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Green Synthesis of Silver Nanoparticles using *Azadirachta indica* Seeds Aqueous Extract and Evaluation of their Anti-diabetic Potentials Through in vitro and *in silico* Analysis

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ABSTRACT

Deficiency and futility of insulin lead to a serious metabolic disorder known as Diabetes. It affects the metabolism of carbohydrates, proteins and lipids. Several therapeutic approaches have been developed to overcome such life-threatening diseases, yet the risk is substantial. Echo-friendly silver nanoparticles have been effectively used in multiple ways. For instance, decomposition of harmful contaminants, water refining, sterilization, food conservation, nano-insecticides and cosmetics. In current research, silver nanoparticles were synthesized using aqueous extract of *Azdirachta indica* seeds, characterized using UV-Visible spectroscopy, FT-IR, XRD, SEM, TEM and AFM analysis.These AgNPs were assessed for their anti-diabetic activities using α-amylase inhibitory and α-glucosidase inhibitory assays. In the alpha-amylase inhibitory assay,the lowest % inhibition was noted as 26.6±1.53% at 10 µg/ml and the highest 79.33±2.21% at 100 µg/ml. Whereas acarbose (standard drug) showed % inhibition at 10 µg/ml 30.50±1.53% and that of 100µg/ml 82 ±3%. The percent inhibitory values of alpha-glucosidase at 10µg/ml were noted 25.3±0.6% and at 100 µg/ml 80±1.51%. Whereas Acarbose, revealed %inhibition at10µg/ml 28.6±2.09% and at100µg/ml 83.13±1.26% respectively. For results confirmation, molecular docking was conducted to assess the biological interaction between the silver nanoparticles, α-amylase and α-glucosidase. The docking score of silver nanoparticles against α-amylase and α-glucosidase was found as -5.6164, -5.0013 and that of Acarbose was -6.9441,-7.1956 respectively.The docking score number of interactions of acarbose is relatively high in comparison to the nanoparticle, which is in agreement with the experimental findings. From the current research, it is concluded that the given silver nanoparticles have considerable inhibitory effects on the key enzymes that are involved in diabetes.

INTRODUCTION

Diabetes mellitus is a prolonged disorder initiated by inherited or acquired deficiency in synthesis of insulin by the beta cells of pancreas, or by the uselessness of the synthesized insulin [1].The insufficiency of insulin consequences in high level of glucose in the blood, which cause damage in several systems of the body, especially nervous and circulatory systems [2].The International Diabetes Federation guesses that 536.60 million individuals worldwide had diabetes mellitus in 2021 and this number is likely to cross 783.20 million by 2045. This indicates that the incidence of diabetes continues to rise globally [3]. According to prior estimates from IDF and other surveys, approximately half of diabetes patients are unaware that they have the metabolic disorder [4].

A key obstacle in the prevention and control of diabetes mellitus is changing one's lifestyle by committing to exercise that is more physical, consume fewer carbohydrates, along with giving up inactive behaviors [5]. Plants are an essential component of sophisticated conventional health care for thousands of years; they still provide humans novel methods to heal themselves. Medical plant treatment is based on empirical evidence of hundreds and possibly thousands years of use [6]. Medicines derived from plants have been used for thousands of years. About 25% of the world's top-selling medications in 2001 and 2002 consisted of natural ingredients themselves or derived from them [7].

Azadirachta indica, commonly referred to as neem tree, a widely used medicinal plant in Africa and Asia. It has been

employed for a variety of medicinal uses since ancient times [8]. Due to its abundance of biologically active ingredients, including nimbin, gedunin, azadirachtin, and nimbidin, which have anti-diabetic, anti-inflammatory, antipyretic, antifungal, antibacterial, antimalarial, immunomodulatory, diuretic, and hypoglycemic properties, it is employed in many traditional remedies [9]. The neem tree has been a popular plant in India and its neighboring states for over 3,000 years. One of the best efficient medical plants, it has a wide-ranging of biological potentials and has been used as remedy in more than fifty different countries [10]. Although synthetic drugs such as acarbose and miglitol exhibit potent inhibitory properties on α-glucosidase and α- amylase, they may cause diarrhea, vomiting, and edema in the intestines [11]. Today's oral antidiabetic medicines don't provide sustained glycemic control. Thus, plant extracts, which are decreasing blood glucose level with negligible side effects, are using as antidiabetic medicines [12]. Numerous findings indicate the essential role that metals play in the metabolism of carbohydrates and the regulation of DMT2 [13]. Oxides of silver, vanadium, chromium, magnesium, and Zinc have been shown to possess multiple therapeutic effects and to be crucial in lowering blood glucose levels and treating diabetes mellitus [14]. Silver nanoparticles have been demonstrated to have antidiabetic properties [15]. It has been shown by recent studies that biosynthesized nanoparticles work more effectively than other nanoparticles. Due to the advantages they offer, which include greater healing potential, solubility and surface area [16, 17]. The utilization of extract has drawn more interest since the creation of Ag nanoparticles using plants has multiple priority over other biosynthesized nanoparticles [18]. Furthermore, the manufacturing of nanoparticles is an efficient process since biomolecules can be used as capping, stabilizing and reducing agents instead of expensive, hazardous chemicals [19, 20].

