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ANTIDIABETIC POTENTIAL OF PLANT-BASED SILVER NANOPARTICLES SYNTHESIZED BY USING THE EXTRACTS OF *Moringa oleifera*, *Abelmoschus esculentus* and *Catharanthus roseus*

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ABSTRACT

***Diabetes mellitus* is an endocrine disorder caused due to inadequacy of insulin or resistance leading to the increase in blood glucose level. This study was aimed to use plant-based silver nanoparticles (AgNPs) for its anti-diabetic potential. The AgNPs of drumstick (*Moringa oleifera*), okra (*Abelmoschus esculentus*) and periwinkle (*Catharanthus roseus*) were prepared using green synthesis approach. Characterization of plant-based AgNPs was performed to confirm the synthesization of nanoparticles by three methods *viz*., UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), and field emission scanning electron microscopy (FESEM). UV-visible spectroscopy showed surface plasmon resonance peak at 400 nm for *M. oleifera*-AgNPs (MO-AgNPs), and 401 nm for both *C. roseus*-AgNPs (CR-AgNPs) and *A. esculentus*-AgNPs (AE-AgNPs). FTIR was used to determine various functional groups present in these AgNPs. FESEM showed spherical nanoparticles of 12-22, 21-46 and 13-49 nm for AE-AgNPs, CR-AgNPs and MO-AgNPs, respectively. The size distribution of AgNPs was done using dynamic light scattering (DLS) analysis which showed maximum AgNPs at 50 nm (CR-AgNPs), 70 nm (AE-AgNPs) and 40 nm (MO-AgNPs). The plant-based AgNPs showed significant antidiabetic potential against carbohydrate digestive enzymes like amylase and glucosidase. All the three synthesized AgNPs showed increase in the inhibitory activity of α-amylase and α-glucosidase enzyme. D-glucose diffusion retardation index (GDRI) of AE-AgNPs and MO-AgNPs was almost same as the standard drug, acarbose, used for diabetes treatment. Hence, the above plant-based AgNPs possess anti-diabetic potential and can be used in diabetic related medicine formulations.**

Keywords: Diabetes, drumstick, green synthesis, okra, periwinkle, silver nanoparticles

INTRODUCTION

Diabetes mellitus is a major non-communicable disease which affects over 100 million people world-wide, and is undeniably one of the top five causes of death globally (Dubey and Mishra, 2018). Diabetes is a growing global concern which has affected over 415 million adults. India has the 2nd highest number of people with type 2 diabetes in the world. The Asian Indian population constitutes over 17% of the world's population has a distinctive physical characteristic of increased levels of intra-abdominal fat and insulin resistance, despite having a low body mass index (BMI) which increases risk of developing type 2 diabetes and experiencing premature coronary heart disease (Mehta *et al.*, 2009; Unnikrishnan *et al*., 2016). The International Diabetes Federation states that over 371 million people worldwide in the age group range of 20-79 have diabetes (Artarini and Adnyana, 2019).

Ayurveda and traditional medicinal systems have long recognized the potential of plants as alternative medicine for diabetes treatment. These herbal drugs offer a safe and cost-effective solution, with minimal side effects. The active components of medicinal plants, possess remarkable properties - they can regenerate pancreatic beta cells, release insulin, and combat insulin resistance (Kavishankar *et al.,* 2011). Plants are incredibly a major source of secondary metabolites and other novel therapeutic compounds that could significantly improve human health with minimal adverse effects. The bioactive clusters found in plants, like alkaloids, flavonoids, saponins, steroids, terpenoids, polysaccharides, and tannins, have broadly been studied and are majorly recognized for their contribution to various traditional and therapeutic practices. It's safe to say that the potential benefits of these plant-derived compounds are truly remarkable (Rehana, 2017).

