

***In vitro* anti diabetic activity of PVA encapsulated silver nanoparticles of ethanolic extract of *Pisonia grandis* leaves**

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Abstract

*Diabetes mellitus is a chronic metabolic ailment that progresses beyond a person's lifetime. It is brought on by a relative shortage of insulin synthesis, leading to variable degrees of insulin resistance. The present study involves biosynthesis of poly vinyl alcohol (PVA) encapsulated silver nanoparticles of ethanolic extract of *Pisonia grandis* leaves (PVA AgNPs Pg) along with the evaluation of their potential biomedical applications. Analytical techniques like UV spectroscopy, XRD, SEM, EDX, TEM, FTIR and Zeta potential measurement were employed to assess the separated silver nanoparticles. PVA AgNPs Pg was also used to investigate the anti-diabetic effect in vitro.*

These studies have showed significant efficacy in inhibiting the enzymes DPPIV, alpha-amylase and alpha-glucosidase. Therefore, it is reasonable to conclude that silver nanoparticles fabricated by green synthesis might be used as a phytomedicine to treat diabetes.

Keywords: Diabetes, Nanoparticles, Encapsulation, Spectroscopy, Anti-diabetic activity

Introduction

Diabetes mellitus is an alarming metabolic disorder that can be either acute or long-term. The most common signs and symptoms are thirst, hunger and frequent urination. Severe health problems might arise from untreated diabetes mellitus like cancer, stroke, neuropathy, vision loss, chronic renal disease and cardiovascular problems. An estimated 25% of people worldwide suffer from diabetes mellitus, a condition that affects both developed and developing countries. The bulk of drugs used to treat diabetes such as biguanides, thiazolidinediones, sulphonyl urea and alpha-glucosidase inhibitors, lower blood sugar. Despite the lengthy history of usage of antidiabetic drugs, the primary issue with them is that individuals with diabetes may become resistant to medications, which might have dreadful impacts such as weight gain, lactic acidosis, gastrointestinal disturbance and in certain situations, liver damage.¹⁶

Diabetes kills more than a million people annually and ranks as the ninth chief basis of death. The prevalence of diabetes mellitus is rising universally with the biggest rise occurring

in Western Europe and other industrialised regions.¹³ Diabetes is a terrible disorder distinguished by abnormally high blood sugar levels triggered by a mix of environmental and hereditary factors. It has various treatment alternatives, but none of them completely cured the disease. Several plants and vegetables have been examined and proved to have anti-diabetic qualities in animal models, implying that researchers are exploring for plant-based anti-diabetic drugs with fewer side effects.¹⁹

Patients are asked to begin antidiabetic medication therapy when diet and exercise are insufficient to manage hyperglycaemia. These medications do have a few side effects, which may shorten the length of the treatment. The primary drawbacks of the oral modalities used to treat type 2 diabetes are their limited bioavailability and instantaneous release of the medication, which necessitates more frequent dosage. During the last several years there has been a significant development of innovative delivery mechanisms using nanotechnology which may increase the effectiveness of anti-diabetic regimens. For optimal effects, the medication can be gradually and carefully encapsulated into a nano-carrier system.²⁸ One of the topics that has received the greatest research attention in recent years is silver nanoparticles, or AgNPs. Applications for AgNPs include the ability to construct them utilising a variety of synthetic methodologies depending on the desired properties and application area. One of the most promising synthesis approaches is green creation of silver nanoparticles. AgNPs are frequently applied in food packaging, therapeutic equipment and other uses due to their strong antibacterial properties.²¹

Medicinal plants contain important phytochemicals, they hold great potential for treating a wide range of ailments. Herbal medications are gaining relevance since they are cost-effective and provide better therapeutic results with few adverse effects.¹² Nanoscience and nanotechnology have significantly improved illness detection, treatment and prevention in different sectors.

Since ancient times, silver has been used to cure wounds and illnesses. Silver nanoparticles (AgNPs) are popular among metal nanoparticles due to their inimitable effects.²² Since silver nanoparticles (AgNPs) have unique properties and potential therapeutic applications, the science of nanomedicine has focused a great deal of interest on them.

Administering drugs may be done with a variety of nanoparticles including AgNPs. To decrease side effects and to increase the effectiveness of medication therapy for diabetes, nanoparticles can be engaged to administer insulin or additional anti-diabetic medications in a targeted and regulated style. One of the uses for AgNPs is the progress of glucose sensors. This will afford real-time blood glucose detection, which is critical for diabetes control.¹¹

Pisonia grandis R. Br (Nyctaginaceae) is one such popular evergreen lettuce tree growing widely in India. It is highly adapted to seacoasts and flourishes in gardens in Chennai and other coastal places of both the east and west coasts. Native Americans extensively use of this species' leaves, stems and roots to manufacture a range of indigenous medicines. Numerous experts have studied the plant, focusing on its medical benefits. Pinitol and allantoin are the two major bioactive compounds derived from the plant.²⁷

Current study aimed on the anti-diabetic effect of polyvinyl alcohol encapsulated silver nanoparticles of ethanolic extract of *Pisonia grandis* leaves (PVA AgNPs Pg).

Material and Methods

Preparation of Leaf Extract: The leaves of the plant were washed and dried under shade for weeks. The dried leaves were crushed and a nice powder was made. 10 grams of the powdered sample were mixed with 100 ml of ethanol subjected to ethanolic extraction using the cold maceration method. Filtration was carried out using Whatmann no.1 filter paper to separate the unextractable matter. After filtration, it was evaporated to crude form by a rotatory evaporator under reduced pressure.

Silver nanoparticles synthesis (Ag NPsPg): Silver nanoparticles (Ag NPs) were prepared from ethanolic extract of *Pisonia grandis* leaves. After preparing the crude extract, 200mg of the dried samples was mixed with 100ml of water. 10 ml sample was then blended using 90 ml of 1mM AgNO₃. (0.017g AgNO₃ in 100ml water).

