Assessment of in vitro antidiabetic properties of synthesized silver nanoparticles using ethanolic extract of Boerhavia diffusa

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Research Article

Keywords: Diabetes Mellitus, Boerhavia diffusa, silver nanoparticle, Antidiabetic activity

Posted Date: February 16th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2581001/v1

Abstract

The *in vitro* antidiabetic efficacy of ethanolic extract *Boerhavia diffusa (B.diffusa)* synthesized silver nanoparticles (AgNPs) was investigated by inhibition of α-amylase, α-glucosidase, protein glycation assay, non-enzymatic glycosylation of hemoglobin, glucose uptake by yeast cells and glucose diffusion at varying concentrations (10 to 100µg/ml). The alpha-amylase assay shows that the acarbose (standard) and *B. diffusa* had IC50 values of 46.2 µg/ml and 55.4 µg/ml, whereas alpha-glucosidase inhibitory activity was found to be 63.4 µg/ml and 93.0 µg/ml respectively. Further, non-enzymatic glycosylation analysis showed IC50 value of metformin (standard) as 28.6 µg/ml and *B. diffusa* as 63.9 µg/ml. The protein glycation activity was inhibited in non-enzymatic glycosylation of hemoglobin. The glycosylation was induced using pioglitazone (standard) which gives IC50 value of 616.4 µg/ml by which *B. diffusa* showed 756.3 µg/ml. The uptake of glucose by yeast cells was analyzed and the result shows that the glucose concentration increased steadily from 5mM to 25mM (maximum absorption) of both metronidazole (standard) and *B. diffusa*. From 30 to 180 minutes, the glucose diffusion experiment revealed that the concentration of the metformin and *B. diffusa* extract was positively correlated with the time. The ethanolic extract of synthesized AgNPs and the reference medication employed in all experiments both benefit their curative potential for the treatment of insulin resistance. The generated silver nanoparticles can be used for industrial and therapeutic purposes and can be released into the environment without harm. More *in vivo* study can be reviewed, however the green synthesized ethanolic extract of *B. diffusa* exhibits promising affect for the treatment of diabetes mellitus.

Introduction

Nanotechnology is an important revolution in enormous fields of science. Nanoscience and nanotechnology deal with the manufacturing and application of nanoparticles, which may be utilized in various areas (Sukweenadhi et al. 2021). The biosynthesis of silver nanoparticles (AgNPs) has become a great challenge in the past decade in the field of nanobiotechnology. Medicinal plant extracts are widely used in the green synthesis of AgNP as reducing and stabilizing agents, overcoming the disadvantages of traditional physical and chemical synthesis methods (Some et al. 2022). In recent years, the diagnosis and management of diabetes have benefited significantly by nanotechnology. Additionally, it has a proven track record of identifying and managing diabetes problems (Lavanya et al. 2022).

Diabetes mellitus (DM) is a persistent metabolic disorder attributed to hyperglycaemia, hyperlipidaemia and hyperaminoacidemia, together with insulin insufficiency, insulin-receptor resistance or both. Among the differing types of DM, type 2 is greater generic worldwide (Jayappa et al. 2020). Certain plants have been used in herbal remedies for many years. A diverse range of key attributes and bioactive metabolites are discovered in the medicinal plants affiliated with their components. These curative plants are rich in tannins, phenolics and alkaloids. Such biologically active substances found in plants may accelerate the process by which metal ions are transformed into biologically active nanoparticles in a normal biogenesis pathway that is beneficial to the environment (Tundis et al. 2010). One of the therapeutic strategies being researched for the treatment of diabetes is the suppression of α-amylase, α-glucosidase,

non-enzymatic glycosylation of haemoglobin, glucose absorption by yeast cells, glucose diffusion and protein glycation test activity (Jadalla et al. 2022).

In Ayurveda, the herb *Boerhavia diffusa (B. diffusa)*, which belongs to the Nyctaginaceae family, is used to treat a wide range of diseases (Oyebode et al. 2018). The primary extract utilized in this investigation is the whole *B. diffusa* plant which is used for antidiabetic treatments (Tamboli et al. 2021). This research has been focused on the synthesis of silver nanoparticle and *in vitro* antidiabetic effects of the whole plant extract of *B. diffusa*.

