

Formulation of Silver Nanoparticles Using *Gymnema Sylvestre* Leaf Extract and *In-Vitro* Anti-diabetic Activity

ABSTRACT

Aim: Formulation of Silver Nanoparticles Using *Gymnema Sylvestre* Leaf Extract and *In-Vitro* Anti-diabetic Activity.

Place and Duration of the Study: The present work has been carried out between April-2021 to July-2021 and at Mandasur University Mandasaur, Madhya Pradesh-458001.

Methodology: A versatile technique was implemented for the synthesis of silver nanoparticles using aqueous and alcoholic extract of leaves of *Gymnema Sylvestre*. The Ag-NP was subjected to UV, FTIR, XRD, and particle size analysis. The silver nanoparticles *Gymnema Sylvestre* evaluated its anti-diabetic activity by inhibiting the enzymes α -amylase.

Results: The reduction of silver nitrate to silver nanoparticles was confirmed by UV-Vis spectrophotometer showing a typical resonance (SPR) at about 400 nm which is specific for Ag-NPs. In FT-IR analysis the strong band peak at 3355 cm^{-1} in the extract is vibration bands, which may be due to the overlapping of amine N-H stretching bands. SEM and EDX images reveal that the particles are spherical and the relatively uniform shape of the silver nanoparticles was confirmed in the range of 70-100 nm in extract, whereas DLS showed good zeta potential and less particle size.

Conclusions: The silver nanoparticles that were biologically synthesized from *Gymnema Sylvestre* were economical, non-toxic, and environmentally benign. Due to the reducing and capping nature of the bioactive Phyto-compounds, present in the aqueous extracts. This extract is found to be suitable for the green synthesis of silver nanoparticles. Synthesized silver nanoparticles exhibit the highest level of anti-diabetic activity by inhibiting carbohydrate metabolizing enzymes such as α -amylase. Therefore,

synthesized nanoparticles can be a good therapeutic agent for controlling diabetes by inhibiting enzymes that hydrolyze carbohydrates.

Keywords: *In-Vitro* anti-diabetic Activity, silver nanoparticles, green synthesis, α -amylase

ABBREVIATIONS

FTIR: Fourier Transform Infrared Spectroscopy

XRD: X-ray diffraction

SEM: Scanning Electron Microscopic

EDX: Energy Dispersive X-Ray Spectroscopy

GS: *Gymnema Sylvestre*

AgNO₃: Silver Nitrate

Ag-NP: Silver nanoparticles

1. INTRODUCTION

Diabetes mellitus is a chronic disease caused by a partial or total deficiency of insulin, which causes hyperglycemia leading to acute and chronic complications [1]. The incidence of diabetes mellitus is increasing worldwide. Control of plasma glucose concentrations is vital for decreasing the incidence and severity of the long-term effects of diabetes [2]. Synthetic drugs are likely to produce severe effects, as well as being unsuitable for use during conditions such as pregnancy. In addition to conventional diabetes therapy; several studies have shown that some plants used in traditional medicine have beneficial effects in diabetic patients [3, 4]. More than 400 plants worldwide have been documented to be useful for the treatment of diabetes [5-7]. Most traditional anti-diabetic plants await proper medical and scientific evaluation to determine their ability to improve blood sugar control.

Gymnema Sylvestre (GS), a plant used in Indian Ayurvedic medicine for the treatment of diabetes mellitus, has also been known since ancient times to have an anti-saccharin flavoring effect. Many researchers have reported that *G. Sylvestre* lowers blood sugar, stimulates the heart, uterus, and circulatory system, and exhibits anti-sweet and hepatoprotective activities [8]. *Gymnema Sylvestre* is an herb native to the tropical forests of India and has been used in herbal medicine as a treatment for diabetes for nearly two millennia, but there is insufficient scientific evidence to draw firm conclusions on its effectiveness.

Gymnema Sylvestre has been used for centuries in the treatment of diabetes in folk medicine, Ayurvedic and homeopathic systems. In addition, it also has antimicrobial, antitumor, obesity, anti-inflammatory, and anti-hyperglycemic activity. Also in our previous studies, we have reported that the bioactive compounds present in *Gymnema Sylvestre* have anti-stress, anti-

allergic, and anti-ulcer activity. Administration of *Gymnema Sylvestre* extract to diabetic rats increased the activity of superoxide dismutase and decreased lipid peroxide by directly eliminating reactive oxygen species, due to the presence of numerous antioxidant compounds, or by increasing the synthesis of antioxidant molecules (Albumin and uric acid) [9].

Currently, numerous methods, particularly physical, chemical, and natural, and crossover techniques can be found to synthesize various types of precious metal nanoparticles, such as silver, gold, silicon, zinc, and platinum nanoparticles. Although current techniques related to physical and chemical substances tend to be better known and popular concerning the activity associated with nanoparticles, the actual degree of associated environmental toxicity, as well as the non-biodegradable nature of the elements, has limited their programs [10,11].