Encapsulation of silver nanoparticles using poly vinyl alcohol (PVA): For doing encapsulation, the 1% solution of poly vinyl alcohol (PVA) was heated at 80°C for 5 hours. The ratio of leaf extract volume, AgNO₃ and PVA solution of 1:10:3 was applied to synthesize PVA-modified silver nanoparticles. The sample was then agitated by a magnetic stirrer for two hours at 24°C.⁶

Characterization of silver nanoparticles: Produced silver nanoparticles were characterized by employing a variety of methods. UV-visible absorption spectroscopy was introduced to investigate the Ag NPs' optical properties (Shimadzu – BioSpec – nano, Japan) by recording spectra between 200nm and 800 nm. The topography and sizes of the Ag NPs were then studied using transmission electron microscopy. With 2h scans ranging from 30 to 80°, X-ray powder diffraction (XRD) motifs of AgNPs were examined.

FTIR spectra of Ag NPs were attained utilizing an FTIR spectrophotometer. The arrangement of biological particles at the top and the synthesized AgNPs' dispersion were revealed using Fourier transform infrared (FTIR) spectra. The SEM and EDX methods revealed the synthesized AgNP's morphological characteristics along with elemental composition. The particle size analyzer confirmed the Zeta potential value of nanoparticles.

UV-visible absorption spectroscopy of PVA Ag NPsPg:

UV-visible spectral analysis is used to characterize the development and accomplishment of silver nanoparticles. Dilution of 0.1 ml of the sample to 10 times with double distilled water was used for the bio-reduction of Ag ions. UV-visible spectra were recorded with spectrophotometer from 300 to 700 nm wavelength at room temperature.⁹ The analysis of UV- visible spectroscopy absorbance data can provide confirmation of plasmonic resonance and the production of silver nanoparticles.⁴

SEM with EDAX analysis of PVA Ag NPsPg:

SEM and EDX were used to analyze the PVA Ag NPsPg's surface morphology and basic locations. The PVA Ag NPsPg was homogeneously dispersed on the sample possessor by a carbon tape before being coated in platinum for 120 seconds with an ion coater system and evaluated under a Scanning electron microscope. Following the acquisition of SEM images, an EDX detector attached to the SEM equipment was employed to examine the elements present.²³

TEM analysis of PVA Ag NPsPg:

The size, shape and morphology of the silver nanoparticles were ascertained by Transmission electron microscopy examination. At 200 kV, Hitachi H-800 was used to perform TEM measurements. By applying a drop of the bio-reduced diluted solution on a copper grid covered with carbon and exposure to air beneath a light, the TEM grid was created.⁵

FTIR analysis of PVA Ag NPsPg:

The PVA-encapsulated silver nanoparticle production is ensured and the infrared spectra of emission and absorption are obtained using FT-IR analysis. In order to understand how silver might interact with bioactive molecules and generate stable (capping material) silver nanoparticles, it is helpful to consider these interactions. The advantage of this method is that it instantaneously collects spectral data in an extensive array.

The study found that plant extracts include functional groups that lowered Ag⁺ ions throughout the formation of silver nanoparticles. 16 cm⁻¹ resolution and 3500-500 cm⁻¹ range were used for the analysis.²

Zeta Potential Measurement of PVA Ag NPsPg:

Particle size analyzer (Litesizer 500) was inclined to evaluate the surface charge of the PVA Ag NPsPg. Hydrodynamic diameter and polydispersity index were calculated as a function of time. Measurement of polydispersity index (PDI) value is a clear representation of nanoparticle size

distribution. Zeta potential is crucial for the stability of nanoparticles.³

X-ray diffraction (XRD) studies: With a Cu target and a scintillation counter ($\lambda = 1.5406 \text{ \AA}$) operating at 40 kV and 40mA in the range of $2\theta = 30^\circ\text{--}80^\circ$, the high-resolution X-ray diffraction (XRD) patterns were acquired at 3 kW. The acquired pictures were compared to the crystalline structure database of the Joint Committee on Powder Diffraction Standards (JCPDS).²⁵

In vitro anti-diabetic activity

In vitro α -amylase inhibition activity: One millilitre of aqueous extract containing 50 μg –500 μg of the sample was pre-incubated for 30 minutes with 500 μl of α -amylase (0.5 mg/ml of 20 mM sodium phosphate buffer pH 6.9). The starch solution was then added to 1 millilitre of 1% w/v 20 mM sodium phosphate buffer at pH 6.9 followed by incubation.

After adding DNS reagent, boil the entire sample and then add 15 ml of distilled water. The absorbance at 540 nm was measured. Acarbose was used as a standard drug.²⁰

$$\% \text{ Inhibition} = (\text{Abs control} - \text{Abs extract} / \text{Abs control}) \times 100$$

In vitro α -glucosidase inhibitory activity: One millilitre of the aqueous extract containing 50 μg –500 μg of the sample was pre-incubated with 36 μl of 0.1 M pH 6.8 sodium phosphate buffer solution. 17 μl of 5-nitrophenyl-D glucopyranoside (5mM) substrate were heated to 37°C for five minutes. To this, 17 μl of the α -glucosidase solution (pH 6.8, 0.15 U/ml sodium phosphate buffer solution 0.1 M) was added followed by incubation for 15 minutes. Then add 3.3 ml of sodium hydroxide (50 mM) to stop the process. The absorbance at 410 nm was measured. Acarbose was used as reference drug.²⁴

$$\% \text{ Inhibition} = (\text{Abs control} - \text{Abs extract} / \text{Abs control}) \times 100$$

In vitro DPP-IV inhibitory activity: The inhibitory action of dipeptidyl peptidase IV (DPP-IV) was accomplished by the technique described by Gonzalez-Montoya et al¹⁰ with minor changes. DPP-IV (1mU/ml) was prepared in 10mM Tris pH-7.5 at 37°C. The enzyme solution (50 μl) was diluted using the desired concentrations of the given test compounds/std control (25 μl) in a flat bottom 96 well plate and then incubated for 5 minutes at 37°C. Add 100 μl of rebaudioside (substrate) to all the wells and incubate the plate for 15 minutes at 37°C. The process was arrested by incorporating 25 μl of 25% acetic acid and read the absorbance at 405nm by a microplate reader. Sitagliptin was used as the reference standard.³¹

$$\% \text{ Inhibition} = (\text{Abs control} - \text{Abs extract} / \text{Abs control}) \times 100$$