Materials And Methods

Plant material

Plants belonging to the species *B. diffusa* (Fig. 1) were collected from in and around Coimbatore. The entire plant was authenticated by Botanical Survey of India (BSI), TNAU, Coimbatore (BSI/SRC/5/23/2013-14/Tech/1041).

Extraction and synthesis of AgNPs

Ethanolic extract of whole plant of *B. diffusa* was prepared using the Soxhlet equipment. The extract was then subjected with AgNPs and analyzed for *in vitro* antidiabetic activity (Akhtar et al. 2016).

In vitro antidiabetic activities

The α-amylase inhibition assay

From 1 mg/ml stock solution, different concentrations of plant extracts were prepared in phosphate buffer (10, 20, 40, 60, 80 and 100 µg/ml) of *B. diffusa* extract and acarbose were combined with 500µl of α-amylase (0.5 mg/ml) and incubated for 10 minutes at room temperature. Following that, 500µl of 1% starch solution was added and incubated for 10 minutes. The reaction mixture was then treated with 1 ml of dinitro salicylic acid DNS, a colouring reagent, and heated for 15 minutes in a boiling water bath before adding 10 ml of distilled water. A blank was prepared by replacing the enzyme with buffer for each concentration of the sample set to quantify the absorbance of the coloured extracts. The absorbance was determined at 540nm. With the help of the formula, the inhibition percentage was computed (Ishwarya et al. 2022).

% inhibition = Abs Control – Abs Sample/ Abs Control × 100

The alpha-glucosidase Assay

The alpha glucosidase inhibitory activity was determined using the (Palanuvej et al. 2009) technique. Yeast alpha glucosidase was dissolved at 0.1U/ml in 100mM phosphate buffer, pH 7.0, including 2000 mg/l bovine serum albumin and 200 mg/ml sodium azide as an enzyme source. As a substrate, para-nitro phenyl-alpha-D-glucopyranoside was utilized. *B. diffusa* (5%) plant extract was weighed, and serial

dilutions of 62.5, 31.25, 15.6, 7.8, 3.9 and 1.95 mg/ml dimethyl sulfoxide and distilled water was prepared equal volume. For 5 minutes, ten microliters of plant extract dilutions were incubated with a 50microliter enzyme source. Following the incubation, 50 microliters of substrate were added and incubated for 5 minutes at room temperature. A microtitre reader was used to measure the absorbance at 405 nm.

% inhibition = Abs Control – Abs Sample/ Abs Control × 100

Non-Enzymatic Glycosylation of Haemoglobin Method

In phosphate buffer 0.01 M, pH 7.4, solutions of glucose (2%), haemoglobin (0.6%) and gentamycin (0.02%) were made. 1.0 ml of each of the above solutions was combined with 1.0 ml of the *B. diffusa* extract of varying concentrations (10, 20, 40, 60, 80 and 100 µg/ml). The reaction mixture was incubated for 72 hours at room temperature in the dark, and after that it was determined by colorimetry at 520 nm that much the haemoglobin had been glycosylated. Metformin was used as a standard drug by which further the percentage inhibition was determined using the formula, (Gupta et al. 2012).

% inhibition = Abs Sample – Abs Control/ Abs Sample × 100

Inhibition *in vitro* protein glycation

Fructose (1000 mM, in 200 mM, phosphate buffer pH 7.4) (4.0 ml) with 5.0 ml of BSA (20 mg/ml, in 200 mM phosphate buffer, pH 7.4) was incubated for 24 hours, either in the absence or presence of varied 1.0 ml of final concentrations of plant extract *B. diffusa* (250, 500, 750, and 1000 µg). Following the incubation time, the fluorescence intensities of the reaction mixtures were assessed throughout an emission wavelength range of 370–650 nm at an excitation wavelength of 360 nm. The amount of fluorescent advanced glycation end-products (AGEs) produced was correlated with the fluorescence intensity. At final concentrations of 1.25, 0.75, and 0.25 mg/ml, the common anti-glycation drug Pioglitazone served as a positive control. The percentage inhibition of fluorescent AGE formation was calculated using the following equation, (Avwioroko et al. 2022)

% inhibition = (FC- FB) – (FS -FSB)/ (FC- FB) × 100

Where FC is the fluorescence intensity of the control, FCB is the fluorescence intensity of the control blank, FS is the fluorescence intensity of the sample and FSB is the fluorescence intensity of the sample blank.