Nanotechnology is a field of study that focuses on materials with smaller dimensions ranging from 1 to 100 nm. In recent times, green nanoparticles (NPs) have captured the attention of researchers because their distinctive morphological and physicochemical properties. They hold great potential for applications in biology, medicine, and the environment. Nano-materials are rapidly gaining popularity due to their promising biomedical uses. It is evident that researchers are enthusiastic about exploring the vast possibilities that these materials offer. Nanomaterials exhibit unique atom-like behaviour because of their large surface area and wide band gap (Rehana, 2017; Ramachandran *et al.*, 2020).

There have been many methods used for the synthesis of silver nanoparticles by chemical means, which possess many toxic components. Green synthesis is an eco-friendly method of creating nanoparticles that is preferred over chemical synthesis, as it avoids the usage of harmful chemicals. This approach is made possible by the natural properties of plants, which possess both primary and secondary metabolites that can be used for the synthesis process. The AgNPs, produced via green synthesis have numerous beneficial properties, including antidiabetic, antimicrobial, antioxidant, and anticancer effects, and are widely used in various industries (Advances, 2016). AgNPs synthesized by many plants such as lemongrass, grape pomace, japonica leaves, etc. show antidiabetic effect (Agarwal *et al.,* 2018; Saratale *et al.,* 2020). The present study provides more of such innovative synthesized plant silver nanoparticles showing antidiabetic effect. This research study delves into the incredible potential of silver nanoparticles and their ability to combat diabetes, utilizing the phytoconstituents of plant okra (*Abelmoschus esculentus)*, periwinkle (*Catharanthus roseus*) and drumstick (*Moringa oleifera*). It also provides the comparative antidiabetic effect of above-mentioned plants, which could make them suitable to be used for medicine formulations against diabetes.

MATERIALS AND METHODS

Preparation of plant extracts

The fruits of okra (*Abelmoschus esculentus*) and drumstick (*Moringa oleifera*) were purchased from the local market of Surat, Gujarat (India) and the leaves of periwinkle (*Catharanthus roseus*) were collected from Surat. These plant materials were air-dried, crushed and finely ground. The aqueous plant extract was prepared by dissolving 2 g powdered plant material in 100 mL distilled water, the mixture then heated at 40-50ºC with continuous stirring on a hot plate for 15 min and cooled down to normal temperature before filtration. The filtrate was refrigerated when not in use. The methanolic plant extract was prepared by Soxhlet extraction method by packing 5 g powdered plant material in thimble, extracting it with methanol, then heated to evaporate solvent (Yadav and Agarwala, 2011). These extracts were used for phytochemical analysis.

Synthesis of silver nanoparticles using green synthesis approach

Silver nanoparticles synthesis was performed by taking 1.5 g powdered plant extract of okra, drumstick and periwinkle (separately) in beakers containing 100 mL distilled water, which were then placed on magnetic stirrer at 90ºC and filtered using Whatman filter paper. The filtrate was then titrated against 50 mL of 1 mM silver nitrate solution at 60-70ºC on a magnetic stirrer. During titration, the appearance of yellow colour served as a clear indication of successful synthesis of AgNPs (visual identification). The synthesized plant-based AgNPs were used for phytochemical analysis, characterization and antidiabetic assays (Agarwal *et al.,* 2018; Naderi-Samani *et al.,* 2023).

Characterization of silver nanoparticles (for CR-AgNPs, MO-AgNPs and AE-AgNPs)

After yellow colour appearance, the reaction mixture was centrifuge at 10,000 rpm for 5 min and then washed twice with Milli-Q water, followed by another round of centrifugation. The UV-visible spectroscopy for spectral analysis and surface plasmon resonance (SPR) was conducted in the range of 380-470 nm using UV-Vis spectrophotometer (UV-2800). The sample was further kept for 6 months to check its stability (Agarwal *et al.,* 2018). All three plant-based AgNPs solution were centrifuged, the pellets air dried and used for further characterization. To identify the functional groups involved in nanoparticle synthesis, Fourier transform infrared spectroscopy (FTIR) was performed at a wavelength of 400-4000 cm^{-1} using JASCO FT/IR spectrophotometer (Shenoy, 2018). Field emission scanning electron microscopy (FESEM) [FEI Nova NanoSEM-450] was done to examine the morphology and size of plant-based AgNPs (Bala *et al.*, 2014). The particle size distribution of AgNPs was determined with the help of dynamic light scattering (DLS) instrument (ZS-90, Malvern Zetasizer) using the technique of laser scattering (Naderi-Samani *et al.,* 2023).