Glucose diffusion assay: One millilitre of the 10 mg/ml nanoparticle solution and two millilitres of the 22 mM D-glucose solution in 0.15 M NaCl were transferred to a dialysis membrane. The dialysis membrane was knotted at two sides to stop the integrated fluid from leaking. Then it was placed inside a vessel with 40 mL of 0.15 M NaCl and 10 mL of distilled water in it. By substituting clean water for an equivalent volume of the nanoparticle solution, control was preserved. The glucose solution migrated into the outer solution every half an hour while the beaker was at room temperature on an orbital shaker for three hours. The O-toluidine method was used to measure the glucose concentrations at 640 nm.¹

Results and Discussion

UV-visible absorption spectroscopy of PVA Ag NPs Pg:

Production of silver nanoparticles was proved by the leaf extract's colour shift from yellow to vibrant brown which was given in figure 2. The process takes thirty minutes and it specifies that pure silver ions have been converted to metallic silver. After two hours of incubation, the brown colour does not intensify, indicating that Ag^+ has entirely reduced.

Ultraviolet-visible spectrometry was used to determine the absorption of the reaction mixture and to evaluate the reduction of the silver ions and AgNP synthesis by monitoring the peak. The huge SPR peak seen for the solution of PVA-encapsulated silver nanoparticles and the absorption spectra recognized at 430 nm are shown in figure 3. The band's broadness might be explained by the polymer present in the solution.

Increased stability of the silver nanoparticles synthesized using polyvinyl alcohol-based aqueous extract of *Diospyros discolor* wild leaves thoroughly demonstrated the silver nanoparticles' surface plasmon resonance that will not change after modification with polyvinyl alcohol. The PVA-encapsulated silver nanoparticle solution was stable for three months at room temperature.⁶

SEM with EDAX analysis of PVA AgNPs Pg: The Scanning electron microscope (SEM) may examine the surface topography of nanomaterials. PVA AgNPs Pg generated throughout the biosynthetic process are shown morphologically in figure 4.

The results indicate that there may be spherical nanoparticles present which look like spherically shaped nanoparticle clusters with different nano diameters. According to the energy dispersive X-ray analysis (EDAX) of the PVA AgNPs Pg, silver is immobilised on the PVA matrix. The EDX spectrum of PVA AgNPs Pg shows a prominent peak at 3.0 keV that shows the incidence of silver metal (Figure 5). A distinctive optical absorption peak at around 3 KeV is seen in metallic silver nanocrystals because of surface plasmon resonance.¹⁴

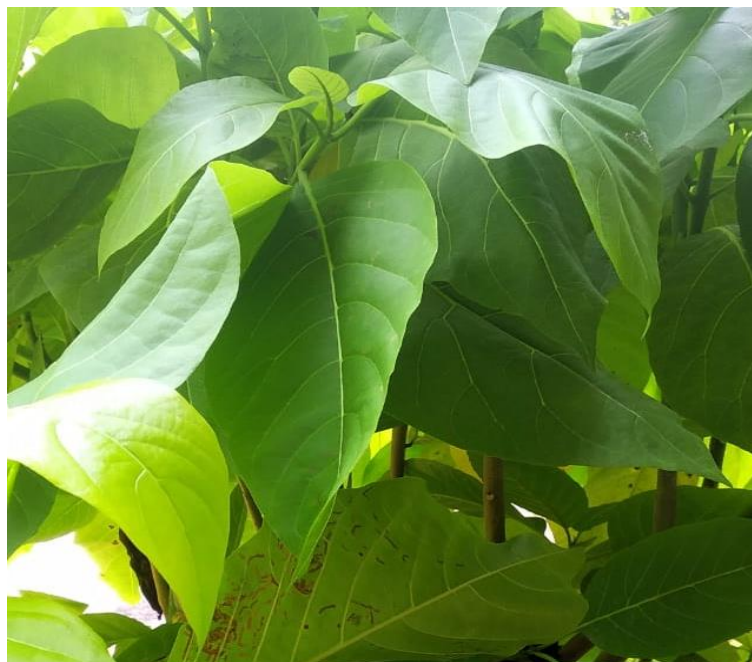


Fig. 1: *Pisonia grandis* R.



Fig. 2: Biosynthesis of PVA-AgNp Pg

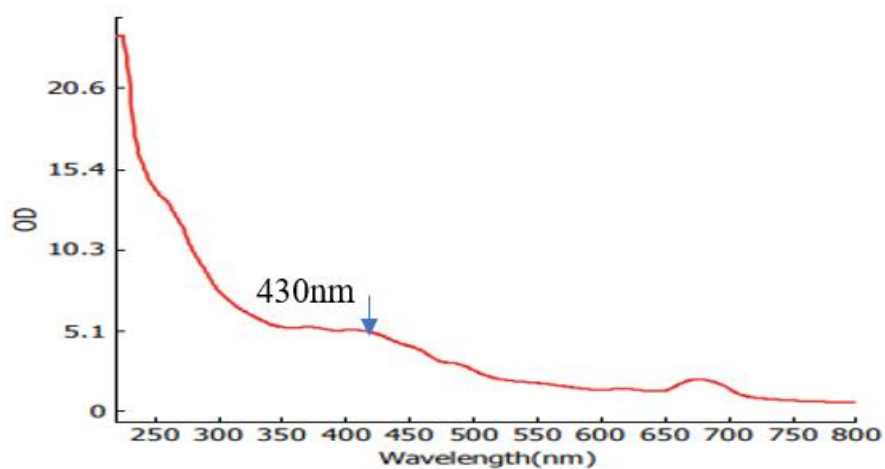


Fig. 3: UV-visible absorption spectra of PVA-AgNp Pg

TEM analysis of PVA Ag NPs Pg: Transmission electron microscopy (TEM) is useful in the characterization of nanoparticles as TEM images are 1000 times more sensitive than SEM images and they contribute additional precise data on the size, shape and crystallography of nanoparticles. Figure 6a and 6b showed the particle size distribution curve of PVA AgNPs Pg by transmission electron microscopy. Using a particle distribution plot, the average particle size of 10.20 nm was examined. TEM image of *trichoderma longibrachiatum* was applied to create spherical nanoparticles having diameters ranging from 5 to 25 nm which were then shown to have an impact on phytopathogenic fungus.²⁶