Glucose uptake by yeast cell

A 10% (v/v) suspension of commercial baker's yeast was made in distilled water after the yeast was repeatedly centrifuged (3,000 × g; 5 min) until the supernatant fluids were clear. 1ml of the glucose solution (5,10 and 25mM) was added to various concentrations of plant extract *B. diffusa* (20, 40, 60, 80 and 100 µg/ml) and incubated for 10 min at 37°C. After adding 100µl of yeast suspension and vortexing the mixture, the reaction was allowed to continue for 60 min at 37°C. The tubes were centrifuged (2,500 ×

g, 5 min) after 60 min to determine the amount of glucose in the supernatant. Metronidazole was taken as a standard drug. The percentage increase in glucose uptake by yeast cells was calculated using the following formula (Ramanathan et al. 2025).

% inhibition = Abs Sample – Abs Control/ Abs Sample × 100

Glucose diffusion assay

To measure the *in vitro* glucose diffusion and absorption, a cellulose ester dialysis tube (CEDT) was filled with 2 mL of 0.15 M NaCl and 0.22 mM glucose in either the presence (treated) or absence (control) of plant extract (50µg/ml). CEDT was transferred into a 50 ml centrifuge tube containing a 45 ml solution of 0.15 M NaCl that had been firmly sealed on both ends and maintained at room temperature in an orbital shaker. Every 60 minutes, the concentration of glucose in the external solution was measured to track the diffusion of glucose (Ansari et al. 2022).

Results

Inhibition of α-amylase activity of synthesized silver nanoparticles of ethanolic extract of *Boerhavia diffusa*

At doses ranging from 10 µg/ml to 100 µg/ml, *B. diffusa* was evaluated for its ability to inhibit α-amylase (Fig. 2). Silver nanoparticles exhibit potent inhibitory efficacy against the enzyme α-amylase in a dose-dependent manner. Silver nanoparticles that have been synthesized from the entire plant have a 30–70% inhibitory impact on amylase. Acarbose and *B. diffusa* maximum doses (100 µg/ml) inhibited 75.31% and 70.4% of the enzymes respectively, with IC50 values of 46.2 µg/ml and 55.4 µg/ml. These results demonstrated that *B. diffusa* successfully suppressed the activity of the α-amylase enzyme in *in vitro* conditions.

Inhibition of α-glucosidase enzyme activity of synthesized silver nanoparticles of ethanolic extract of *Boerhavia diffusa*

The selected effective plant extracts have lower alpha-glucosidase inhibitory activity than acarbose (Fig. 3). The IC50 value of standard drug and *B. diffusa* was found to be 63.4% and 93.0% The plant extracts of *B. diffusa* appear to be relatively potent inhibitors, with the inhibitory activity that could be further improved by separation and purification of the active components.

Inhibition of non-enzymatic glycosylation of synthesized silver nanoparticles of ethanolic extract of *Boerhavia diffusa*

Over a period of 72 hours, there was a dose-dependent rise in the percentage inhibitory impact on hemoglobin glycosylation as the quantity of plant extracts *B. diffusa* increased (Fig. 4). This shows that the plant extracts enhance the quantity of free hemoglobin by reducing the development of the glucose-hemoglobin complex. The largest inhibitions were seen in *B. diffusa* (64.32%) at the highest

concentration (100µg/ml), which was equivalent to Metformin (77.72%). The fact that *B. diffusa* IC50 was 63.97µg/ml and the standard drug metformin showed 28.64µg/ml by which the plant extract is more effective than the common medication at inhibiting hemoglobin glycosylation.

Inhibition of protein glycation of synthesized silver nanoparticles of ethanolic extract of *Boerhavia diffusa*

Using the model system of bovine serum albumin and fructose, the *in vitro* inhibitory efficacy of *B. diffusa* plant of ethanolic extracts on protein glycation was assessed. Figure (5) indicates the increased with increase in concentration from 250–1000µg/ml.

pioglitazone (at a concentrations 1000 µg/ml) showed 58.14% inhibitory effects on the protein glycation activity with an IC_{50} value 856.25µg/ml. The silver nanoparticles from *B. diffusa* of ethanol extracts exhibited 62.79% inhibitory activity with an IC_{50} values of 756.35µg/ml.