Antidiabetic assays

α-Amylase inhibition assay: To assess the effect of synthesized AgNPs on the activity of α-amylase, a mixture of α-amylase (1U mL^{-1}), synthesized AgNP (20-100 µg mL^{-1}), and 0.02M sodium phosphate buffer (0.5 mL) of pH 6.9 was prepared. The mixture was incubated at 37ºC for 20 min, then 1% starch solution (250 µL) added to it and further incubated for 15 min at 37ºC. To stop the reaction, 1mL DNSA (dinitro salicylic acid) was used and then incubated in boiling water-bath for 10 min. The tubes were cooled and absorbance calculated at 540 nm using UV-vis spectrophotometer. To compare the results, acarbose was used as a standard (Saratale *et al.*, 2020).

α-Glucosidase inhibition assay: A mixture of α-glucosidase was prepared by using 150 µL of 0.1M sodium phosphate buffer (pH, 6.9), 1U mL^{-1} α-glucosidase, and 20-100 µg mL^{-1} AgNPs. The mixture was incubated at 37ºC for 10 min. Then 50 µL of 2 mM PNPG (ρ-nitrophenyl α-D-glucopyranoside) prepared in 0.1M sodium phosphate (Na_2HPO_4) buffer was added to the tubes, and the mixture incubated for additional 20 min at 37ºC. After incubation, the reaction was terminated using 50 µL of 0.1M sodium carbonate and absorbance noted at 405 nm using UV-vis spectrophotometer (UV-2800). Acarbose was used as standard (Dattatraya *et al.,* 2017; Saratale *et al.,* 2018).

D-Glucose diffusion assay: To study glucose diffusion, 1 mL synthesized AgNPs solution was mixed with 2 mL of 22 mM D-glucose solution (prepared in 0.15M NaCl). This mixture was poured into a dialysis membrane and tied at the ends. Membrane was dispersed in a 50 mL solution (consisting of 40 mL 0.15M NaCl and 10 mL milli Q water). To ensure accuracy, a membrane with milli Q water instead of synthesized AgNPs was used as control. Acarbose (standard drug) was used instead of synthesized AgNPs as positive control. The beakers were placed on an orbital shaker at 37ºC for 3 h. The diffusion of glucose from the membrane to the external solution was measured using a GOD/POD kit at intervals of 30 min (Agarwal *et al.,* 2018). Glucose diffusion retardation index (GDRI) was calculated by following formula:

$$\text{GDRI} = \frac{100-\text{Glucose concentration in external solution in presence of AgNPs}}{\text{Glucose concentration in external solution without AgNPs}}$$

Statistical analysis

All the experiments were performed in triplicates. One-way analysis of variance (ANOVA) was employed for statistical analysis of (Ramachandran *et al.*, 2020). The IC_{50} values were calculated using nonlinear regression (sigmoidal dose-response model).

RESULTS AND DISCUSSION

Qualitative phytochemical analysis

The phytochemicals analysis of methanolic and aqueous extracts of *A. esculentus, C. roseus* and *M. oleifera*), and plant-derived AgNPs revealed the presence of carbohydrates, glycosides (except in methanolic extract), saponins, flavonoids (except in AgNPs), alkaloids in all the samples (Table 1).