In current research work, attempts have been made to synthesize silver nanoparticles using *Gymnema Sylvestre* leaf extract. The characterization was performed using the different spectral analyzes. The synthesized silver nanoparticles were analyzed for their anti-diabetic activity. Our current results have clearly shown that it is indeed possible to have a much more environmentally friendly way of synthesizing Ag-NP without compromising its medicinal properties and therefore plant extracts can be a good alternative to obtain Ag-NP with various improved properties.

2. MATERIALS AND METHODS

2.1 Chemicals and reagents

Dialysis membrane and 1-4, α -D-glucan-glucanohydrolase (α -amylase) were purchased from HiMedia Laboratories, Mumbai, India. Silver nitrate (pure > 99%) was purchased from Sigma Aldrich, India. All other chemicals, reagents, kits, and solvents used in this study were analytical grade and were sourced locally.

2.2 Selection and Collection Plant

Several plants in the literature have been reported for anti-diabetic activity. The *Gymnema Sylvestre* has been reported for various biological activity and its hypoglycaemic potentials are also reported. *Gymnema Sylvestre* shown in **fig. 1** has been selected for the current research work, the plant materials were collected from the medicinal plant garden of the SGMR Pharmacy College Mahagaon, Kolhapur Maharashtra, India. The leaves were washed with water and deionized water to remove the fine dust materials and then, plant leaves are shaded dried for 1 week to completely remove the moisture. The herbarium sheet of the plant material was prepared [8].



Fig. 1: *Gymnema sylvestre* leaf

Table 1: Details of *Gymnema sylvestre*

Particulars	Details
Name	<i>Gymnema Sylvestre</i>
Common Name	Gymnema, Bedakicha Pala, Gurmar, Madhunashini
Kingdom	Plantae
Family	Apocynaceae
Occurrence	Tropical Asia, China, the Arabian Peninsula, Africa, and Australia
Chemical Constituents	Oleanane, Gymnemic acid, Gymnemasides, Gymnemic acid, Gymnemic acid A1.

2.3 Preparation of *Gymnema Sylvestre* (leaf) extract

1. Aqueous extract

The dried leaves were pulverized well with a domestic mixer to make a fine powder. 2.5 g of powder sample was mixed into 100 ml of deionized water and the mixture was boiled at 60⁰C on a hot magnetic stirrer for 30 min. After cooling the leaf extract was filtered with Whatman filter paper. The extract was filtered again by a vacuum filtration unit. The filtrate was stored for further use.

2. Alcoholic extract

The dried leaves were pulverized well with a domestic mixer to make a fine powder. The powdered plant material was subjected to solvent extraction using ethanol as solvent. After cooling the leaf extract was filtered with Whatman filter paper. The extract was filtered again by a vacuum filtration unit. The filtrate was stored for further use [8].

2.4 Synthesis of silver nanoparticles from *Gymnema Sylvestre*

For the synthesis of silver nanoparticles, 2 ml of the extract (aqueous and alcoholic) of *Gymnema Sylvestre* was taken into a round bottom flask containing 60 ml of 1mM AgNO₃ solution and kept for incubation at 50°C for 2-8 hrs. All the reaction process was carried out in a dark room. The progress of the reaction was monitored via color change [12].

2.5 *In-vitro* anti-diabetic activity (α -Amylase inhibition activity)

Anti-diabetic activity via α -Amylase inhibition technique. *In-vitro* α -amylase inhibition was studied by the DNS method [13]. In brief, 500 μ L of the test compound (10, 30, 100 μ g/ml) was allowed to react with 500 μ L of 0.1M phosphate buffer pH 6.9 containing α -amylase enzyme (fungal diastase (0.5%). After 10-minute incubation at 25°C, 500 μ L of 1% starch solution in 0.1M phosphate buffer pH6.8 was added and incubated at 25°C for 10 min. The same was performed for the controls where 500 μ L of the enzyme was replaced by the buffer. After incubation, 1000 μ L of dinitrosalicylic acid reagent was added to both control and test. They were kept in a boiling water bath for 10 min and cooled. The blank was prepared by replacing the enzyme with buffer for each set of concentrations of a test sample. The control was maintained without the addition of a sample that represented 100% enzyme activity. The absorbance was recorded at 540nm using a spectrophotometer and the percentage inhibition of α -amylase enzyme was calculated using the formula. Acarbose was used as the positive control.

$$\text{Inhibition (\%)} = \frac{\text{Abs 540 (control)} - \text{Abs 540 (extract)}}{\text{Abs 540 (control)}} * 100 \text{ ----- (1)}$$

2.6 Characterization of the synthesized Ag-Silver Nanoparticles

The Synthesized Silver Nanoparticles from *Gymnema Sylvestre* Leaf Extract were characterized using the following spectroscopic techniques.

UV-Vis characterization

Mixing of the *Gymnema Sylvestre* Leaf Extract in the AgNO₃ solution makes the color change of the solution which is an indication of the formation of the silver nanoparticles in the stabilized form. Confirmation of the silver nanoparticle generation is carried out via estimation of the UV spectra which indicated a typical silver surface Plasmon resonance at about 400 nm [8, 12].