Glucose uptake assay by yeast cells of synthesized silver nanoparticles of ethanolic extract of *Boerhavia diffusa*

The impact of the *B. diffusa* samples on glucose transport across yeast cell membranes was investigated in an *in vitro* method that included yeast cells suspended in variable amounts of glucose solution (5, 10 and 25mM) in the presence of extracts at various concentrations (Table 1). The standard metronidazole was higher as compared to that of silver nanoparticles of ethanolic extract of *B. diffusa*.

Table 1
Antidiabetic activity of Glucose uptake assay by yeast cells

Glucose uptake at 5mM glucose concentration		
Concentration (µg/ml)	Standard (%)	AgNPs (%)
20	5.17 ± 0.294	14.2 ± 0.274
40	19.5 ± 0.312	32.4 ± 0.49
60	38.2 ± 0.664	43.4 ± 0.262
80	60.2 ± 0.64	56.5 ± 0.0987
100	65.2 ± 2.43	66.5 ± 0.0867
Glucose uptake at 10mM glucose concentration		
20	15.5 ± 0.0917	22.2 ± 0.63
40	32.3 ± 1.01	36.7 ± 2.35
60	49.3 ± 1.97	53.1 ± 1.79
80	50.4 ± 4.98	55.7 ± 2.54
100	62.3 ± 2.67	64.5 ± 1.13
Glucose uptake at 25mM glucose concentration		
20	32 ± 0.947	21.3 ± 0.643
40	44.5 ± 1.33	36.6 ± 1.04
60	49.9 ± 1.98	33.8 ± 2.15
80	62.3 ± 1.87	41.9 ± 0.898
100	63 ± 1.76	52.4 ± 1.1

Inhibition of glucose diffusion of synthesized silver nanoparticles of ethanolic extract of *Boerhavia diffusa*

The effects of the *B. diffusa* extract on the inhibition of glucose diffusion across the dialysis membrane were studied using synthesized silver nanoparticle extract (Table 2). The 30, 60, 90, 120 and 180 minutes were used to measure the glucose diffusion inhibition. When compared to silver nanoparticles from an ethanolic extract of *B. diffusa*, the effect of conventional metformin on glucose diffusion measurements at various time intervals was less significant than the sample.

Table 2
Antidiabetic activity of Glucose Diffusion Assay

Sample	Glucose in external solution (mM)				
	30mins	60mins	90mins	120mins	180mins
Absence of drug (Control)	51.9 ± 0.638	61.5 ± 0.57	61.6 ± 0.521	107 ± 0.831	135 ± 0.944
Metformin	18.6 ± 0.309	19.3 ± 0.483	19.8 ± 0.823	19.3 ± 0.248	20.7 ± 0.36
AgNPs	19 ± 0.28	20.4 ± 0.527	21.2 ± 0.351	21.6 ± 0.416	26.5 ± 0.343

Discussion

The distinguishing characteristic of the broad group of chronic metabolic illnesses collectively known as diabetes mellitus is hyperglycemia. Blood sugar levels can be managed with insulin, oral hypoglycemic drugs or inhibitors of carbohydrate hydrolyzing enzymes. Researchers focus on identifying alternative sources for innovative anti-diabetic medications due to the adverse consequences of utilizing insulin and oral hypoglycemics. Determining and evaluating the natural enzyme inhibitors is therefore extremely important. The enzyme's alpha amylase controls how carbohydrates are metabolized and absorbed (Malik et al. 2022). In this work, alpha-amylase was successfully inhibited by a synthesized ethanolic extract of *B. diffusa* at doses between 10 to 100µg/ml. At the concentration of 100µg/ml the IC50 value of *B. diffusa* was 55.4 µg/ml. Acarbose, positive control, has an IC50 of 46.2 µg/ml. Previous research that was comparable to this one found concentration- dependent inhibitions (1000 µg/ml) in *Bersama abyssinica fresen.* The IC50 of the extracts ranged from 13.33 ± 0.57, 20.34 ± 0.67 and 30.97 ± 0.84 µg/ml for the aqueous fraction, ethyl acetate fraction and chloroform fraction, respectively. The crude extract of *Bersama abyssinica fresen* had an IC50 of 88.34 ± 0.81µg/ml. Acarbose, the positive control, has an IC50 of 95.94 ± 0.64 µg/ml (Kifle et al. 2020).