In present research work, the antidiabetic activities of silver nanoparticles (AgNPs) were evaluated by using alpha amylase inhibitory and alpha glucosidase inhibitory assays. To ascertain the biological interaction between the α-amylase and α-glucosidase and silver nanoparticles molecular docking was performed. No previous research was done by green synthesis of silver nanoparticles using seeds of *A. indica*, to best of my knowledge this was the first in vitro and *in silico* antidiabetic research.

MATERIALS AND METHODS

Aqueous Extract Preparation

Azadirachta indica seeds were bought from an authorized medical plants shop in District Mardan, KP, Pakistan and instantly brought to the laboratory for subsequent process. The seeds were wash away through tap water, dried and make its powder with grinder. Approximately 50 gm of the Neem seeds powder was submerged in 100.00 milliliters of distal water in a 500 ml flask and heated for 10 to15 minutes using nonstop stirrer. The same was then kept at 25°C until cooling and then filtered through Whitman Number 1 filter paper [21]. The filtrate was collected in a clean flask, obtained its paste form through water bath and kept at 4 degree Centigrade for further process.

Green Synthesis of Silver Nanoparticles

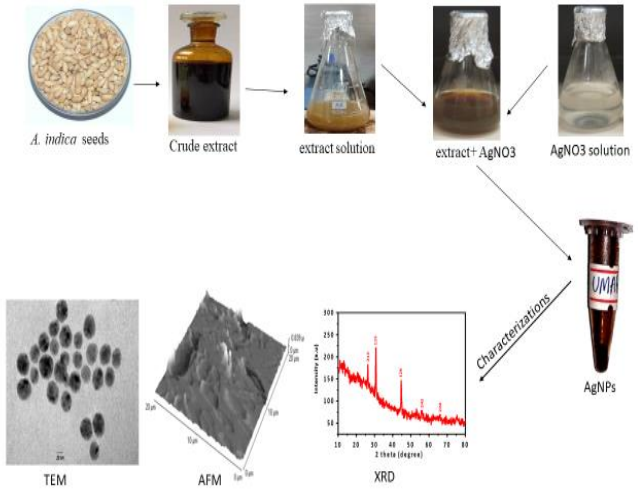
In order to synthesize silver nanoparticles Sulaiman *et al* protocol was followed [22]. In an Erlenmeyer flask, 100 mL (1 mM) solution of silver nitrate (AgNO3) was made. The Ag-NPs were generated by mixing 50 mL of a 1 mM aqueous AgNO3 solution at room temperature with 20 grams of *A. indica* (neem) aqueous extract, vigorously stirring for 20 minutes. The resulting liquid was kept at room temperature and dark chamber to prevent the silver nitrate from auto-oxidizing. The color change of the solution from reddish to dark brown, which indicates the synthesis of AgNPs. The solution was then centrifuged at 10,000 rpm for 30 minutes using a high-speed centrifuge. After that, the supernatants were eliminated, and the pellets were washed three times through double-distilled water, passed from another centrifugation step to eliminate any traces of boundless plant compounds and the same were collected in a Petri dish, dried at ambient temperature and kept for further process.

Characterization of the Green Synthesized Silver Nanoparticles

After synthesis, the given silver nanoparticles were subjected to characterization to determine their morphological, chemical and physical, properties through UV-VIS spectroscopy, FTIR, SEM, TEM, AFM and XRD analysis using concern apparatuses.

Figure 1

Preparation and Characterization of Silver Nanoparticles (AgNPs)



UV-VIS Spectroscopy

To check the influence of the light on the green synthesized AgNPs, the absorption spectra of the solutions. UV-visible spectroscopy was employed to track color changes in the mixture. In the UV spectrophotometer, the UV–Vis spectral analysis was conducted between 200 and 800 nm in wavelength.

FT-IR Examination

After being dried, the pellets were examined using Perkin Elmer FT-IR instrument working at 400–4000 cm in 21% transmittance mode to record FT-IR spectra. The main objective of the FT-IR examination was to examine the functional groups that take part in the AgNP formation. Therefore, the technique was applied to the qualitative evaluation of the produced nanomaterial. The results were

recorded in the form of values in the Origin software was used to plot the relevant graph.

X-RD Examination

X-RD was used to examine accurate information about AgNPs's chemical components, its physical features and crystallographic structure. X-ray diffractometer (DMAX-2500 XRD) (Rigaku, Tokyo, Japan) was used to carry out the experiment.

