M., & Elsayed, K. N. M. (2022). In vitro antioxidant, anticancer, anti-inflammatory, anti-diabetic and anti-Alzheimer potentials of innovative macroalgae bio-capped silver nanoparticles. *Environmental Science and Pollution Research International*. https://doi.org/10.1007/S11356-022-20039-X

Badawi, A. K., Abd Elkodous, M., & Ali, G. A. M. (2021). Recent advances in dye and metal ion

removal using efficient adsorbents and novel nano-based materials: an overview. *RSC Advances*, *11*(58), 36528–36553. https://doi.org/10.1039/D1RA06892J

Barani, M., Rahdar, A., Sargazi, S., Amiri, M. S., Sharma, P. K., & Bhalla, N. (2021). Nanotechnology for inflammatory bowel disease management: Detection, imaging and treatment. *Sensing and Bio-Sensing Research*, *32*, 100417. https://doi.org/10.1016/J.SBSR.2021.100417

Bernatoniene, J., & Kopustinskiene, D. M. (2018). The Role of Catechins in Cellular Responses to Oxidative Stress. *Molecules 2018, Vol. 23, Page 965*, *23*(4), 965. https://doi.org/10.3390/MOLECULES23040965

Cade, W. T. (2008). Diabetes-Related Microvascular and Macrovascular Diseases in the Physical Therapy Setting. *Physical Therapy*, *88*(11), 1322. https://doi.org/10.2522/PTJ.20080008

Chandrasekaran, S., Anusuya, S., & Anbazhagan, V. (2022). Anticancer, anti-diabetic, antimicrobial activity of zinc oxide nanoparticles: A comparative analysis. *Journal of Molecular Structure*, *1263*, 133139. https://doi.org/10.1016/J.MOLSTRUC.2022.133139

Dawadi, S., Katuwal, S., Gupta, A., Lamichhane, U., Thapa, R., Jaisi, S., Lamichhane, G., Bhattarai, D. P., & Parajuli, N. (2021). Current Research on Silver Nanoparticles: Synthesis, Characterization, and Applications. *Journal of Nanomaterials*, *2021*. https://doi.org/10.1155/2021/6687290

Diabetes. (n.d.). Retrieved December 20, 2021, from https://www.who.int/news-room/fact-sheets/detail/diabetes

Es-Haghi, A., Taghavizadeh Yazdi, M. E., Sharifalhoseini, M., Baghani, M., Yousefi, E., Rahdar, A., & Baino, F. (2021). Application of Response Surface Methodology for Optimizing the Therapeutic Activity of ZnO Nanoparticles Biosynthesized from Aspergillus niger. *Biomimetics*, *6*(2). https://doi.org/10.3390/BIOMIMETICS6020034

Eswaraiah, G., Peele, K. A., Krupanidhi, S., Kumar, R. B., Venkateswarulu, T. C., Eswaraiah, G., Peele, K. A., Krupanidhi, S., Kumar, R. B., & Venkateswarulu, T. C. (2020). Identification of bioactive compounds in leaf extract of Avicennia alba by GC-MS analysis and evaluation of its in-vitro anticancer potential against MCF7 and HeLa cell lines. *Journal of King Saud University – Science*, *32*(1), 740–744. https://doi.org/10.1016/J.JKSUS.2018.12.010

Ghosh, S., More, P., Nitnavare, R., Jagtap, S., Chippalkatti, R., Derle, A., Kitture, R., Asok, A., Kale, S., Singh, S., Shaikh, M. L., Ramanamurthy, B., Bellare, J., & Chopade, B. A. (2015). Antidiabetic and Antioxidant Properties of Copper Nanoparticles Synthesized by Medicinal Plant Dioscorea bulbifera. *Journal of Nanomedicine & Nanotechnology*, *0*(0), 0–0. https://doi.org/10.4172/2157-7439.S6-007