FTIR analysis

Fourier transform infrared (FTIR) spectral measurements are commonly utilized for the estimation of the functional group. The FTIR analysis was utilized in the confirmation of the silver nanoparticles via estimation of the peak values which accounts for the various functional groups which are normally responsible for reducing and capping these silver nanoparticles [12].

Scanning electron microscopic (SEM) analysis and energy-dispersive X-ray spectroscopy (EDX)

Surface morphology and size of biosynthesized Ag-NPs using both extracts of *Gymnema Sylvestre* were studied by SEM images at higher resolution with different magnifications as shown in the figure below. The SEM images showed the existence of small spherical nanoparticles. The chemical composition and crystalline nature of the biosynthesized Ag-NPs were obtained by the EDX analysis. Generally, it is known that Ag-NPs give a typical optical absorption peak in the range 2.1–3.7 KeV due to surface Plasmon resonance [12].

X-ray diffraction (XRD)

The synthesized silver nanoparticles were characterized by XRD estimate the crystal and nanostructure of silver particles. XRD pattern of silver nanoparticles was represented at optimal conditions. The results of the XRD analysis were in line with the results which are reported in the literature [13].

Particle size and zeta potential Analysis

The aqueous suspension of the synthesized nanoparticles was filtered through a 0.22 μm syringe driven filter unit and the size of the distributed nanoparticles were measured by using the principle of Dynamic Light Scattering (DLS) technique made in a Nanopartica (HORIBA, SZ-100) compact scattering spectrometer [12, 13].

3. RESULTS AND DISCUSSION

3.1 Synthesis of silver nanoparticles from *Gymnema Sylvestre*

Aq. Extract: The synthesis of the silver nanoparticles was ascertained by the color change from faint yellow color to the dark brown color solution. After completion of the incubation period, the color of the mixture of silver nitrate solution and *Gymnema Sylvestre* leaf extract was changed to dark brown color which ascertained the formation of the silver nanoparticles. The confirmation of the silver nanoparticles was carried out via UV-visible characterization. The silver surface was known for typical Plasmon resonance at 400 nm, the UV visible spectra of synthesized silver nanoparticles showed Plasmon resonance peak at 400 nm.

Alcoholic extract: The green synthesis of silver nanoparticles was done by using alcoholic extract of *Gymnema Sylvestre* leaf as a reducing agent. The confirmation of the formation of the silver nanoparticles was done with the formation of the dark color after the reaction

3.2 UV-Visible spectral analysis

It is well known that silver nanoparticles exhibit a brown color, which results from the excitation of Plasmon vibrations from the surface of the silver nanoparticles. After the addition of a 1 mM silver nitrate solution to the aqueous extract, the color of the composition had changed to a dark brown color. The maximum absorbance peak is observed at 390 nm for *the Gymnema Sylvestre* (**Fig. 2a and 2b**). General observations suggest that the bio-reduction of Ag^+ (silver ions) to $\text{Ag}^{(0)}$ has been confirmed by UV-Visible spectroscopy [8].