SEM / Scanning Electron Microscopy

The scanning electron microscope was used to characterize the surface morphology of Ag nanoparticles. After 6 hours of reaction, the sample was prepared by centrifuging the colloidal solution for four minutes at 10,000 rpm. After being re-dispersed in deionized water, the pellet was centrifuged once again. After the three rounds of the process, acetone was used to wash the material. After sonicating the refined silver nanoparticles for ten minutes to form a suspension, a drop of the suspension was applied to the copper grid coated with carbon. The given sample was examined using scanning electron microscope (Jeol JSM-6490A) at Centralized Resource Laboratory University of Peshawar, KP, Pakistan.

TEM / Transmission Electron Microscopy

TEM is a useful and significant method for characterizing nanomaterials. TEM (Philips, CM 12) was used to measure the distribution, size, morphology, and particle and/or grain sizes quantitatively. TEM offers two benefits: superior three-dimensional resolution and the opportunity to do extra systematic measures. Hence, sample preparation is crucial to achieving the best possible image quality.

Atomic Force Microscope Analysis

The external morphology of the given nanoparticles were visualized using Atomic force microscope in standard atmospheric environment. The observed sample was spread on tiny slide and explored on contact approach of the apparatus.

Anti-Diabetic Efficacy Applying *in vitro* Study

Alpha-Amylase Inhibition Assay

Applying the 3, 5-dinitrosalicylic acid technique, to evaluate the alpha-amylase inhibition [24]. The test mixture consisted of 10 to 100 µg/mL of AgNPs, 500-µl buffer of sodium phosphate (0.02 M) with alpha-amylase (500µl). For twenty minutes, at 37°C, the blend was incubated. Initially, the tubes were filled with 250 µl of 1% starch and incubated for 15 minutes at 37°C. Dinitrosalicylic acid (1 ml) was added to terminate the reaction, and it was at that time incubated in water bath up to 10 minutes. After cooling the tubes, the absorbance at 540 nm was find. The % of α-amylase inhibition was calculated by the given equation.

$$\%Inhibition = \frac{Absorbance\ of\ blank - Absorbance\ of\ \text{sample}}{Absorbance\ of\ \ blank} \times 100$$

Alpha-Glucosidase Inhibitory Activity

By following the method of Riyaphan with some modification [25]. Alpha-glucosidase (500µl), AgNPs (10–100 µg/mL), and sodium phosphate (0.1 M) were all mixed in 150µL of the test mixture. After 10 minutes of intubation at 37°C, 50µL of paranitrophenyl alpha-D-glucopyranoside was added to the mixture and re-incubated for 20 minutes at 37°C in sodium phosphate buffer (0.1 M). Then add 50 µL (0.1 M) of sodium carbonate to stop the reaction and the absorbance at 405 nm was measured.

Percentage inhibition of enzyme by of AgNPs was calculated using the formula:

$$\%Inhibition = \frac{Absorbance\ of\ blank - Absorbance\ of\ \text{sample}}{Absorbance\ of\ \ blank} \times 100$$

Anti-Diabetic Efficacy Applying *in silico* Study

Molecular Docking Analysis

Using the molecular operating environment (MOE), a molecular docking investigation of a synthesized nanoparticle was carried out [26]. Alpha-amylase and alpha-glucosidase's 3D structures, with PDB IDs 3BAJ and 3W37, respectively, were obtained from the Protein Databank Database (PDB) [27, 28] before energy minimization, every water molecule was eliminated. Using MMFF94's force field, energy minimization was carried out up to 0.05 gradients [29]. All of the synthesized nanoparticles' 3D structural coordinates were created in ChemDraw and placed in a new MOE database. Both target proteins' substrate-binding sites were identified for molecular docking. The MOE SVL script was used to test the docking process before docking analysis [30]. During docking, every ligand atom was made flexible in order to produce the low-energy ligand protein complex. To rank the compounds, the GBVI/WSA dG score function was employed. At last, Pymol v.1.7 was employed for the ligand-protein complex interaction analysis [31]. Redocking was carried out to assess the super impossibility of docking postures in order to confirm the docking results.

Statistical Analysis

GraphPad Prism software 10 was used for the statistical analysis of the data. The obtained triplicates data has been expressed as the mean ± standard deviation. By applying Tukey's post hoc comparison and One-way ANOVA, the p-value was found less than 0.05, the results were considered significant [32].

RESULTS

UV Analysis

The UV absorption peak of AgNPs ranges from 350 – 400 nanometers [33]. **Fig. 2** shows the UV absorption peaks of *A. indica* UV-Vis spectra approximately at 380.00nm, evidently signifying the creation of AgNPs in the *A. indica* aqueous extract. The appearance of the peak at 380 nm happens from the surface Plasmon resonance phenomenon, which is brought about by the applied electromagnetic field excitation of surface plasmons on the outer surface of the silver nanoparticles.