In this work, we looked at plant-derived α-glucosidase inhibitors. So, we examined at how ethanolic extracts affected the *B. diffusa* derived α-glucosidase enzyme's ability to inhibit. Standard medication and *B. diffusa* were found to have IC50 values of 63.4% and 93.0% respectively. Diabetes is often treated by blocking carbohydrate-hydrolyzing enzymes like α-glucosidase to delay the absorption of glucose. The most popular α-glucosidase inhibitor, acarbose produces gastrointestinal adverse effects. Since medicinal plants may be used to make medications, α -glucosidase inhibitors screened from plants have undergone much research recently. An anti-glucosidase activity test revealed that *T. nelsonii* extract significantly inhibited the enzyme, with an IC50 value of 193 ± 20 µg/ml in comparison to acarbose 120 ± 20 µg/ml (Cruz-Jiménez et al. 2022).

The chemical process of non-enzymatic chemical bonding between hemoglobin (Hb) and carbohydrates, such as glucose results in the development of glycated Hb and under hyperglycemic circumstances, the quantity of glycated Hb considerably increases (Saxena et al. 2021). This demonstrates how the plant

extracts increase the amount of free hemoglobin by decreasing the formation of the glucose-hemoglobin complex. Regardless of metformin (77.72%), *B. diffusa* (64.32%) at the greatest concentration (100 g/ml) exhibited the biggest inhibitions. The fact that metformin IC50 was 28.64 µg/ml while *B. diffusa* was 63.97 µg/ml indicates that the plant extract is more potent in inhibiting hemoglobin glycosylation than the conventional drug. A similar investigation was conducted on a plant extract from *Ficus platyphylla* and *Sterculia setigera* (Ukwuani-Kwaja et al. 2021). While the IC50 value was discovered to be (3.18 mg/ml) and (5.97 mg/ml) in 25 mg/ml concentration for 72 hours, were relatively lower than metformin (8.84 mg/ml), they nevertheless showed that the plant extract is more effective than the typical medication against hemoglobin glycosylation.

The protein glycation activity was 58.14% inhibited by pioglitazone (at 1000 µg/ml concentrations), with an IC50 value of 856.25 µg/ml. With an IC50 value of 756.35 µg/mL, the silver nanoparticles from the ethanol extracts of *B. diffusa* showed 62.79% of protein glycation inhibitory action. As further reference in comparison to the widely used anti-glycation medication aminoguanidine (IC50 = 138 mg/L), the extracts of *dandelion* (EE), *roseroot* (EE), and *Myrica gale* (WE) showed a considerable suppression of AGE production (IC50 = 69.4, 74.0 and 70.4 mg/ml, respectively) (Sekhon-Loodu et al. 2019). The most potent inhibitors of protein glycation were found to be the leaf extracts of *C. bullatus*, *C. zabelii* and *C. integerrimus* (IC50 = 32.6–36.5 µg/ml), which had a capacity twice as high as that of aminoguanidine (IC50 = 71.1 µg/ml), a synthetic drug used to treat diabetic complications and known to stop the formation of AGEs. The conventional polyphenols, particularly procyanidin B2 and (-) -epicatechin, had substantial anti-AGE action in addition to the extracts, greatly outperforming aminoguanidine in this regard (Kicel et al. 2022).

An *in vitro* approach that comprised yeast cells suspended in varying levels of glucose solution (5, 10 and 25mM) in the presence of extracts at varied concentrations was used to examine the effect of the *B. diffusa* samples on glucose transport across yeast cell membranes (Table 1). The regular metronidazole was more potent than the *B. diffusa* ethanolic extract's silver nanoparticles. In 5mM the drug and the plant extract showed 65.2 ± 2.43 and 66.5 ± 0.0867 under the concentration of 100µg/ml. Whereas in 25mM it showed 63 ± 1.76 and 52.4 ± 1.1. According to a prior study, *Centella asiatica* Below is a representation of the results for the glucose absorption in the experiment at 5 mM concentration. In yeast cells, the rate of glucose transport through the cell membrane was investigated. A decrease was seen at five distinct concentrations of CANPs and acarbose (40, 80, 120, 160 and 200 g/ml). The maximum inhibition for CANPs and acarbose at 200 g/ml was 65.1 ± 2 0.64 and 76.34 ± 0.29, whereas the minimum inhibition for CANPs and acarbose at 200 g/ml was 25.41 ± 0.23 and 57.17 ± 0.36 and at 10mM glucose concentration, compared to 5mM, it exhibits reduced suppression of parentage growth (19.73 ± 0.34 to 63.27 ± 0.57) (Wilson et al. 2015). The available findings indicate that the plant extract may effectively increase glucose absorption, which in turn shows that it can successfully increase glucose utilization at various needed concentrations, managing blood glucose levels as also stated by previous research. Metronidazole diffuses through selective absorption, although it still has a stronger hypoglycemic impact (Ronquillo et al. 2020).