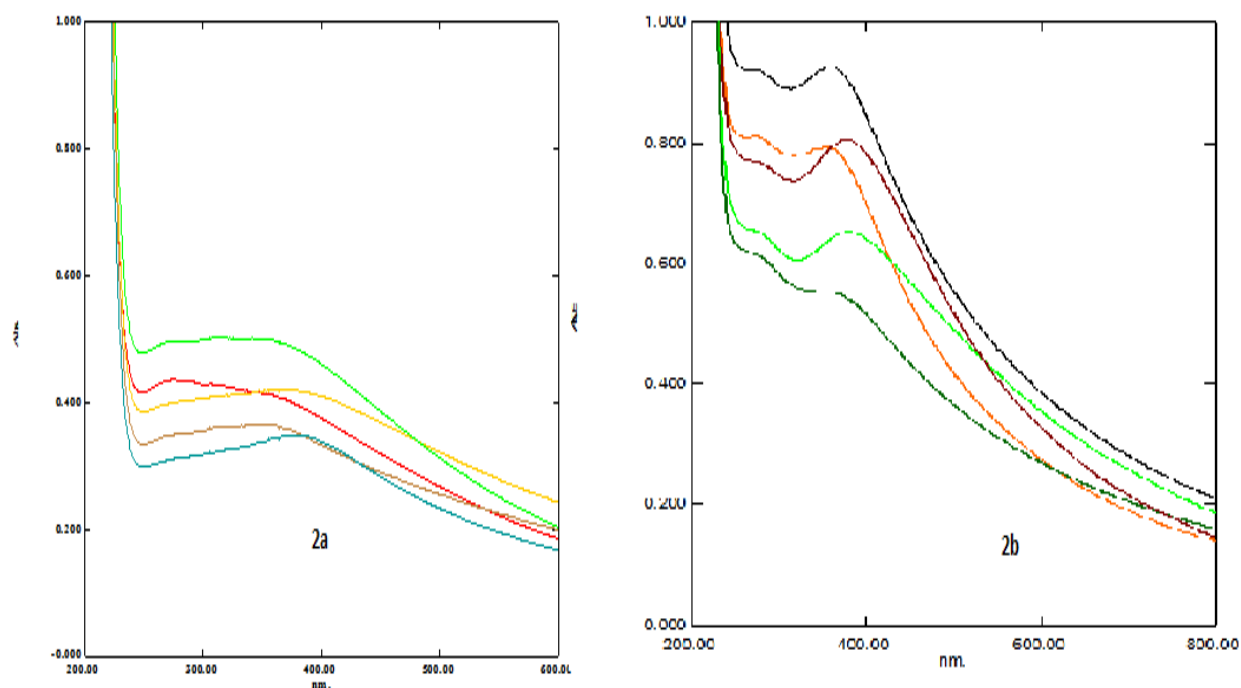


Fig. 2: (2a) UV spectra of aq. Extract and (2b) alcoholic extract

3.3 FTIR analysis

The FTIR spectrum of silver nanoparticles biosynthesized using *Gymnema Sylvestre* (**Fig.3a and 3b**) shows absorption peaks at 3355, 2989, 2885, 2822, 2104, 1772, 1637, 1406, and 1049 cm^{-1} . The peak at 3355 cm^{-1} reveals the presence of NH stretching vibration, indicating the group of primary and secondary amino proteins, 2989 cm^{-1} reveals the presence of CH stretching vibration, indicating the presence of alkenes, 2885 cm^{-1} reveals the presence of stretching CH vibration, which indicates the presence of alkanes, 2822 cm^{-1} reveals the presence of CH stretching vibration, which indicates the presence of aldehydes, 2104 cm^{-1} reveals the presence

of -C=C- vibration of stretching, indicates the presence of alkynes, 1772 cm^{-1} reveals the presence of stretching vibration $\text{C}=\text{O}$, indicating the presence of carbonyls in proteins, 1637 cm^{-1} reveals the presence of stretching vibration NH bending, indicating the presence of primary amines, 1406 cm^{-1} reveals the presence of DC stretching vibration, indicating the presence of aromatics, 1049 cm^{-1} reveals the presence of CC stretching vibrations, indicating the presence of aliphatic amines, respectively, indicating the participation of proteins to the reduction and stabilization of silver ions. The position of these bands was comparable to that of phenols, flavonoids, and tannins. Therefore, we can confirm that the nano tamponing of *Gymnema Sylvestre* extract is responsible for the reduction and subsequent stabilization of Ag-NPs. *Gymnema Sylvestre* extract was coated with phytochemicals and secondary metabolites such as saponins, terpenoids, and gymnemic acid derivative of gymnemagenin which contains the functional groups of amines, aldehydes, carboxylic acids, and alcohols [8].