Functional Group Analysis by FTIR Spectroscopy

In the FTIR spectrum of *A. indica* based AgNPs, the peaks were observed at 3,300cm^{-1}-which are associated to OH stretching, CH stretching, 2097cm^{-1}-C=N stretching, 1637 cm^{-1}-N-H stretching 575cm^{-1}-C-X stretching [34]. The AgNPs revealed broad peak at 3300cm^{-1} which specify the existence of OH group or carboxyl groups and subsequently production of AgNPs there is an alteration in

the wide peak to the right at 1637cm^{-1} signifying the NH stretching. These carboxyl and amide groups signify the existence of secondary amines, a protein signature marker that confirms the protein or phytochemical's bio fabrication of the nanoparticles [35]. **Fig.3** clearly illustrates the bio fabrication of the *A. indica* mediated AgNPs by the activity of phytochemicals such alkaloids, flavonoids, phenols, and terpenoids [36].

Figure 2

UV-Visible Absorption Peak of A. indica Mediated AgNPs

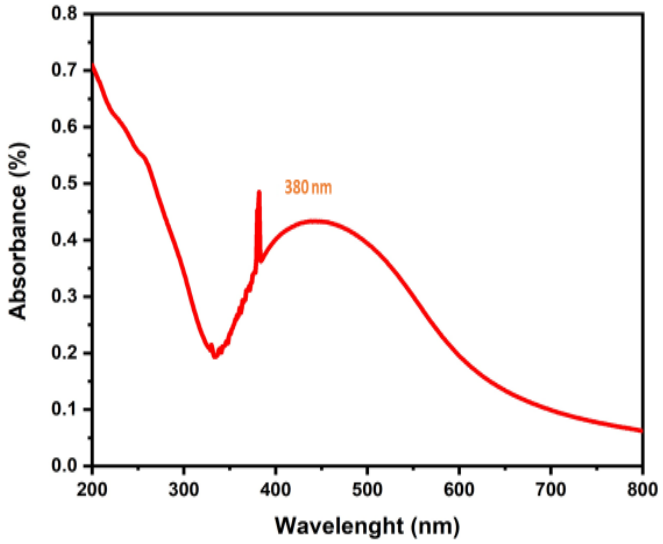
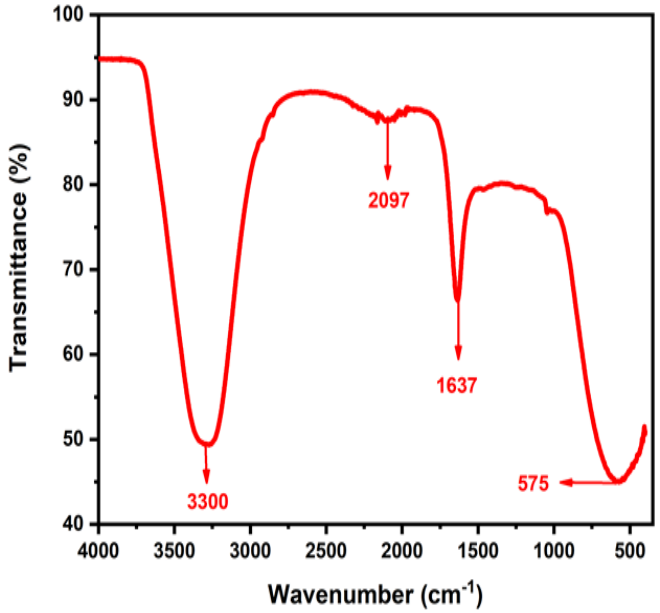


Figure 3

FTIR Spectrum of A. indica Mediated AgNPs

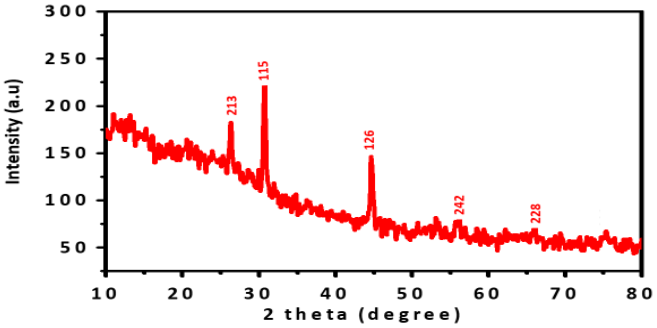


XRD Analysis

XRD analysis given as **Fig 4** Shows that in the 2θ range of 10° to 80°, there are different diffraction peaks. Prominent peaks in the XRD spectrum appeared at 26.62°, 31.28°, 45.1°, 55.2°, 66.28°, and 75.31°, that indexed the cubic face centered silver's planes. The consequence validated the AgNPs' crystalline structure and this is in agreement with the previously reported green synthesized AgNPs used for biomedical applications [37].