Table 1: Qualitative phytochemical analysis of three lichen extracts and their AgNPs

Phytochemicals	Tests performed	*Abelmoschus esculentus*			*Catharanthus roseus*			*Moringa oleifera*		
		ME	AQ	AgNPs	ME	AQ	AgNPs	ME	AQ	AgNPs
Carbohydrates	Benedict	-	+	-	-	+	+	-	+	+
	Fehling	-	+	-	-	-	-	-	-	-
	Molisch	-	+	+	-	+	+	-	+	+
Phenols	Ferric chloride	-	-	-	+	-	-	+	-	-
Tannins	Braymers	-	-	-	+	-	-	+	-	-
Flavonoids	Alkaline reagent	+	+	-	+	+	-	+	+	-
Saponins	Distilled water	+	+	-	+	+	-	+	+	-
Glycosides	Borntrager	-	+	+	-	+	+	-	+	+
	Kellar Kilani	-	-	-	-	+	-	-	+	-
Steroids	Chloroform	-	-	-	-	-	-	-	-	-
Terpenoids	Salkowski	-	-	+	-	+	-	-	-	+
Alkaloids	Hager	+	+	+	+	+	+	+	+	+
	Wagner	+	+	+	+	+	+	+	+	+
	Mayer	+	+	+	+	+	+	+	+	+

ME - Methanolic extract; AQ - Aqueous extract; AgNPs - Silver nanoparticles; + indicates presence and - indicates absence

Characterization of synthesized AgNPs

Visual identification: The appearance of yellow colour is the preliminary confirmatory test for nanoparticle synthesis. The test was performed using a 1 mM AgNPs solution that was titrated against a plant extract. The results showed a clear and distinct colour change from transparent to yellow, and then to brown, after being kept for a long incubation period. These results were similar to those obtained during the confirmation process in (Agarwal *et al.*, 2018), which further reinforces the accuracy and reliability of this method.

UV-visible spectroscopic analysis: Surface plasmon resonance (SPR) of AgNPs showed peaks in the range of 380-470 nm. The UV-visible analysis spectroscopic absorbance peaks were observed at 400 nm for MO-AgNP, and 401 nm for CR-AgNP and AE-AgNP. The stability of AgNP was monitored for 6 months. During storage, no aggregation or SPR changes were observed. The synthesis of AgNPs remained stable during further experiments, which revealed it to be useful for various biomedical uses.

FESEM analysis: The micrographs of surface morphology of AgNPs, examined by FESEM, (Fig. 1a-c) revealed irregular surface morphology in all the three synthesized AgNPs, which on magnification

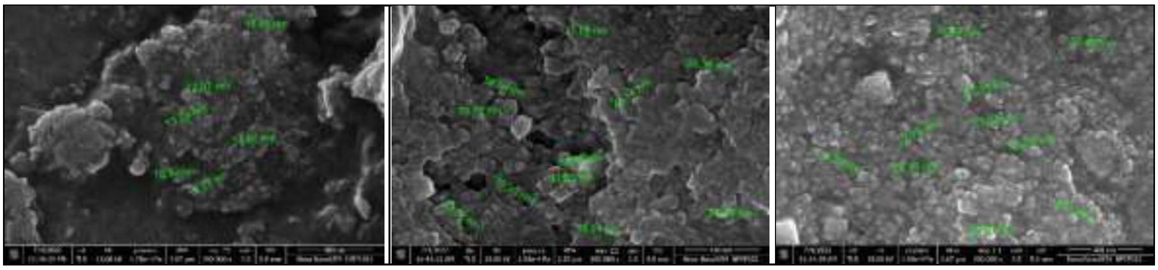


Fig. 1: FESEM micrographs of synthesized plant-derived AgNPs with the size of nanoparticles; a) AE-AgNPs (ranging between 12-22 nm); b) CR-AgNPs (ranging between 21-46 nm); c) MO-AgNPs (ranging between 13-49 nm) at 400 nm scale