Using manufactured silver nanoparticle extract, the effects of the *B. diffusa* extract on the suppression of glucose diffusion across the dialysis membrane were investigated (Table 2) the level was increasing from initial to final minutes. The duration of the measurements for the glucose diffusion inhibition were 30, 60, 90, 120, and 180 minutes. The effect of conventional acarbose on glucose diffusion assays at various time intervals was somewhat lesser when compared to silver nanoparticles from an ethanolic extract of *B. diffusa*. To investigate the impact of various leaf extract fractions on its glucose retardation activity throughout the dialysis tube, a glucose diffusion experiment was carried out. For the future reference (Konappa et al. 2020) Significant differences between the fractions were seen in the glucose diffusion at the various periods measured. It was shown that glucose diffusion increased from 30 to 180 minutes in *A. nilgiricum* leaves.

Conclusion

The activities of α-amylase, α-glucosidase, non-enzymatic glycosylation of hemoglobin, glucose absorption by yeast cells, glucose diffusion and protein glycation test activities, which play a significant role in diabetes mellitus. It was found to be inhibited by the AgNPs of ethanolic extract of *B. diffusa*. By integrating all the data presented above, it has been demonstrated that the *B. diffusa* might be employed in the treatment of diabetes mellitus. *In vitro* tests found that the *B. diffusa* assisted in identifying the presence of antidiabetic qualities. However, more research to be carried out by *in vivo* assay and clinical trials must be carried out to verify precisely that the diabetic illness may be entirely healed.

Declarations

Author contribution Reena joy A performed the research and collected the materials and reagents drafted the manuscript and Gayathri Devi S revised and finalized the manuscript.

Funding Not applicable

Data availability the datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics statement This article does not contain any studies with animals performed by any of the authors.

Conflict of interest the authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Akhtar MN, Mahalingam MG (2016) Antioxidant, anti-microbial and glucose diffusion inhibition activities of the aqueous and chloroform extract of *Phyllanthus urinaria*. International Journal of Pharmacy and Pharmaceutical Sciences 8:278-80.