Figure 4

XRD Patterns of A. indica mediated AgNPs

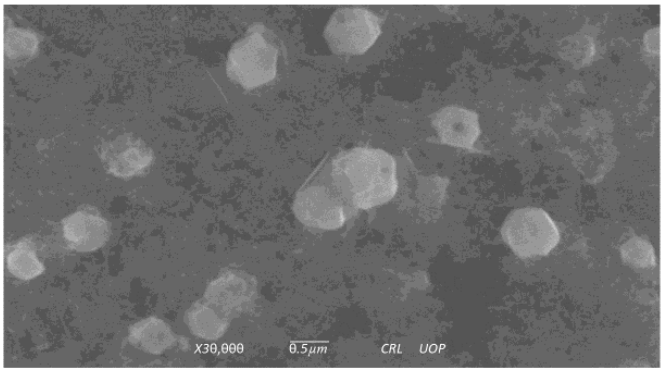


SEM Analysis

SEM analysis was used to examine the structural physical characteristics of the bio-synthesized AgNPs. The synthesized AgNPs emerged as moveable groups and were smooth, spherical and generally homogeneous in size. These demonstrate the stability of AgNPs, their spherical morphology and small in size **Fig 5** [38]. The dehydration processes that was used to prepare the samples for SEM examination apparently assisted the AgNPs cluttering.

Figure 5

SEM Image of A.indica Mediated AgNPs

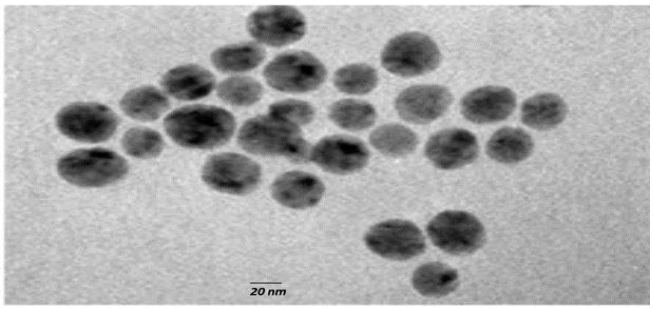


TEM Analysis

TEM image showing AgNPs synthesized by *A. indica* seed extract. The particles have an average size of 20 nm and predominantly spherical, triangular, cuboidal and tetrahedral in shape **Fig 6**. The majority of the AgNPs had smooth edges and a generally round shape. During the production of AgNPs, phytochemical components found in *A. indica* seeds, such as saponin glycoside, alkaloids, proteins and flavonoids, may become entrapped and function as reducing agents [39].

Figure 6

TEM Image of AgNPs Synthesized using A.indica extract

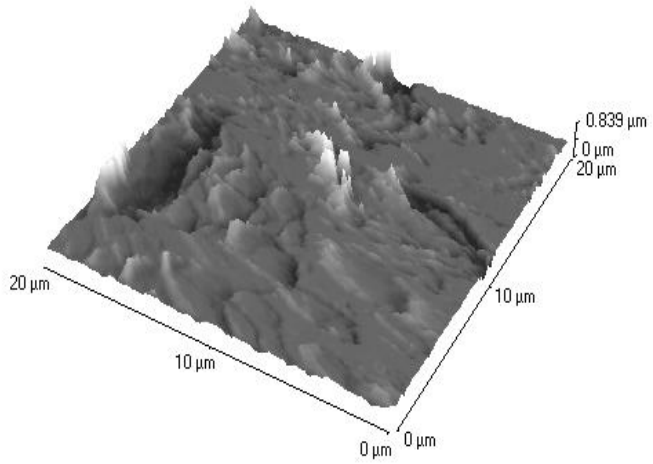


Atomic Force Microscope (AFM) Analysis

The morphology and external roughness of the silver particles (AgNPs) produced from *A. indica* seeds aqueous extract were evaluated using AFM analysis **Fig 7**. According to the AFM image, the synthesized AgNPs have spherical shape and uniformly packed surface.