Gorrini, C., Harris, I. S., & Mak, T. W. (2013). Modulation of oxidative stress as an anticancer strategy. *Nature Reviews Drug Discovery 2013 12:12*, *12*(12), 931–947. https://doi.org/10.1038/nrd4002

Hassanisaadi, M., Barani, M., Rahdar, A., Heidary, M., Thysiadou, A., & Kyzas, G. Z. (2022). Role of agrochemical-based nanomaterials in plants: biotic and abiotic stress with germination improvement of seeds. *Plant Growth Regulation*, *97*(2), 375–418. https://doi.org/10.1007/S10725-021-00782-W

Hassanisaadi, M., Bonjar, G. H. S., Rahdar, A., Pandey, S., Hosseinipour, A., & Abdolshahi, R. (2021). Environmentally Safe Biosynthesis of Gold Nanoparticles Using Plant Water Extracts. *Nanomaterials 2021, Vol. 11, Page 2033*, *11*(8), 2033. https://doi.org/10.3390/NANO11082033

Hu, F., Sun, D. S., Wang, K. L., & Shang, D. Y. (2022). Nanomedicine of Plant Origin for the Treatment of Metabolic Disorders. *Frontiers in Bioengineering and Biotechnology*, *9*, 1466.

https://doi.org/10.3389/FBIOE.2021.811917/BIBTEX

Jain, A., Soni, M., Deb, L., Jain, A., Rout, S. P., Gupta, V. B., & Krishna, K. L. (2008). Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of Momordica dioica Roxb. leaves. *Journal of Ethnopharmacology*, *115*(1), 61–66. https://doi.org/10.1016/J.JEP.2007.09.009

Jamkhande, P. G., Ghule, N. W., Bamer, A. H., & Kalaskar, M. G. (2019). Metal nanoparticles synthesis: An overview on methods of preparation, advantages and disadvantages, and applications. *Journal of Drug Delivery Science and Technology*, *53*, 101174. https://doi.org/10.1016/J.JDDST.2019.101174

Kaniyala Melanthota, S., Yury, ·, Kistenev, V., Borisova, E., Ivanov, D., Zakharova, O., Boyko, A., Denis Vrazhnov, ·, Dharshini Gopal, ·, Chakrabarti, S., Prasada, S., & Mazumder, N. (2022). Types of spectroscopy and microscopy techniques for cancer diagnosis: a review. *Lasers in Medical Science 2022*, 1–18. https://doi.org/10.1007/S10103-022-03610-3

Kharroubi, A. T., & Darwish, H. M. (2015). Diabetes mellitus: The epidemic of the century. *World Journal of Diabetes*, *6*(6), 850. https://doi.org/10.4239/WJD.V6.I6.850

lakshmi, J. B. (2022). "Evaluation of Antidiabetic Activity of Aqueous Extract of Bark of Pterocarpus Marsupium Silver Nanoparticles Against Streptozotocin and Nicotinamide Induced Type 2 Diabetes in Rats." *Biomedical Journal of Scientific & Technical Research*, *43*(1). https://doi.org/10.26717/BJSTR.2022.43.006853

Lan Chi, N. T., Narayanan, M., Chinnathambi, A., Govindasamy, C., Subramani, B., Brindhadevi, K., Pimpimon, T., & Pikulkaew, S. (2022). Fabrication, characterization, anti-inflammatory, and anti-diabetic activity of silver nanoparticles synthesized from Azadirachta indica kernel aqueous extract. *Environmental Research*, *208*, 112684. https://doi.org/10.1016/J.ENVRES.2022.112684

Mahmoud, A. M., Ahmed, O. M., Mohamed, I. B., Soliman, H. A., & Mohamed, B. M. (2021). The Preventive Effects and Mode of Actions of Ulva Fasciata Synthesized Silver Nanoparticles in Doxorubicin-Induced Hepatotoxicity in Wistar Rats. *Journal of Pharmaceutical Research International*, *33*(24A), 24–48. https://doi.org/10.9734/JPRI/2021/V33I24A31429

Mauricio, M. D., Guerra-Ojeda, S., Marchio, P., Valles, S. L., Aldasoro, M., Escribano-Lopez, I., Herance, J. R., Rocha, M., Vila, J. M., & Victor, V. M. (2018). Nanoparticles in medicine: A focus on vascular oxidative stress. *Oxidative Medicine and Cellular Longevity*, *2018*. https://doi.org/10.1155/2018/6231482

Meyts, P. De. (2016). The Insulin Receptor and Its Signal Transduction Network. *Endotext*.