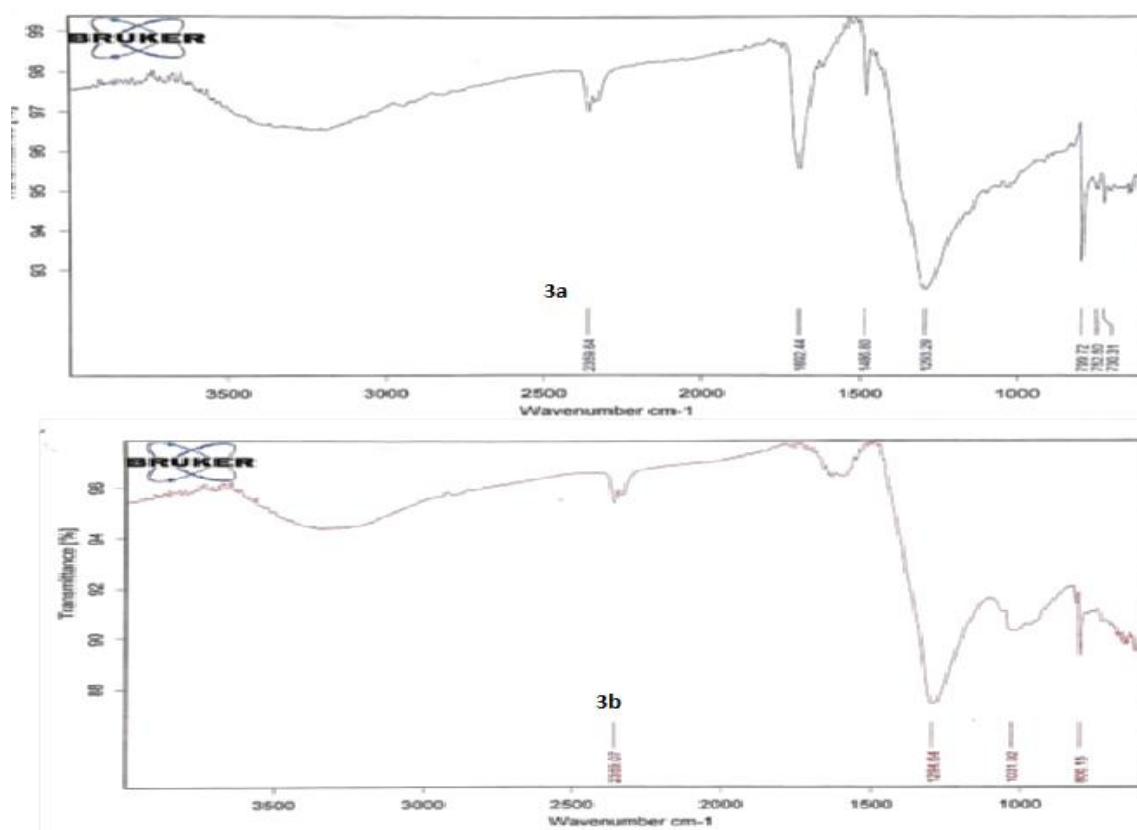


Fig. 3: (3a) FTIR spectra of aq. Extract and (3b) alcoholic extract

3.4 Scanning electron microscopic (SEM) analysis and energy-dispersive X-ray spectroscopy (EDX)

Surface morphology and size of biosynthesized Ag-NPs using both extracts of *Gymnema Sylvestre* were studied by SEM images at higher resolution with different magnifications as shown in figure (**fig. 4a, and 4b**). The SEM images showed the existence of small spherical

nanoparticles. The chemical composition and crystalline nature of the biosynthesized Ag-NPs were obtained by the EDX analysis. Generally, it is known that Ag-NPs give a typical optical absorption peak in the range 2.1–3.7 KeV due to surface Plasmon resonance [8, 13].

Synthesized Ag-NPs were further characterized for the surface morphology and metallic composition with SEM with EDX analyses. The Scanning electron microscopic (SEM) analysis of the high-density Ag-NPs synthesized alcoholic extract of *Gymnema Sylvestre* leaf is shown in **Fig 4a**. The SEM analysis indicated the morphology and size of the nanoparticles were dependants on the biomolecules which are present in the extract. The microscopic images indicated silver nanoparticles synthesized are having spherical shapes and the size of the particles is in the range 70–100 nm. A number of the results reported the possible effects of the extracting solvent on the size and shape of the silver nanoparticles which can be attributed to the concentration and type of the biomolecules which are present in the extract. The type and concentration of the biomolecules in the extract led to the formation of the hydrogen bond and electrostatic interaction between biomolecules and Ag as these molecules are capping the silver molecules. This capping phenomenon is one of the reasons for the stabilization of the nanoparticles. FE-SEM analysis showed surface morphology and topography of the silver nanoparticles. The formation of Spherical SNPs without agglomeration was evidenced. Energy-dispersive X-ray spectroscopy (EDX) analysis was performed to analyze the chemical composition of the synthesized silver nanoparticles using extract of *Gymnema Sylvestre* leaf. Generally, it is known that Ag-NPs give a typical optical absorption peak in the range 2.1–3.7 KeV due to surface Plasmon resonance. The EDX spectra of the synthesized Ag-NP showed a typical Plasmon resonance peak at 2.9 KeV which confirmed the formation of the Ag-NP. The EDX spectra also showed the presence of the other elements which might be due to the presence of the biomolecules in the ethanolic extract of the *Gymnema Sylvestre* leaf as shown (**fig 4c and 4d**).