Fig. 7

Shows AFM Image of A.indica Mediated AgNPs

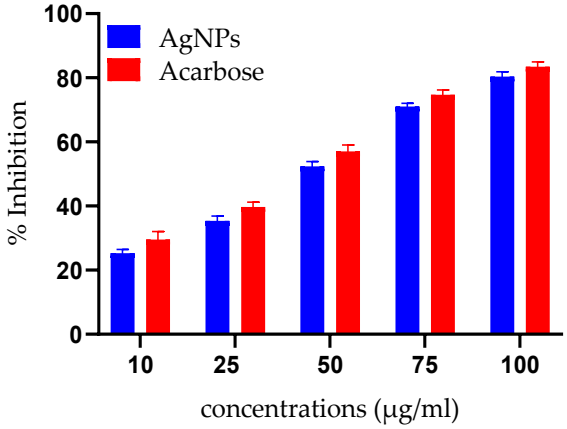


α-Amylase Inhibitory Assay

The inhibitory effect of AgNPs on alpha amylase using concentrations 10µg/ml, 25µg/ml, 50µg/ml, 75µg/ml, and 100µg/ml were noted as 26.6±1.53%, 36.33±1.6%, 50±2.48%, 68.5±1.32%, and 79.33±2.21% respectively. Whereas Acarbose (standard drug), showed % inhibition at 10µg/ml, 25µg/ml, 50µg/ml,75 µg/ml, and 100µg/ml as 30.50±1.53%, 39.66±1.53%, 54.3±2.37%, 71.2±1.31%, 79.5±2.121% and 82 ±3%respectively.

Figure 8

Represents the Percentage Inhibition of Alpha Amylase

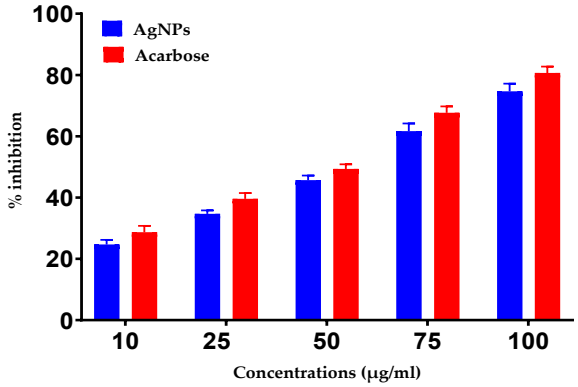


Alpha-Glucosidase Inhibitory Assay

The inhibitory effect of AgNPs (10-100µg/ml) on alpha-glucosidase was ascertained. The percent inhibitory values of AgNPs at 10µg/ml, 25µg/ml ,50µg/ml, 75µg/ml and 100µg/ml were noted as 25.3±0.6%, 34.67±1.55%, 52.3±1.528%, 71±1.51%, and 80±1.51% respectively. Whereas Acarbose used as a standard drug, revealed % inhibition at10µg/ml, 25µg/ml, 50µg/ml, 75µg/ml, and 100µg/ml as 28.6±2.09%, 39.58±1.90%, 57±2%, 74.66±1.53, & 83.13±1.26% respectively.

Figure 9

Represents the % Inhibition of Alpha Glucosidase



Antidiabetic Activity by in silico Analysis

Docking studies of synthesized nanoparticles revealed a variety regarding probable interactions with the binding site of substrate **(Fig.10)** of both receptor proteins, the binding site residues entangled in the interactions includes Tyr 62, Gln 63, Glu 233, His 299, Asp 300, His 305 and ARG 319. The docking score and interaction details are enlisted in table 1. The synthesized nanoparticle demonstrated a considerable interaction with the amylase binding site residues i.e., Glu 233, Tyr 62 form hydrogen bond donor and acceptor interactions, with the catalytic core residues of alpha-amylase, while His 305, and Glu was found in metal and ionic contacts with synthesized nanoparticles having docking score of -5.6164. Similar to Alpha-amylase, the synthesized nanoparticle was docked to the substrate binding site residues of α -glucosidase. The protein ligand interactions analysis revealed that nanoparticle forms significant interaction with key catalytic core residues of the target protein, Asp568 and Met 470 was found in the hydrogen donor interactions, Arg 552 forms hydrogen bond donor while Asp 469 was found in metal contact with the synthesized nanoparticle. The docking score of nanoparticles was found as -5.0013 and the interactions details are shown in table 2. Furthermore, Acarbose was also docked to the active site of both targets, the docking score number of interactions of acarbose is relatively high in comparison to the nanoparticle which is in agreement with the experimental findings.

Table 1

Reveals the Interactions Details of Nanoparticles and Acarbose in the Active Site of α-amylase

Name of Compounds	Interacting Residues	Interactions Type	Distance	Energy	Docking Score
Nanoparticle	ASP 568 MET 470 ARG 552 ASP 469	H-donor H-donor H-acceptor metal	3.19 3.77 2.78 3.13	-0.7 -4.4 -2.3 -2.0	-5.6164

	ASP 469	H-donor H-	2.99	-1.9	
	ASP 232	donor H-	2.99	-0.7	
Acarbose	LYS 506	acceptor H-	3.14	-1.1	-6.9441
	TRP 432	acceptor H-	2.98	-0.8	
	PHE 476	pi	4.38	-0.8	