Nagaraja, S., Ahmed, S. S., R., B. D., Goudanavar, P., M., R. K., Fattepur, S., Meravanige, G., Shariff, A., Shiroorkar, P. N., Habeebuddin, M., & Telsang, M. (2022). Green Synthesis and Characterization of Silver Nanoparticles of Psidium guajava Leaf Extract and Evaluation for Its Antidiabetic Activity. *Molecules 2022, Vol. 27, Page 4336*, *27*(14), 4336. https://doi.org/10.3390/MOLECULES27144336

Nair, S. S., Kavrekar, V., & Mishra, A. (2013). *In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts*. *3*(1), 128–132.

Oves, M., Ahmar Rauf, M., Aslam, M., Qari, H. A., Sonbol, H., Ahmad, I., Sarwar Zaman, G., & Saeed, M. (2022). Green synthesis of silver nanoparticles by Conocarpus Lancifolius plant extract and their antimicrobial and anticancer activities. *Saudi Journal of Biological Sciences*, *29*(1), 460–471. https://doi.org/10.1016/J.SJBS.2021.09.007

Parveen, R., & Akhtar, N. (2013). In vitro evaluation of antioxidant activity of phytochemical extracts from different varieties of various plants. *Asian Journal of Chemistry*, *25*(18), 10561–10564. https://doi.org/10.14233/ajchem.2013.15924

Perry, S. W., Norman, J. P., Barbieri, J., Brown, E. B., & Gelbard, H. A. (2011). Mitochondrial membrane potential probes and the proton gradient: a practical usage guide. *BioTechniques*, *50*(2), 98–115. https://doi.org/10.2144/000113610

Perumalsamy, R., & Krishnadhas, L. (2022). Anti-Diabetic Activity of Silver Nanoparticles Synthesized from the Hydroethanolic Extract of Myristica fragrans Seeds. *Applied Biochemistry and Biotechnology*, *194*(3), 1136–1148. https://doi.org/10.1007/S12010-022-03825-8/TABLES/2

Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. *Indian Journal of Clinical Biochemistry*, *30*(1), 11. https://doi.org/10.1007/S12291-014-0446-0

Prietzel, J., Harrington, G., Häusler, W., Heister, K., Werner, F., & Klysubun, W. (2016). Reference spectra of important adsorbed organic and inorganic phosphate binding forms for soil P speciation using synchrotron-based K-edge XANES spectroscopy. *Journal of Synchrotron Radiation*, *23*(2), 532–544. https://doi.org/10.1107/S1600577515023085/HF5306SUP1.PDF

Satalkar, P., Elger, B. S., & Shaw, D. M. (2015). Defining Nano, Nanotechnology and Nanomedicine: Why Should It Matter? *Science and Engineering Ethics 2015 22:5*, *22*(5), 1255–1276. https://doi.org/10.1007/S11948-015-9705-6

Servat-Medina, L., González-Gómez, A., Reyes-Ortega, F., Sousa, I. M. O., Queiroz, N. de C. A., Zago, P. M. W., Jorge, M. P., Monteiro, K. M., de Carvalho, J. E., San Román, J., & Foglio, M. A. (2015). Chitosan–tripolyphosphate nanoparticles as Arrabidaea chica standardized extract carrier: synthesis, characterization, biocompatibility, and antiulcerogenic activity. *International Journal of Nanomedicine*, *10*, 3897. https://doi.org/10.2147/IJN.S83705