2. Ansari P, Choudhury ST, Abdel-Wahab YH (2022) Insulin secretory actions of ethanol extract of *Eucalyptus citriodora* leaf, including plasma DPP-IV and GLP-1 levels in high-Fat-Fed rats, as well as characterization of biologically effective phytoconstituents. Metabolites 12(8):757.
3. Avwioroko OJ, Anigboro AA, Otuechere CA, Atanu FO, Dairo OF, Oyetunde TT, Ilesanmi OB, Apiamu A, Ejoh AS, Olorunnisola D, Alfred MO (2022) α-Amylase inhibition, anti-glycation property and characterization of the binding interaction of citric acid with α-amylase using multiple spectroscopic, kinetics and molecular docking approaches. Journal of Molecular Liquids 119454.
4. De La Cruz-Jiménez, L., Hernández-Torres, M. A., Monroy-García, I. N., Rivas-Morales, C., Verde-Star, M. J., Gonzalez-Villasana, V and Viveros-Valdez, E. (2022). Biological activities of seven medicinal plants used in Chiapas, Mexico. *Plants*, *11*(14), 1790.
5. Gupta D, Lobo R, Gupta N (2012) *in-vitro* antidiabetic activity of stem bark of *Bauhinia purpurea* Linn. Der Pharmacia Lettre 4(2):614-9.
6. Ishwarya R, Jeyavani J, Jayakumar R, Alarifi S, Govindarajan M, Nicoletti M, Vaseeharan B (2022) *Citrullus lanatus*-encased zinc oxide nanoparticles as potential anti-diabetic, anti-inflammatory and antibacterial agents: A new strategy towards biocompatible nano-drugs. Journal of the Indian Chemical Society 99(10):100703.
7. Jadalla BM, Moser JJ, Sharma R, Etsassala NG, Egieyeh SA, Badmus JA, Marnewick JL, Beukes D, Cupido CN, Hussein AA (2022) *in Vitro* Alpha-Glucosidase and Alpha-Amylase Inhibitory Activities and Antioxidant Capacity of *Helichrysum cymosum* and *Helichrysum pandurifolium* Schrank Constituents. Separations 9(8):190.
8. Jayappa MD, Ramaiah CK, Kumar MA, Suresh D, Prabhu A, Devasya RP, Sheikh S. (2020) Green synthesis of zinc oxide nanoparticles from the leaf, stem and *in vitro* grown callus of *Mussaenda frondosa L.*: Characterization and their applications. Applied Nanoscience 10(8):3057-74.
9. Kicel, A., Magiera, A., Skrzywanek, M., Malczuk, M and Olszewska, M. A. (2022). The Inhibition of α-Glucosidase, α-Amylase and Protein Glycation by Phenolic Extracts of *Cotoneaster bullatus*, *Cotoneaster zabelii* and *Cotoneaster integerrimus* Leaves and Fruits: Focus on Anti-Hyperglycemic Activity and Kinetic Parameters. *Molecules*, *27*(20), 7081.
10. Kifle ZD, Enyew EF (2020) Evaluation of *in vivo* antidiabetic*, in vitro* α-amylase inhibitory and *in vitro* antioxidant activity of leaves crude extract and solvent fractions of *Bersama abyssinica* fresen (melianthaceae). Journal of Evidence-Based Integrative Medicine 25:2515690X20935827.
11. Konappa, N., Arakere, U. C., Krishnamurthy, S., Gangadharaiah, K. C., Gubbiveeranna, V., Shiviaha, N and Siddapura, N. (2020). Evaluation of *in vitro* antioxidant and antidiabetic activities from *Amomum nilgiricum* leaf extract. *Plant Science Today*, *7*(4), 638-648.
12. Lavanya K (2022) Anti-Diabetic Activity of Silver Nanoparticles Synthesized from the Hydroethanolic Extract of *Myristica fragrans* Seeds. Applied Biochemistry and Biotechnology.
13. Malik MN, Haq IU, Fatima H, Ahmad M, Naz I, Mirza B, Kanwal N (2022) Bioprospecting *Dodonaea viscosa* Jacq.; a traditional medicinal plant for antioxidant, cytotoxic, antidiabetic and antimicrobial potential. Arabian Journal of Chemistry 15(3):103688.

14. Oyebode OA, Erukainure OL, Chukwuma CI, Ibeji CU, Koorbanally NA, Islam S (2018) *Boerhaavia diffusa* inhibits key enzymes linked to type 2 diabetes *in vitro* and *in silico*; and modulates abdominal glucose absorption and muscle glucose uptake *ex vivo*. Biomedicine and pharmacotherapy 106:1116-25.
15. Palanuvej C, Hokputsa S, Tunsaringkarn T, Ruangrungsi N (2009) *in vitro* glucose entrapment and alpha-glucosidase inhibition of *mucilaginous substances* from selected Thai medicinal plants. Scientia Pharmaceutica 77(4):837-50.
16. Ramanathan S, Vijaya PP, Yogananth N, Syed Ali M (2025) *in vitro* evaluation of anti-diabetic potential of *Gymnema sylvestre* and *Dregea volubilis* a medicinally important asclepiadaceae plant.
17. Ronquillo, J. E. B., Aro, M. G., Herrera, R. J. P., Lat, M. I., Ramos, M. K. H., Magbojos-Magtibay, C. R and Tadiosa, J. P. B (2020) *in vitro* anti-diabetic activity of *Crescentia cujete* (Calabash tree) leaves through alpha-amylase inhibition assay and glucose uptake by yeast cell.
18. Saxena, S., Kumar, S., Hajare, S. N., Gupta, S., Gautam, S and Ghosh, S. K. (2021). 'BhAVI-23'-A spice-herb based dietary infusion possessing *in-vitro* anti-viral potential. *Journal of Ayurveda and integrative medicine*, *12*(2), 312-319.
19. Sekhon-Loodu, S and Rupasinghe, H. V. (2019). Evaluation of antioxidant, antidiabetic and antiobesity potential of selected traditional medicinal plants. *Frontiers in nutrition*, *6*, 53.
20. Some S, Mondal R, Dam P, Mandal AK (2022) Synthesis of biogenic silver nanoparticles using medicinal plant extract: A new age in nanomedicine to combat multidrug-resistant pathogens. In Green Synthesis of Silver Nanomaterials (pp. 359-387).
21. Sukweenadhi J, Setiawan KI, Avanti C, Kartini K, Rupa EJ, Yang DC (2021) Scale-up of green synthesis and characterization of silver nanoparticles using ethanol extract of *Plantago major* L. leaf and its antibacterial potential. South African Journal of Chemical Engineering 38(1):1-8.
22. Tamboli FA, More HN (2021) *In vitro* screening of Anti-diabetic activity and Anti-inflammatory activity of leaves extract of *Barleria gibsoni* Dalz. Research Journal of Pharmacy and Technology 14(3):1289-92.
23. Tundis R, Loizzo MR, Menichini F (2010) Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. Mini Reviews in Medicinal Chemistry 10(4):315-31.
24. Ukwuani-Kwaja, A. N., Nwaogu, J., Abba, A and Yahaya, M. (2021). Inhibitory Effects of Methanol Stem Bark Extracts of *Sterculia Setigera* and *Ficus Platyphylla* on Hemoglobin Glycosylation and α-amylase. *Diabetes and Metabolism*, *9*(2), 13-18.
25. Wilson, S., Cholan, S., Vishnu, U., Sannan, M., Jananiya, R., Vinodhini, S and Rajeswari, D. V. (2015). *In vitro* assessment of the efficacy of free-standing silver nanoparticles isolated from *Centella asiatica* against oxidative stress and its antidiabetic activity. *Der Pharmacia Lettre*, *7*(12), 194-205.