XRD analysis of PVA AgNP Pg: With the use of an XRD diffractogram, AgNPs' crystalline orientation was confirmed. Miller indices (111), (200), (220) and (311) are correlated with the Bragg's diffraction peaks at 2θ values of

38.1° , 44.2° , 64.4° and 77.3° (Figure 7). These peaks show the establishment of face-centred cubic crystalline elemental AgNPs. Using the JCPDS database (File no. 87-0717), the acquired data were indexed. The average crystalline size of the synthesised AgNPs was determined using the Scherrer equation $D = k\lambda/\beta\cos\theta$, where D is the average particle size, k is the shape factor (constant 0.9), λ is the X-ray wavelength (1.5406 Å), β is the complete width at half maximum of the peak and θ is diffraction angle. The acquired diffraction pattern matched the earlier reports.

Ocimum sanctum leaf extract also showed the three peaks with XRD pattern at $2\theta = 12^\circ$, 32° and 38° indicating the occurrence of organic substance and silver nanoparticles.⁸ The obtained XRD pattern was in agreement with the literature on AgNPs synthesis as well as the pattern of common metallic silver (JCPDS Nos. 00-004-0783 and 89-3722).

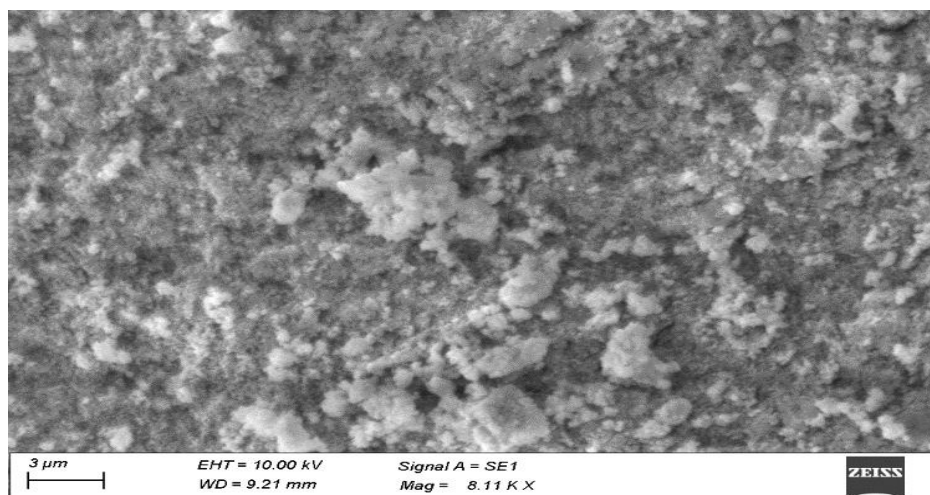


Figure 4: Scanning electron microscopy (SEM) images of PVA-AgNP Pg

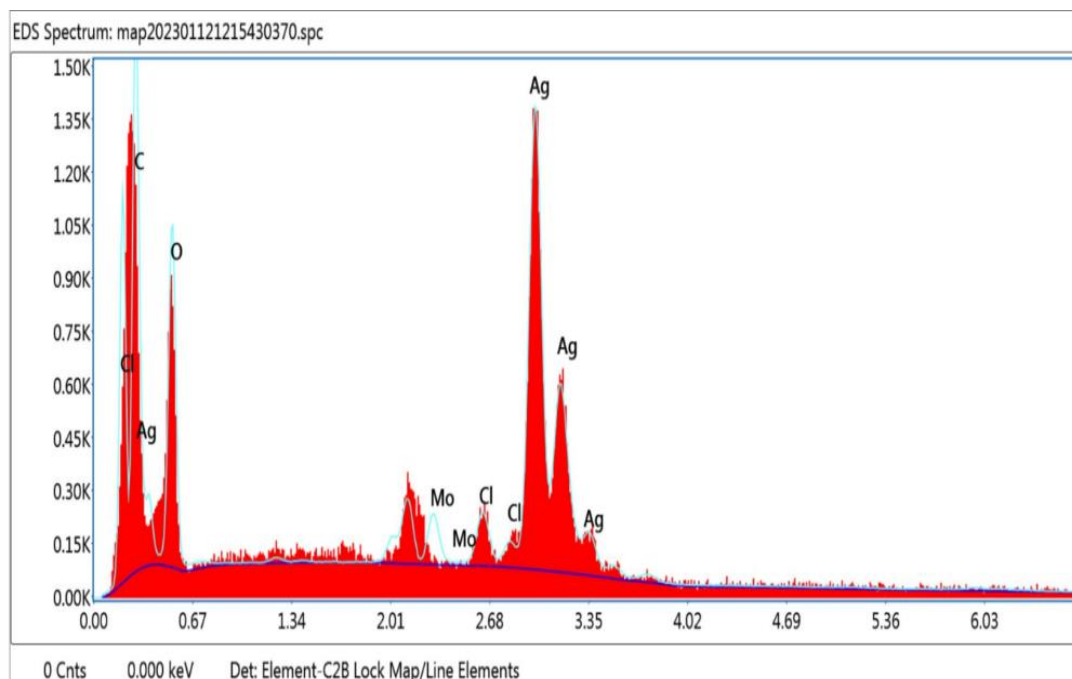


Figure 5: Energy dispersive X-ray spectroscopy (EDX) analysis of PVA AgNP Pg

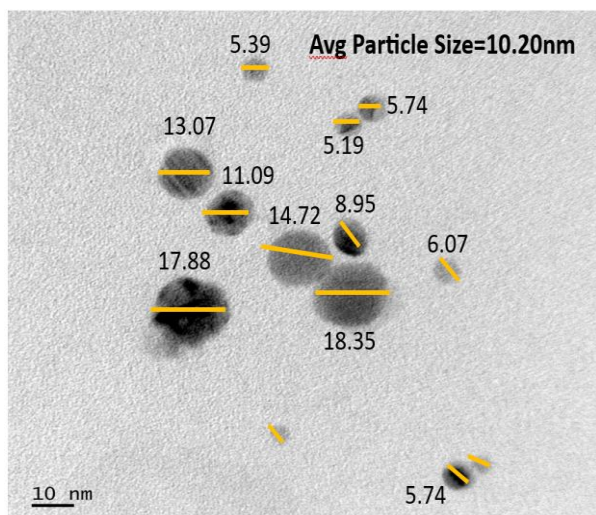


Figure 6a: TEM analysis of PVA -AgNPs Pg

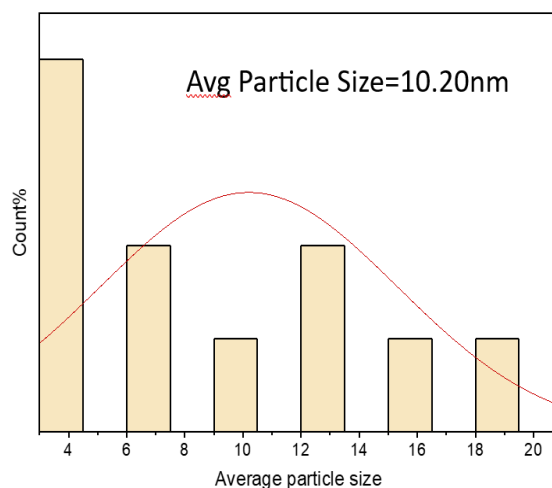


Figure 6b: Particle size distribution curve

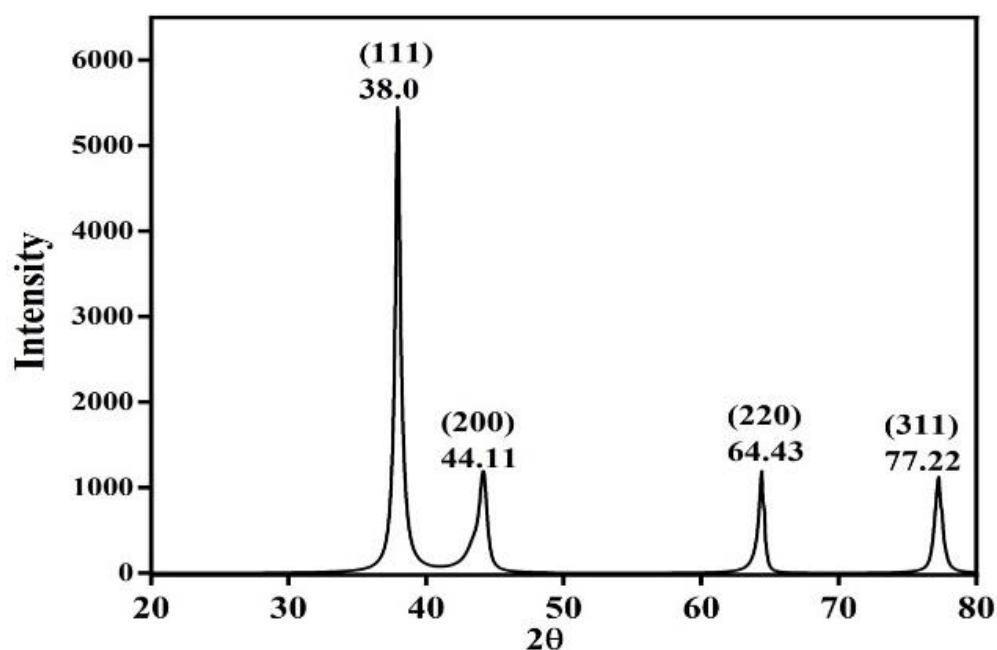


Figure 7: X-ray diffraction (XRD) analysis of PVA AgNP Pg

Table 1
The FTIR spectra results of PVA AgNPs

S.N.	Retention Time cm^{-1}	Functional group	Phyto compounds Identified
1	3302.01	O-H stretching (alcohol)	Poly Hydroxy compound
2	1635.64	C=C stretching (Alkene)	Ketone compounds
3	1404.18	O-H bending (Carboxylic acid)	Phenol or tertiary alcohol
4	1327.03	O-H bending (Alcohol)	Phenol or tertiary alcohol
5	1226.73	C-O stretching (alkyl aryl ether)	Cyclic ethers