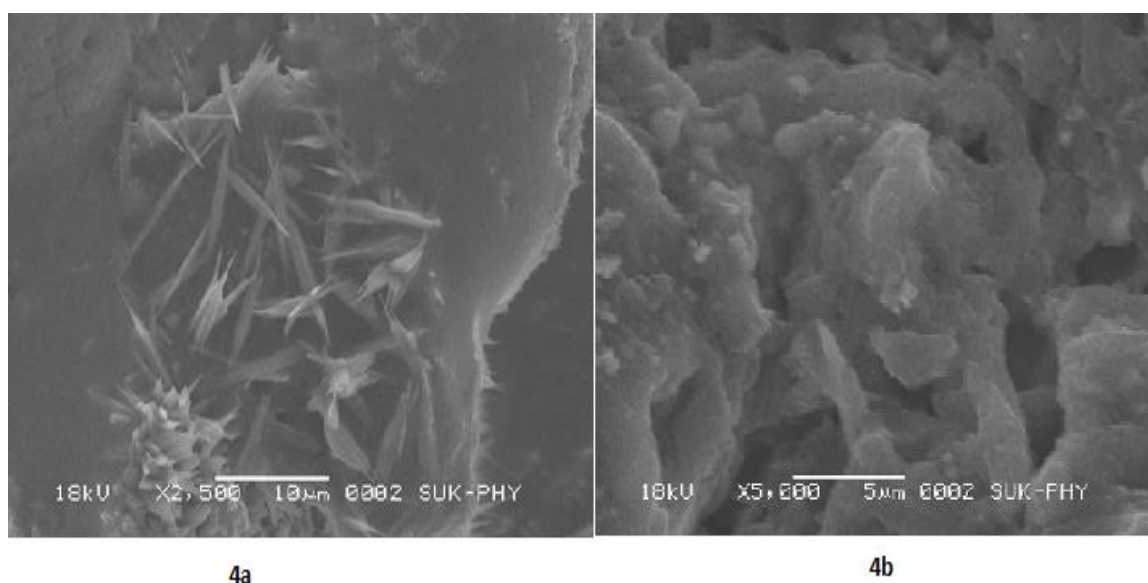


Fig. 4: (4a) SEM image of aq. Extract and (4b) alcoholic extract

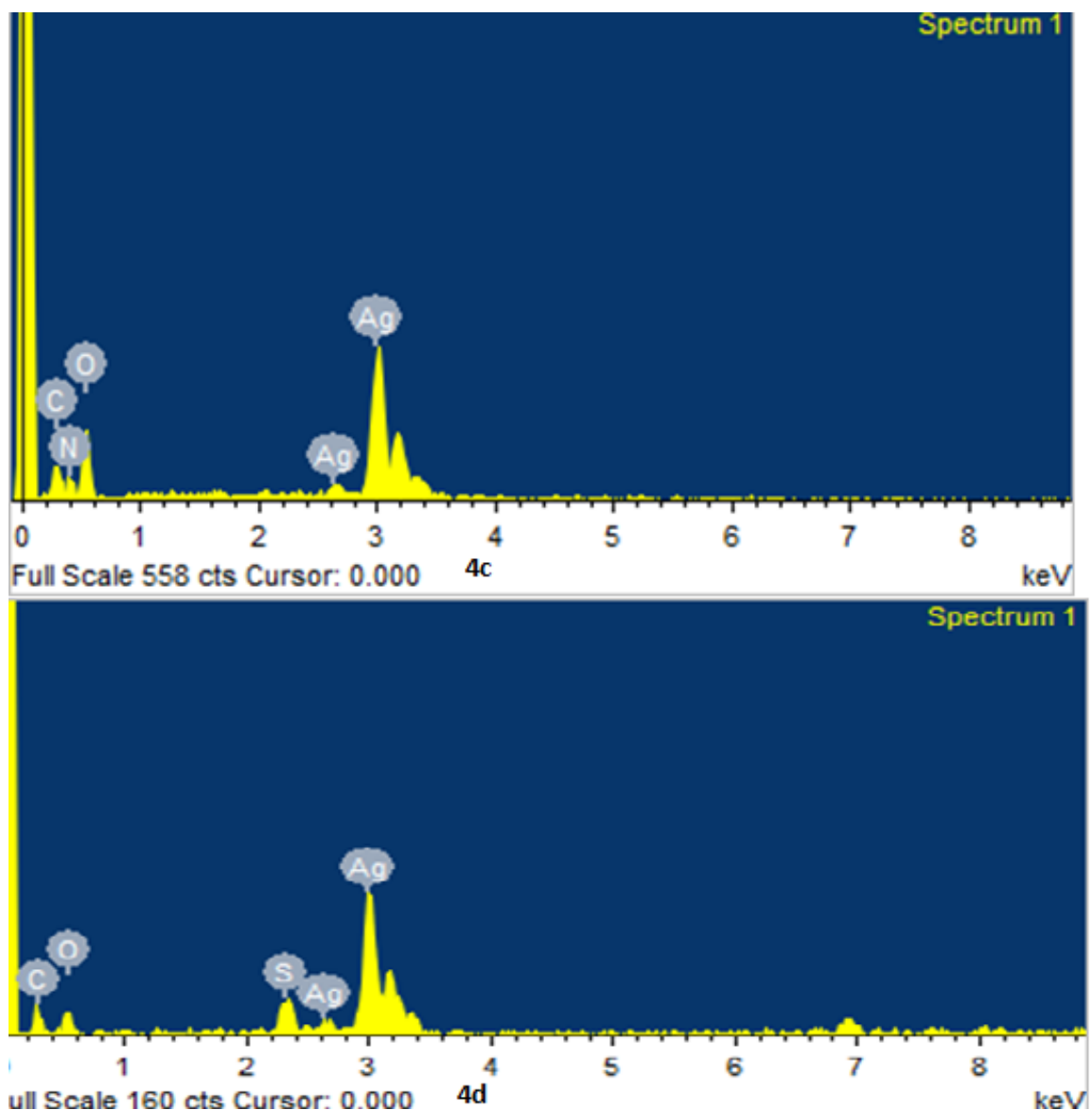


Fig. 4: (4c) EDS spectra of aq. Extract and (4d) alcoholic extract

3.5 XRD analysis

The XRD analysis of Ag-NP shows several size-dependent characteristics that lead to the regular position, height, and width of the peak. The XRD was mainly conducted to study the crystalline nature of the silver nanoparticles of *Gymnema Sylvestre* synthesized in green. The diffraction intensities were recorded from 10° - 80° to 2θ angles (**Fig. 5a and 5b**). Five different and important characteristic peaks were observed in the 2θ of 38.6° , 45.8° , 58.7° , 64.8° and 78.6° corresponding to the planes (111), (200), (220), (311), and (222) which indicate that the SNPs are highly crystalline, respectively [8].