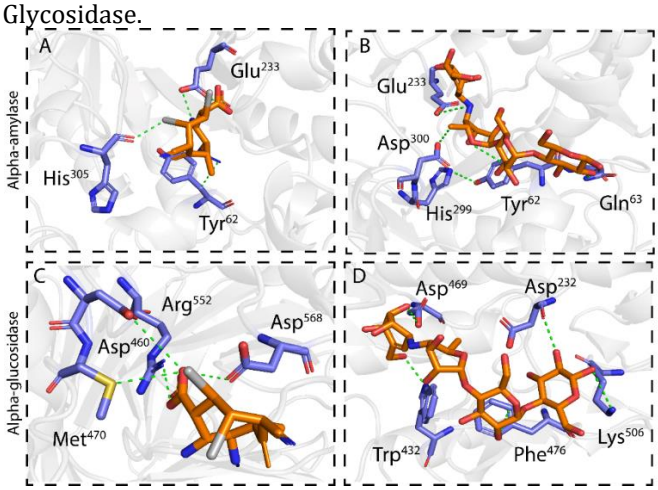
Table 2

Reveals the Interactions Details of Nanoparticles and Acarbose in the Active Site of α-glucosidase

Name of Compounds	Interacting Residues	Interaction Type	Distance	Energy	Docking Score
Nanoparticle	GLU 233	H-donor H-acceptor metal ionic	2.89	-9.0	-5.0013
	TYR 62		3.57	-0.7	
	HIS 305 GLU 233		2.93	-2.1	
			2.94	-4.9	
Acarbose	ASP 300	H-donor	3.31	-0.5	-7.1956
	GLU 233	H-donor H-	3.24	-0.7	
	HIS 299	acceptor H-	3.43	-0.5	
	GLN 63	acceptor H-	3.04	-1.4	
	TYR 62	pi	4.78	-0.5	

Figure 10

3D Interaction Pattern of Ag Nanoparticle and Acarbose in the Substrate-Binding Site of Alpha-Amylase and Alpha Glycosidase.



DISCUSSION

The World Health Organization claimed that 15 million people globally suffer from Diabetes type 2, a number that might double by the year 2025. It is approximately 90 percent of all incidents of diabetes. Presently affecting approximately 170 million people around the world and may reach more than 365 million before 2030 [40]. Plants are known to have the ability to block certain enzymes such as α-glucosidase & α-amylase. Oils derived from a variety of plants were utilized to create various metal nanoparticles in a sustainable way for medical purposes [41]. A study claims that cumin oil-mediated silver nanoparticles have an outstanding α-amylase inhibitory action, proving their antidiabetic efficacy. Inhibitors of the enzymes that break down carbohydrates are an excellent remedy for post-meal hyperglycemia regulation, which raises serum sugar levels [43]. Nanotechnology is concerned with nanoparticles that are 1-100 nm in size in one dimension. Nanoparticles are now widely employed for covering a wide range of products, including medications, electronics, energy interactions and actions. Commercial uses of these nanoparticles in the fields of pharmaceutical and other medical sciences heavily rely on NPs [44]. Due to their higher biocompatibility over chemically produced AgNPs, produced by biosynthesis are more suitable for use in medical applications. Green chemistry, which uses microorganisms and plant extract, is safe for the environment, non-toxic and affordable [45]. Combining pharmaceuticals with metal nanoparticles for the control of diabetes is a novel method to increase the efficacy of medications [46]. Reducing mediators are necessary for the production of nanoparticles in order to improve their biological effects by lowering their harmfulness and improving its bioavailability and biocompatibility in living organisms. Moreover, they enhance the colloidal stability of the particles, inhibit uncontrolled growth of the particles, and prevent clusters or clumping of the particles [47]. Plant mediated AgNPs have been proven to possess antidiabetic activity. A number of studies within the field of plant-mediated nanomedicine has proven that green manufactured nanoparticles, have advantages over crude extracts due to their wide surface area, better solubility and stronger therapeutic activity [48]. The size of nanoparticles is expressively control by natural substances exist in the extract of plants that function as potent reducing agents. A stronger reductant in the extract leads to a greater rate of reaction, which in turn produces smaller nanoparticles [49].