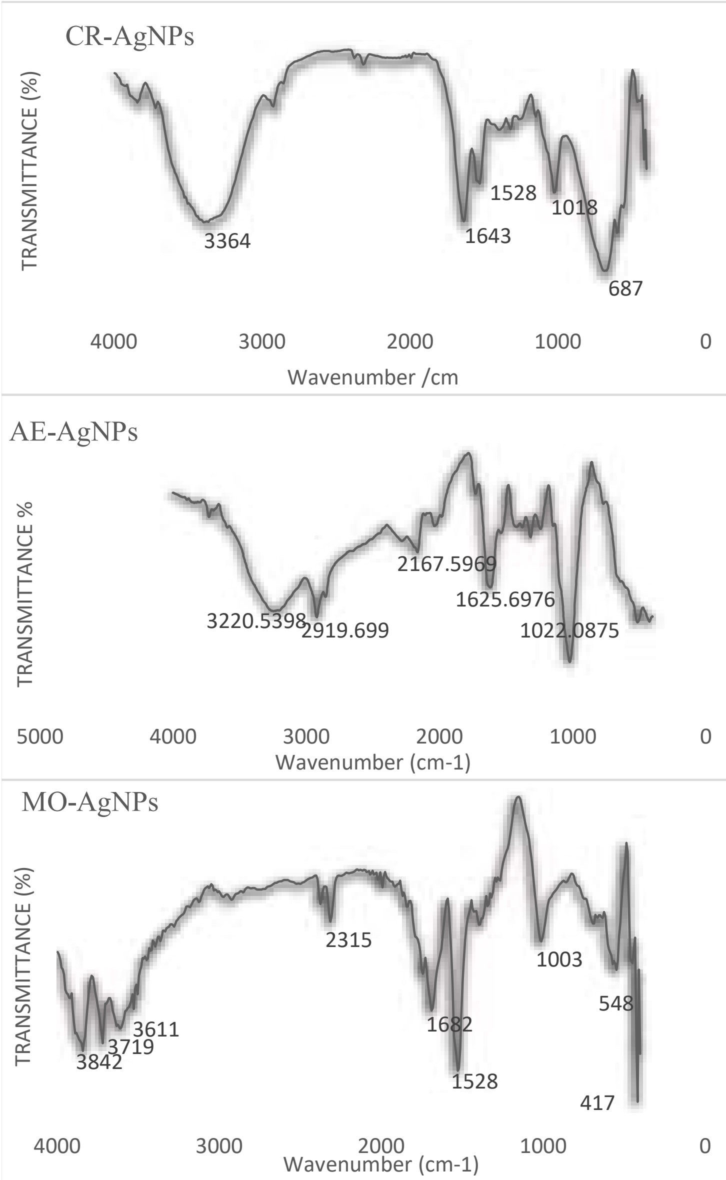


Fig. 2: FTIR of CR-AgNPs; AE-AgNPs; MO-AgNPs

of 200000x showed aggregation of group of smaller spherical particles ranging from 12-22 nm in AE-AgNPs, 21-46 nm in CR-AgNPs, and 13-49 nm in MO-AgNPs.

FTIR analysis of synthesized AgNPs: The FTIR spectroscopy is used to identify the chemical bonds and functional groups in a molecule, and it produces an infrared absorption spectrum that acted as a unique "fingerprint" of the molecule's composition. Fig. 2a-c shows the respective FTIR graphs of synthesized AgNPs and Table 2 shows the absorption bands of all the synthesized AgNPs with their wave number and their corresponding functional groups indicating the presence of respective chemical compounds (Register *et al.,* 2016).