6	686.66	C-Br stretching	halo compound
7	601.79	C-I stretching (halo compound)	halo compound
8	563.21	C-I stretching	halo compound
9	493.78	C-I stretching	halo compound
10	470.63	C-I stretching	halo compound
11	439.77	S-S stretching	Aryl disulfides
12	416.62	S-S stretching	Aryl disulfides

FTIR Analysis of PVA AgNP Pg: The functional groups existing in the plant leaf extract were recognized using FTIR spectra. These components of PVA AgNP Pg which lead to the reduction of silver ions for silver nanoparticle synthesis are shown in figure 8. The different absorption peaks are given in table 1. The peak at 3302 corresponds to -OH stretching while the 1635 cm peaks are allotted to -CH stretching. The other peaks were assigned to phenol or tertiary alcohol at 1404 cm and 1327 cm, cyclic ethers at 1226.73 cm and aliphatic bromo compounds at 686.66 cm and 601. Aryl disulfides are indicated in the regions of 439.77 and 416.

The leaf extract's phytochemicals including amino acids, triterpenoids, alkaloids, phytosterol and flavonoids, may contribute to the formation of nanoparticles, according to the FTIR spectra. Our findings were in consistent with the previous studies that found that the biomolecules included in

the leaf extract including phenolics, terpenoids, sesquiterpenes and flavonoids, were essential for changing silver from its ionic form to its metallic nano form.²⁹

Zeta potential analysis of PVA AgNP Pg: The stability of colloidal particles is one of the main properties of nanoparticles. Measurement of polydispersity index (PDI) value is a clear representation of nanoparticle size distribution. Particles will stay suspended in the liquid for a longer amount of time, preventing rapid agglomeration, according to the Zeta potential value of -30 to $+30$ mV.³ Compared to pure silver nanoparticles which have a mean zeta potential of -20 mV, the PVA-coated silver nanoparticles (PVA AgNP Pg) have an improved mean Zeta potential of -7.4 mV, which is a powerful signal of the stability of silver nanoparticles originated by electrostatic repulsion. The results are given in figure 9 and table 2.