Shah, M., Fawcett, D., Sharma, S., Tripathy, S. K., & Poinern, G. E. J. (2015). Green Synthesis of Metallic Nanoparticles via Biological Entities. *Materials*, *8*(11), 7278. https://doi.org/10.3390/MA8115377

Sharif, A., Akhtar, M. F., Akhtar, B., Saleem, A., Manan, M., Shabbir, M., Ashraf, M., Peerzada, S., Ahmed, S., & Raza, M. (2017). Genotoxic and cytotoxic potential of whole plant extracts of Kalanchoe laciniata by Ames and MTT assay. *EXCLI Journal*, *16*, 593. https://doi.org/10.17179/EXCLI2016-748

Suganthy, N., & Pandima Devi, K. (2016). In vitro antioxidant and anti-cholinesterase activities of Rhizophora mucronata. *Pharmaceutical Biology*, *54*(1), 118–129. https://doi.org/10.3109/13880209.2015.1017886

Sultana, S., Akhtar, N., & Asif, H. M. (2013). Phytochemical screening and antipyretic effects of hydro-methanol extract of Melia azedarach leaves in rabbits. *Undefined*, *8*(2), 214–217. https://doi.org/10.3329/BJP.V8I2.14708

Tao, Z., Shi, A., & Zhao, J. (2015). Epidemiological Perspectives of Diabetes. *Cell Biochemistry and Biophysics 2015 73:1*, *73*(1), 181–185. https://doi.org/10.1007/S12013-015-0598-4

Tran, N., Pham, B., & Le, L. (2020). Bioactive Compounds in Anti-DiabetiAc Plants: From Herbal Medicine to Modern Drug Discovery. *Biology 2020, Vol. 9, Page 252*, *9*(9), 252. https://doi.org/10.3390/BIOLOGY9090252

Veeragoni, D., Deshpande, S., Rachamalla, H. K., Ande, A., Misra, S., & Mutheneni, S. R. (2022). In Vitro and in Vivo Anticancer and Genotoxicity Profiles of Green Synthesized and Chemically Synthesized Silver Nanoparticles. *ACS Applied Bio Materials*. https://doi.org/10.1021/ACSABM.2C00149/ASSET/IMAGES/LARGE/MT2C00149_0007.JPEG

Veiseh, O., Tang, B. C., Whitehead, K. A., Anderson, D. G., & Langer, R. (2014). Managing

diabetes with nanomedicine: challenges and opportunities. *Nature Reviews Drug Discovery 2014 14:1*, *14*(1), 45–57. https://doi.org/10.1038/nrd4477

Xu, L., Wang, Y. Y., Huang, J., Chen, C. Y., Wang, Z. X., & Xie, H. (2020). Silver nanoparticles: Synthesis, medical applications and biosafety. *Theranostics*, *10*(20), 8996. https://doi.org/10.7150/THNO.45413

Zheng, Y., Ley, S. H., & Hu, F. B. (2017). Global aetiology and epidemiology of type 2 diabetes mellitus and its complications. *Nature Reviews Endocrinology 2017 14:2*, *14*(2), 88–98. https://doi.org/10.1038/nrendo.2017.151

Zia-Ul-Haq, M., Stanković, M. S., Rizwan, K., & Feo, V. De. (2013). Grewia asiatica L., a Food Plant with Multiple Uses. *Molecules 2013, Vol. 18, Pages 2663-2682*, *18*(3), 2663–2682. https://doi.org/10.3390/MOLECULES18032663

Zubair, M., Azeem, M., Mumtaz, R., Younas, M., Adrees, M., Zubair, E., Khalid, A., Hafeez, F., Rizwan, M., & Ali, S. (2022). Green synthesis and characterization of silver nanoparticles from Acacia nilotica and their anticancer, antidiabetic and antioxidant efficacy. *Environmental Pollution*, *304*, 119249. https://doi.org/10.1016/J.ENVPOL.2022.119249