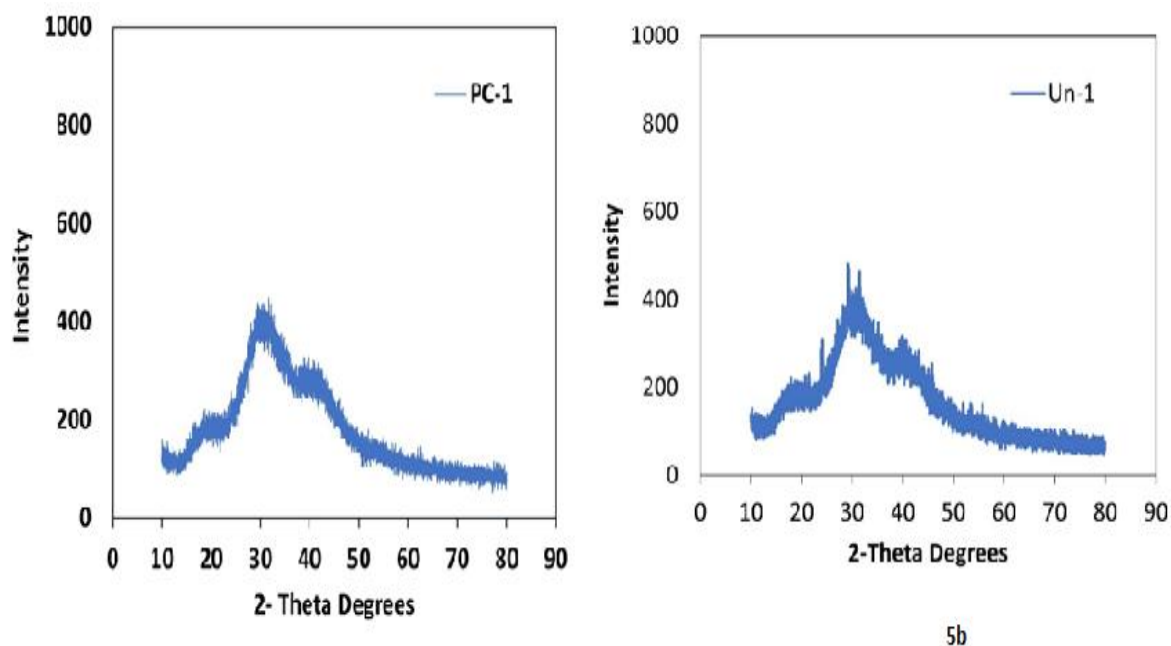


Fig. 5: (5a) XRD spectra of aq. Extract and (5b) alcoholic extract

3.6 Particle size and zeta potential Analysis

The particle size of the developed nanoparticles was determined using the Horiba SZ-100 system. The results of the particle size determination indicated the developed nanoparticles are having the desired size with a mean size of the particles being 87.9 nm. The zeta potential of the developed nanoparticles was found to be -21.8 mV, a negative value of the Zeta potential of the developed nanoparticles indicated these are stable. The results of the particle size and zeta potential are given in (fig. 6a and 6b) respectively.

The results of the particle size determination indicated the developed nanoparticles are having the desired size with a mean size of the particles being 145.1 nm. The zeta potential of the developed nanoparticles was found to be -15.4 mV, a negative value of the Zeta potential of the developed nanoparticles indicated these are stable. The results of the particle size and zeta potential are given in (fig. 6c and 6d) respectively [8, 13].