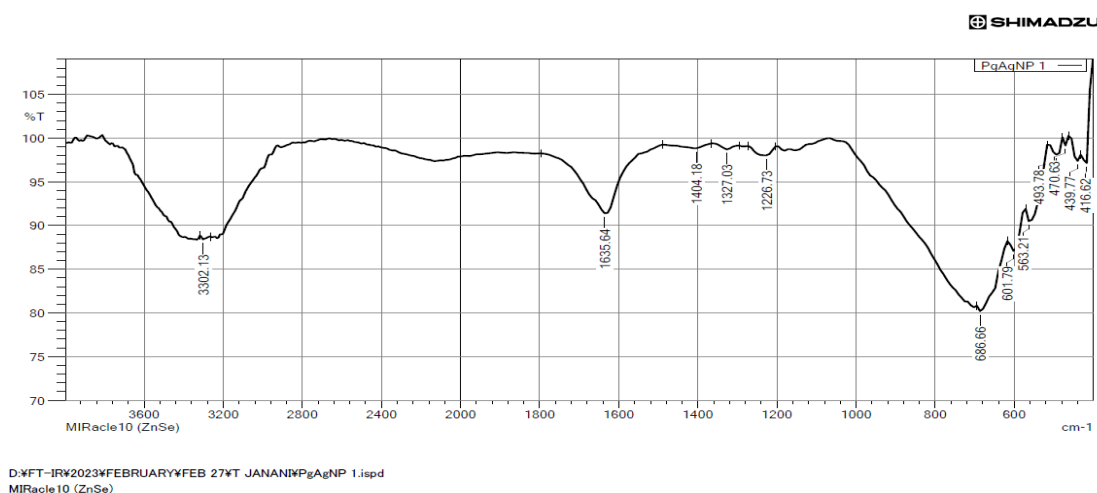


Figure 8: Fourier transform infrared spectrophotometer (FTIR) analysis

Zeta potential distribution

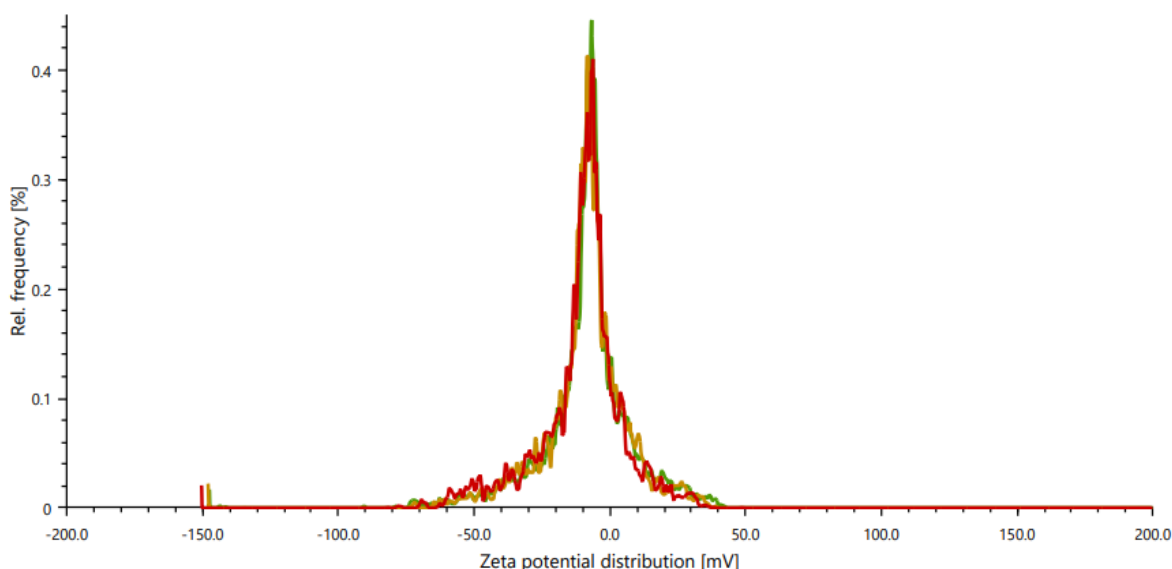


Figure 9: Zeta potential analysis of PVA AgNP Pg

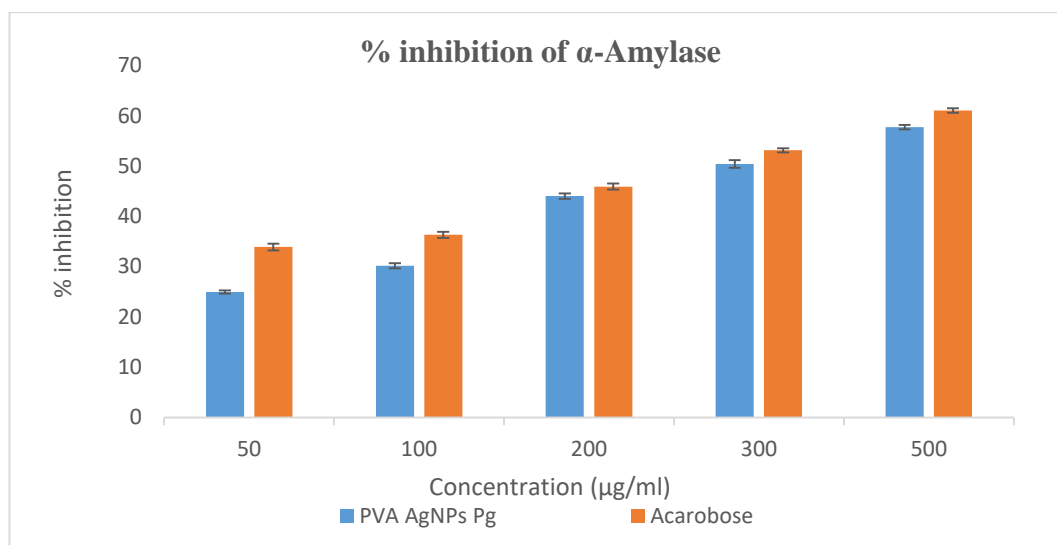


Figure 10: Alpha-amylase inhibitory activity of PVA AgNPs Pg and acarbose

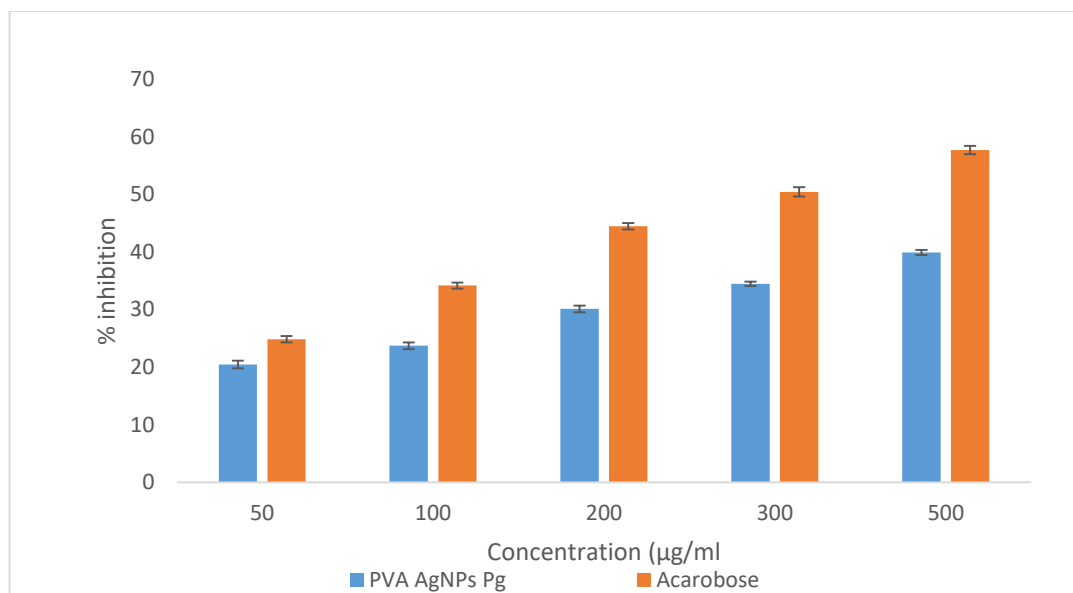


Figure 11: Alpha-glucosidase inhibitory activity of PVA AgNPsPg and acarbose

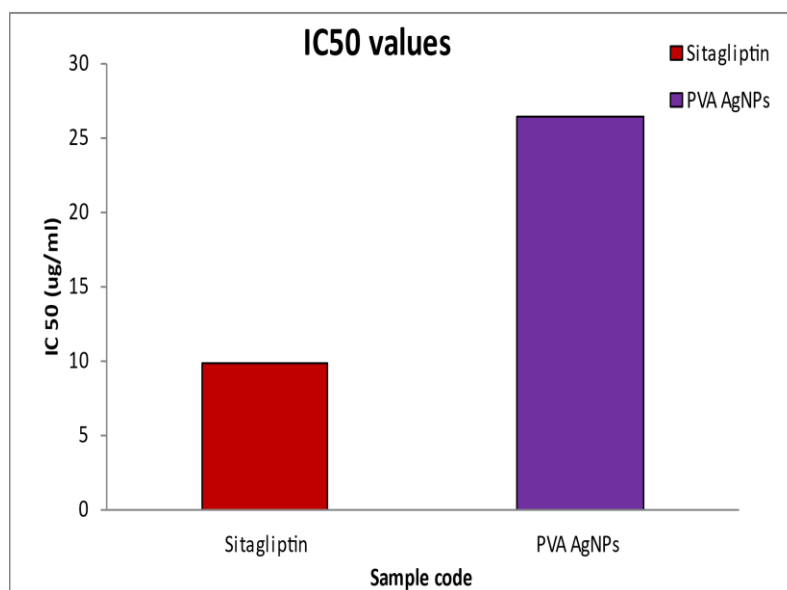


Figure 12: DPP- IV inhibitory activity of PVA AgNPsPg and sitagliptin

Table 2

Mean zeta potential, Hydro dynamic diameter and Polydispersity Index of Pure AgNPs and PVA- AgNPs Pg

No	Sample	Mean zetapotential	Hydro dynamic diameter	Poly dispersity Index
1	Pure AgNPs	-20mv.	91.32 nm	0.20
2	PVA AgNPs Pg	-7.4 mV	159.76nm	0.10

Table 3

IC₅₀ of Alpha-amylase

Alpha- amylase IC ₅₀ (µg/ml)	PVA AgNP Pg	Acarbose
	345.90	271.00

In vitro antidiabetic activity

Alpha- amylase inhibitory activity: Alpha-amylase, a digestive enzyme, degrades and consumes glucose and carbohydrates. Delay in the digestion of starch and oligosaccharides causes blood glucose levels and glucose absorption to drop when digestive enzymes such as α -amylase are blocked.³¹ Figure 10 shows the inhibitory impact of PVA AgNPs Pg on the α -amylase enzyme at concentrations ranging from 50 to 500 µg/ml. Inhibition levels varied between 24.94 and 57.72%, the highest inhibitory doses for PVA AgNPs Pg (500µg/ml) and acarbose were 57.72% and 61.06% respectively. Table 3 displays the IC₅₀ values of the PVA AgNPs Pg, which were determined to be comparable to the traditional acarbose. *In vitro* α -amylase activity was efficiently suppressed by the synthesized nanoparticles.