DLS analysis: Dynamic light scattering (DLS) is the most reliable widely used method for measuring size distribution of nanoparticles (Sagadevan *et al.*, 2020). Fig. 3 shows the size distribution of synthesized plant AgNPs. The highest peak was observed for CR-AgNPs (at 50 nm) which depicts maximum AgNPs formed were of size 50 nm. In case of AE-AgNPs, the highest peak observed was at 70

Table 2: FTIR analysis of synthesized AgNPs with their functional group of respective wavenumbers

Wavenumber (cm^{-1})	Functional group		Wavenumber (cm^{-1})	Functional group	
417	-	-	1682	C=O stretching	Conjugated ketone
548	C-I stretching	Halo compound	2167	N=N=N stretching	Azide
687	C-Br stretching	Halo compound	2315	-	-
1003	C-F stretching	Halo compound	2919	C=H stretching	Alkane
1018	C-F stretching	Halo compound	3220	O-H stretching	Carboxylic group
1022	C-O stretching	Vinyl ether	3364	N-H stretching	Aliphatic primary amine
1528	N-O stretching	Nitro compound	3611	O-H stretching	Alcohol
1625	C=C stretching	Conjugated alkene	3719	O-H stretching	Water
1643	C=C stretching	Monosubstituted alkene	3842	-	-

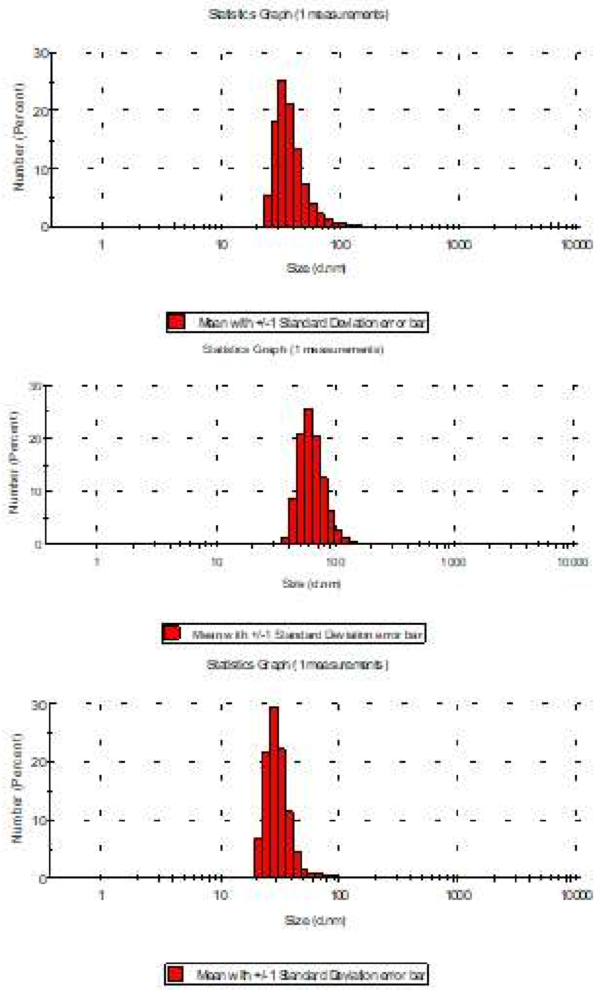
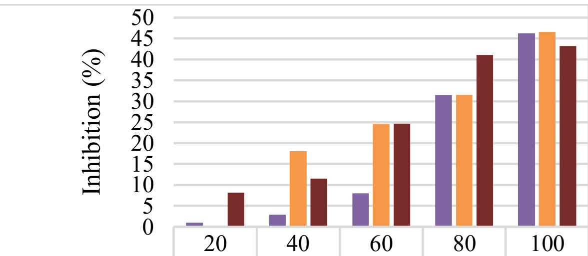


Fig. 3: DLS analysis of synthesized AgNPs: CR-AgNPs (top figure), AE-AgNPs (middle one) and MO-AgNPs (bottom figure)



	20	40	60	80	100
AE-AgNPs	1.01	2.91	7.97	31.51	46.2
CR-AgNPs	0	18.1	24.55	31.51	46.58
MO-AgNPs	8.16	11.54	24.66	41.08	43.12

Concentration (µg mL^{-1})

Fig. 4: α-amylase inhibition by plant based AgNPs

nm and in case of MO-AgNPs at 40 nm which reveal their maximum size.