In the current research work, silver nanoparticles have synthesized through biological method using *Azadirachta indica* seeds aqueous extract that were characterized by ultraviolet–visible (UV–vis) spectrophotometry (**fig. 2**), and Fourier Transform Infrared (FTIR) (**fig. 3**). Formation of AgNPs was predominantly detected by variation in colour of reaction mixture from light brown to dark brown after treatment with 1 mM silver salt (AgNO3) soloution. UV–vis spectroscopy showed peak at 380 nm that confirmed the formation of AgNPs [50]. FTIR showed the presence of carboxyl and amide group indicate the presence of secondary amines which is a signature marker of proteins confirming the bio-fabrication of the nanoparticles by the action of the protein or phytochemicals, which increases the stability of AgNPs in the colloids [51]. XRD analysis **(Fig 4)** shows that in the 2θ range of 10° to 80°, there are different diffraction peaks. Prominent peaks in the XRD spectrum appeared at 26.62°, 31.28°, 45.1°, 55.2°, 66.28°, and 75.31°, which indexed the cube shape face centered silver's planes. This result supported the existence of crystals of given Ag NPs and this is in agreement with the previously re-ported green synthesized Ag NPs used for biomedical applications [52]. In SEM analysis the synthesized AgNPs emerged as moveable groups and were smooth, spherical, and generally homogeneous in size (**Figure 5**). These demonstrate the stability of AgNPs, their spherical morphology and small in size, similar results were observed in earlier research in which *Cucumis prophetarum* extracts were used to biosynthesized AgNPs [53]. In TEM result, the nanoparticles have an average size of 20.0 nm and predominantly spherical, triangular, cuboidal, and tetrahedral in shape (**Figure 6**). The majority of the AgNPs had smooth edges and a generally round shape. During the production of AgNPs, phytochemical components found in *A. indica* seeds, such as saponin glycoside, alkaloids, proteins, and flavonoids, may become entrapped and function as reducing agents

[54]. The morphology and external roughness of the silver particles (AgNPs) generated from *A. indica* seeds aqueous extract were evaluated using AFM analysis. According to the AFM image, the AgNPs have spherical shape and uniformly packed surface (**Fig. 7**). To check and confirm the antidiabetic activity, alpha amylase inhibitory assay and glucosidase inhibitory assay was also performed.The inhibitory effect of AgNPs on alpha-amylase using concentrations 10µg/ml, 25µg/ml, 50µg/ml,75µg/ml, and 100µg/ml were noted as 26.6±1.53%, 36.33±1.6%, 50±2.48%, 68.5±1.32%,and 79.33±2.21% respectively Whereas Acarbose (standard drug), showed % inhibition at 10µg/ml, 25µg/ml, 50µg/ml,75 µg/ml, and 100µg/ml as 30.50±1.53%, 39.66±1.53%, 54.3±2.37%, 71.2±1.31%, 79.5±2.121% and 82 ±3%respectively (**Fig. 8**).

The inhibitory effect of AgNPs (10-100µg/ml) on alpha glucosidase was ascertained. The percent inhibitory values of AgNPs at 10µg/ml, 25µg/ml ,50µg/ml, 75µg/ml, 100µg/ml were noted as 25.3±0.6%, 34.67±1.55%, 52.3±1.528%, 71±1.51%, and 80±1.51% respectively Whereas Acarbose used as a standard drug, revealed % inhibition at10µg/ml, 25µg/ml, 50µg/ml, 75µg/ml, and 100µg/ml as 28.6±2.09%, 39.58±1.90%, 57±2%, 74.66±1.53, & 83.13±1.26% respectively (**Fig.9**). Molecular docking studies of synthesized nanoparticles revealed a variety regarding probable interactions with the binding site of substrate (**Fig.10**) of both receptor proteins, the binding site residues intangled in the interactions includes Tyr 62, Gln 63, Glu 233, His 299, Asp 300, His 305 and ARG 319. The docking score and interaction details are enlisted in table.1. The synthesised nanoparticle demonstrated a considerable interaction with the amylase binding site residues i.e., Glu 233, Tyr 62 form hydrogen bond donor and acceptor interactions, with the catalytic core residues of al-pha-amylase, while His 305, and Glu was found in metal and ionic contacts with synthe-sized nanoparticles having docking score of -5.6164. Similar to Alpha-amylase, the synthesized nanoparticle was docked to the substrate binding site residues of α -glucosidase. The protein ligand interactions analysis revealed that nanoparticle forms significant interaction with key catalytic core residues of the target protein, Asp568 and Met 470 was found in the hydrogen donor interactions, Arg 552 forms hydrogen bond donor while Asp 469 was found in metal contact with the synthesized nanoparticle. The docking score of nanoparticles was found as -5.0013 and the interactions details are shown in table 2. Furthermore, Acarbose was also docked to the active site of both targets, the docking score number of interactions of acarbose is relatively high in comparison to the nanoparticle which is in agreement with the experimental findings.

CONCLUSIONS

The aqueous extract of *A. indica* was successfully used to synthesize the silver nanoparticles through the biological reduction of AgNO3. These nanoparticles were properly characterized using standard techniques, such as UV-Vis spectroscopy, XRD, FT-IR, SEM, TEM and AFM analysis. To confirm their antidiabetic potentials, in vitro and in silico studies were conducted. The in vitro and in silico results of the current investigation confirmed that green synthesized AgNPs have considerable anti-diabetic activities as compared to previous studies. However, there is a need for advanced studies on these AgNPs using in vivo models of higher mammals and molecular work.

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