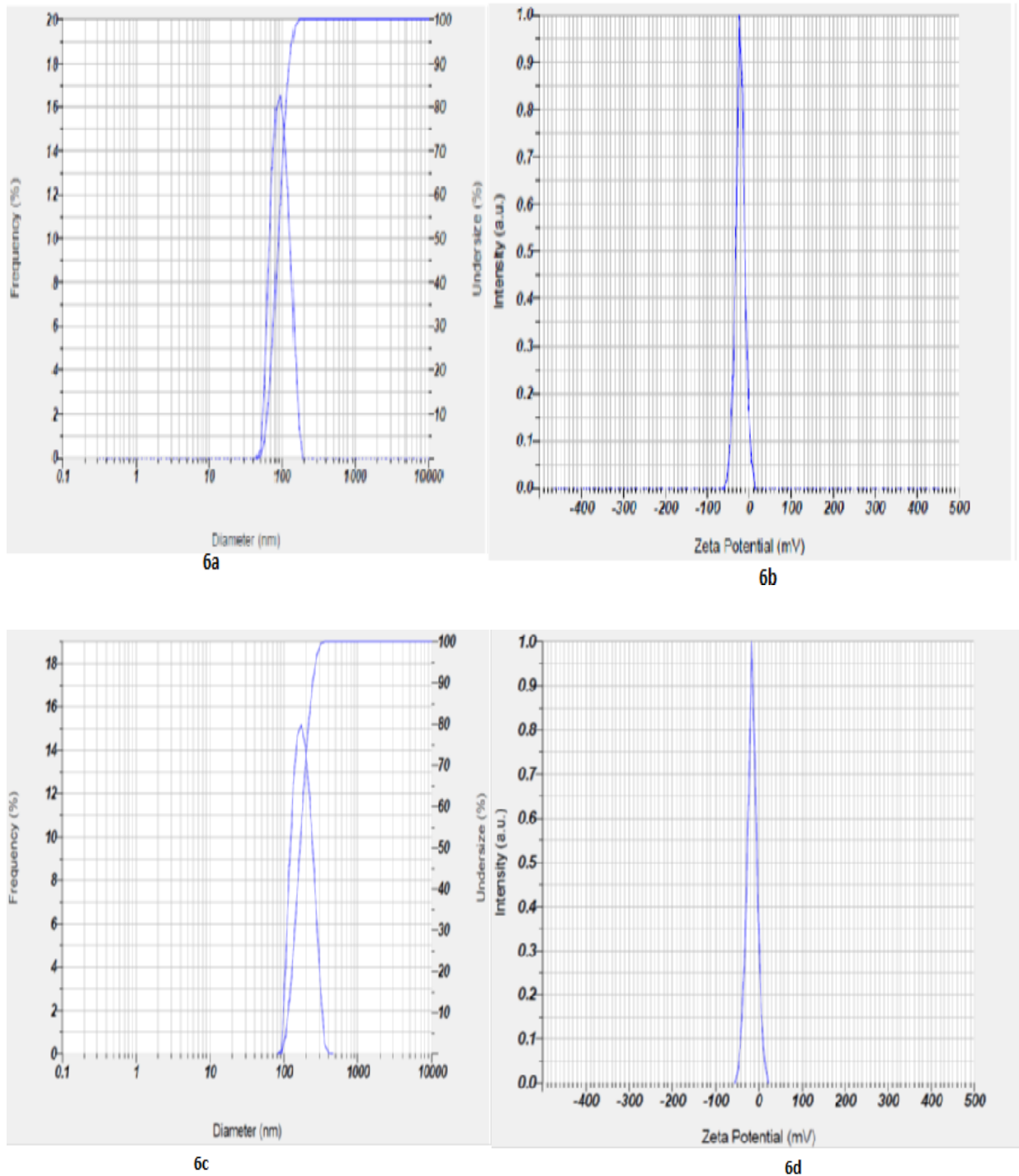


Fig. 6: (6a) particle size image of aq. Extract and (6b) zeta potential image, 6(c) particle size image of aq. Extract and (6d) zeta potential image

3.7 *In-vitro* anti-diabetic activity (α -Amylase inhibition activity)

α -amylase is a key enzyme in carbohydrate metabolism. Inhibition of α -amylase is one of the best ways to lower blood sugar levels. Amylase inhibitors or starch blockers prevent the body from absorbing starch in the diet. Therefore, it is possible to reduce the rise in blood sugar by consuming carbohydrates [14]. Green synthesized silver nanoparticles have been reported to act

as amylase inhibitors to lower blood sugar levels [15]. The silver nanoparticles synthesized by *Gymnema Sylvestre* indicated a higher level of α -amylase inhibitory activity than acarbose at all concentrations tested, as shown in **Tables 2 and 3**. Since the concentration of silver nanoparticles, the percentage of inhibition is also increased by dose-dependent manner. Similarly, silver nanoparticles synthesized by other medicinal plants have been exposed to the inhibitory activity of α -amylase [16].

Table 2: α -amylase enzyme inhibition assay of silver nanoparticles developed from the aqueous extract

Sample code	Concentration ($\mu\text{g/ml}$)	Absorbance at 540nm	% inhibition
Standard Acarbose	100	0.21	58
Aq. Ag-NP	10	0.33	34
	30	0.31	38
	100	0.29	42

Table 3: α -amylase enzyme inhibition assay of silver nanoparticles developed from the alcoholic extract

Sample code	Concentration ($\mu\text{g/ml}$)	Absorbance at 540nm	% inhibition
Standard Acarbose	100	0.21	58
Alc. Ag-NP	10	0.32	36
	30	0.32	36
	100	0.27	46

4. CONCLUSION

The present study focused on the green synthesis of silver nanoparticles by *Gymnema Sylvestre* and the evaluation of its anti-diabetic activity by inhibiting the enzymes that hydrolyze carbohydrates. Synthesized silver nanoparticles exhibit the highest level of anti-diabetic activity by inhibiting carbohydrate metabolizing enzymes such as α -amylase. Therefore, synthesized nanoparticles can be a good therapeutic agent for controlling diabetes by inhibiting enzymes that hydrolyze carbohydrates. Further studies are needed to confirm its precise mechanism in animal and human samples to prescribe this product as a pharmacological agent for the treatment of diabetes.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we

do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

NOTE:

The study highlights the efficacy of "ayurvedic medicine" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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