α-Amylase inhibition

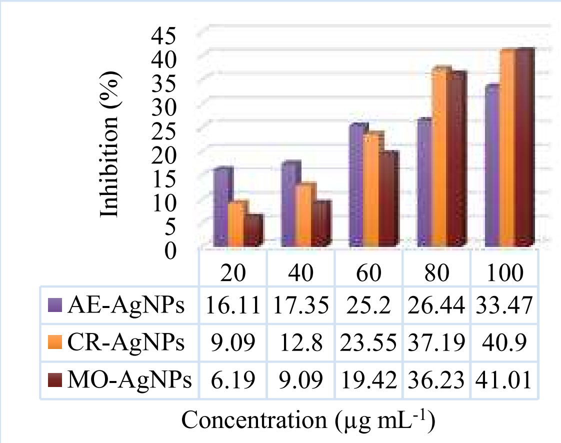
The α-amylase inhibition potential of acarbose (positive control), CR-AgNPs, AE-AgNPs and MO-AgNPs was assessed at the concentrations ranging from 20 to 100 µg mL^{-1}. All the samples demonstrated a dose-dependent inhibition pattern, with varying degrees of potency (Fig. 4). For all the three types of AgNPs α-amylase inhibition increased with increase in concentration. The findings suggest that higher concentration of AgNPs are more effective in inhibiting the enzyme. AE-AgNPs showed lowest inhibition at most concentrations, but its inhibition increased significantly at higher concentrations (80 and 100 µg mL^{-1}). MO-AgNPs showed moderate level of inhibition at lower concentrations, and its inhibition increased steadily with concentration. CR-AgNPs and MO-AgNPs are more effective α-amylase inhibitors, especially at lower concentrations, while AE-AgNPs become more effective only at higher concentrations.

Nonlinear regression was used to determine IC_{50} values. The IC_{50} values for acarbose, CR-AgNPs, and AE-AgNPs were 92.0, 80,08, and 83.65 µg mL^{-1}, respectively; while such values for MO-AgNPs were erraneous and unreliable.

One-way ANOVA performed on absorbance values at 100 µg mL^{-1} indicated significant differences between the groups ($p = 1.44 \times 10^{-17}$), confirming that CR- and AE-derived AgNPs were significantly more potent than control and MO-derived AgNPs.

α-Glucosidase inhibition assay

The inhibitory potential of CR-AgNPs, AE-AgNPs, and MO-AgNPs in comparison to acarbose, a standard *α*-glucosidase inhibitor, revealed that all the AgNPs across the concentrations exhibited a dose-dependent increase in *α*-glucosidase enzyme inhibition Fig. 5), similar to the pattern noted in control acarbose. Control showed a maximum inhibition of 34.71% at 100 µg mL^{-1}, while CR-AgNPs, MO-AgNPs and AE-AgNPs gave 40.50, 42.84, and 33.22%, inhibition, respectively. These findings indicated that the biosynthesized AgNPs