Hydroethanolic extract of *Myristica fragrans* seed's silver nanoparticles exhibit antidiabetic activity. Through diffusion and absorption studies, this study examined the efficiency of glucose transport across membranes and discovered an inhibitory action on α -amylase and α -glucosidase.²⁴ Antidiabetic activity of black cumin seed extract capped AgNPs was carried out by analysing its alpha- amylase inhibitory activity. This finding suggested that increased phenolic component concentrations in the seed extract must have contributed to the significant α -amylase inhibitory action.³⁰

Alpha-glucosidase inhibitory activity: Alpha-glucosidase's(α -glucosidase) main function is to digest and degrade complex carbohydrates into little, readily absorbed bits. Its inhibition is an active strategy to decrease the rate of glucose absorption and to control high postprandial blood glucose levels, that will hinder diabetes development.¹⁷ At concentrations of 50, 100, 200, 300 and 500µg/ml, the percentage inhibition of α -glucosidase by PVA AgNPs Pg improved in a concentration-dependent way and was found to be 20.43 to 39.89. The results were shown in figure 11 and table 4. The green-synthesized silver nanoparticles produced from *Fagonia cretica* (F. cretica) leaves extract showed a strong α -glucosidase enzyme repressive action.¹⁵

Glucose Diffusion Assay: The *in vitro* capacity of *Pisonia grandis* leaf-derived biosynthesized silver nanoparticles to obstruct glucose flow across the dialysis membrane was

examined. As the silver nanoparticles stuck to the glucose molecule, they significantly reduced the glucose's ability to traverse the membrane Table 5 shows the absorbance of the control and PVA AgNPs Pg over three hours. The inhibitory activity might be attributable to the glucose molecule interacting with the PVA AgNPs Pg components. Both control and Ag-NPs displayed potential in obstructing the glucose mobility through the membrane for a period of 3 hrs having maximum concentration of 0.7 and 0.15 mg/ml respectively.

Previous studies reported the inhibition of glucose transport by hydroethanolic extract of *Myristica fragrans* seeds (MFHE). The glucose molecule and the elements in the MFHENP may create a complex, which might be the cause by the inhibitory activity showing the absorbance values of the control and MFHENP to be 0.18 and 0.38 respectively of time period of 3hrs.²⁴ Management of diabetes mellitus was proven by glucose diffusion studies of silver nanoparticles using the aqueous leaf extracts of *Piper betle* (BL). Glucose diffusion was found to be inhibited after 150 min with the concentration of *Piper betle* (BL) at 80 µg/ml.¹⁸

DPP-IV Enzyme inhibition study of the PVA AgNPsPg:

According to the statistical results from the DPP-IV enzyme inhibition investigation by Elisa reader, the PVA AgNPsPg showed good DPP-IV enzyme inhibition activity in the concentration range of 12.5-200ug/ml. PVA AgNPsPg had IC₅₀ values of 26.39ug/ml, while the reference drug sitagliptin had an IC₅₀ concentration of 9.8ug/ml (Table 6). The highest percentage of DPP-IV inhibition observed for PVA AgNPsPg of concentration 200 ug/ml was 96.18% and inhibition observed for sitagliptin of concentration 100 uM/m was 99.37% respectively (Figure 12 and table 6). From the review reports on DPP-IV inhibition studies the *Withania coagulans* root extract showed better inhibition efficacy of 8.76 µg/ml and 21.03 µg/ml against DPP-IV at different percentage of methanol extract (100% and 80n%).⁷

The anti-diabetic potential of bioactive peptides from germinated soybean was carried out by the suppression of dipeptidyl peptidase. Along with intestinal α -glucosidases and α -amylase, the study also revealed the activity of peptides produced from the gastrointestinal digestion of germinated soybean proteins as DPP-IV inhibitors.

Table 4
IC₅₀ of Alpha- glucosidase

Alpha-glucosidase IC ₅₀ (μg/ml)	PVA AgNP Pg	Acarbose
	700.00	339.60

Table 5
Glucose Diffusion Assay by PVA-AgNPs Pg

Time Interval (Min)	Control at 640 nm	PVA-AgNPsPg at 640 nm	Control Concentration (mg/ml)	PVA-AgNPsPg Concentration (mg/ml)
30	0.098	0.041	0.05	0.02
60	0.182	0.091	0.11	0.04
90	0.271	0.139	0.15	0.08
120	0.331	0.178	0.19	0.11
150	0.339	0.234	0.19	0.13
180	0.421	0.269	0.7	0.15

Table 6
IC₅₀ of DPP-IV

DPP-IV IC ₅₀ (μg/ml)	PVA AgNP Pg	Sitagliptin
	26.39	9.8

The study's findings showed that peptide fractions ranging from 5 to 10 kDa that were separated from 6DSPD, may have anti-diabetic effects (IC₅₀ = 0.91).¹⁰

The potential of plant-based DPP-IV inhibitors to control incretin activity by the attainment of polyherbal formulations and nano phytomedicines, as well as their ability to endure oxidative stress under diseased circumstances associated to diabetes, were studied. When glucose is taken orally as contrasting to intravenously, the body makes more insulin with respect to meals due to insulinotropic action of incretin hormones, even in situations when plasma glucose levels remain constant.⁷

Conclusion

Green synthesis is advocated in this study as an inexpensive and sustainable way to prepare nanoparticles. It has been established by several characterization methods that PVA AgNP Pg is engendered. A peak at 430 nm is seen by UV-visible spectroscopy, particle size and shape are confirmed through SEM, silver ions are apparent in EDX spectra, the crystalline structure is revealed by XRD, functional groups that serve as capping material towards PVA AgNP Pg are identified by FTIR and stability is shown by Zeta potential and shape and particle size by TEM.

The study found that PVA AgNP Pg inhibits α -amylase, α -glucosidase and DPP-IV activities and effectively prevents glucose transfer around membranes, as evidenced via glucose diffusion and absorption tests. It follows that they are valuable in the administration of diabetes mellitus. It will require a more extensive examination into a variety of features of *in vitro* and *in vivo* measures to show PVA AgNP Pg's competitiveness dealing type 2 diabetes mellitus,

demonstrating enhanced curative efficacy and reduced unpleasant consequences.

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