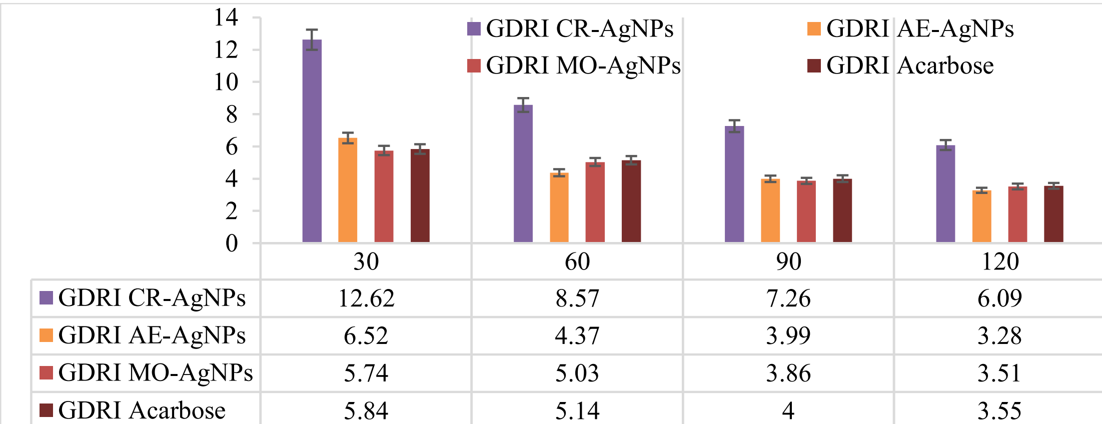
	20	40	60	80	100
AE-AgNPs	16.11	17.35	25.2	26.44	33.47
CR-AgNPs	9.09	12.8	23.55	37.19	40.9
MO-AgNPs	6.19	9.09	19.42	36.23	41.01

Fig. 5: α-glucosidase inhibition by plant-based AgNPs

possessed significant inhibitory activity against *α*-glucosidase, with some surpassing that of acarbose at higher concentrations. To quantify potency, IC_{50} values were calculated using nonlinear regression (sigmoidal dose-response model). Among the AgNPs, CR-AgNPs exhibited the lowest IC_{50} of 51.86 μg mL^{-1}, suggesting it's most effectiveness as *α*-glucosidase inhibitor of test nanoparticles. Notably, other two nano-particle samples AE-AgNPs and MO-AgNPs also demonstrated lower IC_{50} values of 56.85 and 63.21 μg mL^{-1} in comparison to acarbose (76.27 μg mL^{-1}), indicating their higher inhibitory efficiency at lower concentrations.

D-Glucose diffusion assay

This assay evaluates the effect of biologically synthesized AgNPs on the release of glucose through a dialysis membrane into the external medium. The proposed mechanism is that AgNPs can bind or adsorb glucose, thereby lessen its diffusion, a promising strategy for managing non-insulin-dependent diabetes. Several studies support glucose diffusion inhibition via interaction with particle surfaces or entrapment within porous structures (Agarwal *et al.,* 2018; Li *et al.,* 2022). Biological coatings or plant-derived polymers on AgNPs likely enhance GDRI by adding binding sites or creating physical barriers. Fig. 6 compares glucose diffusion retardation index (GDRI) over time for various AgNPs and the standard drug acarbose. AE-AgNPs and MO-AgNPs exhibited GDRIs comparable to acarbose, indicating a similar degree of glucose diffusion retardation. In contrast, CR-AgNPs exhibited highest glucose diffusion rates across all time points, reflecting the lowest retardation effect. AE-AgNPs demonstrated a moderate but progressively decreasing diffusion rate over time. All treatments showed decreased glucose diffusion over time, suggesting a time-dependent glucose-binding absorption effect.



	30	60	90	120
GDRI CR-AgNPs	12.62	8.57	7.26	6.09
GDRI AE-AgNPs	6.52	4.37	3.99	3.28
GDRI MO-AgNPs	5.74	5.03	3.86	3.51
GDRI Acarbose	5.84	5.14	4	3.55

Fig. 6: D-glucose diffusion assay with GDRI of some plant-based AgNPs

Conclusion: Discovering effective antidiabetic treatments is an ongoing process in pharmaceutical industry. The present study showed great promise in the use of AgNPs derived from plant extracts. The study confirmed the presence of essential phytochemicals through phytochemical analysis, forming a strong foundation for antidiabetic potential. Furthermore, the characterization of AgNPs confirmed their synthesis, morphology, size, and functional group. The results of the antidiabetic assays demonstrated the inhibitory effect of synthesized AgNPs, making them a potent candidate for medicinal use. This finding may pave the way for a new and effective antidiabetic treatment.

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