Figures

Figure 1

Boerhavia diffusa

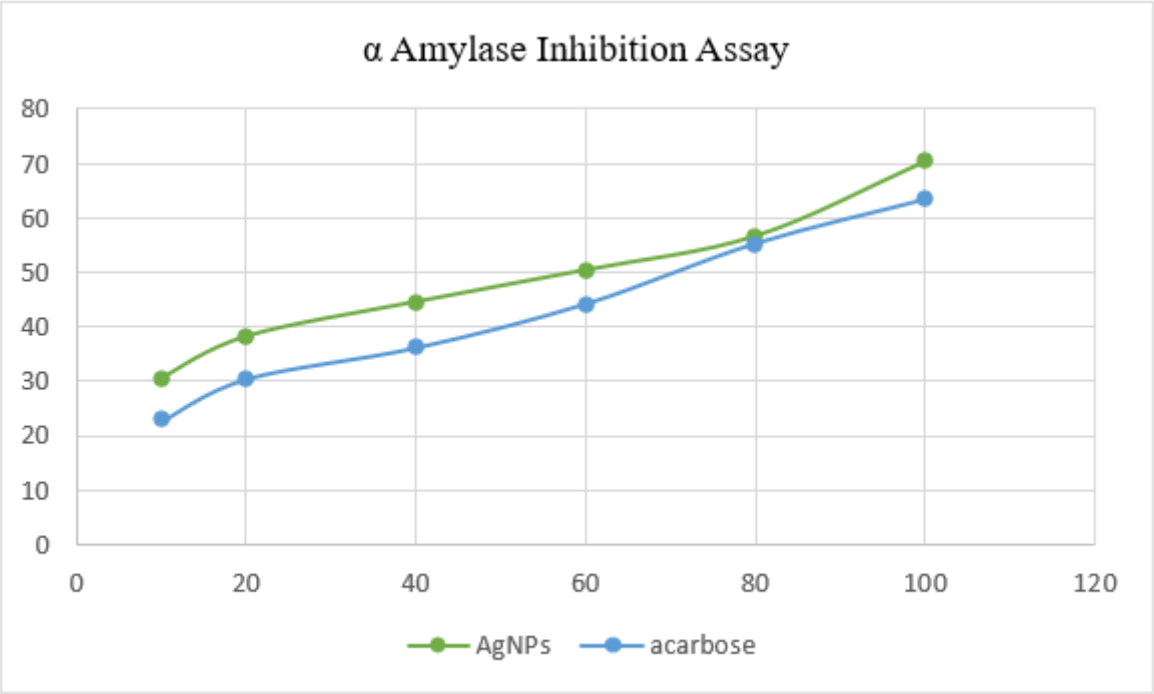

Figure 2

Inhibition of alpha-amylase

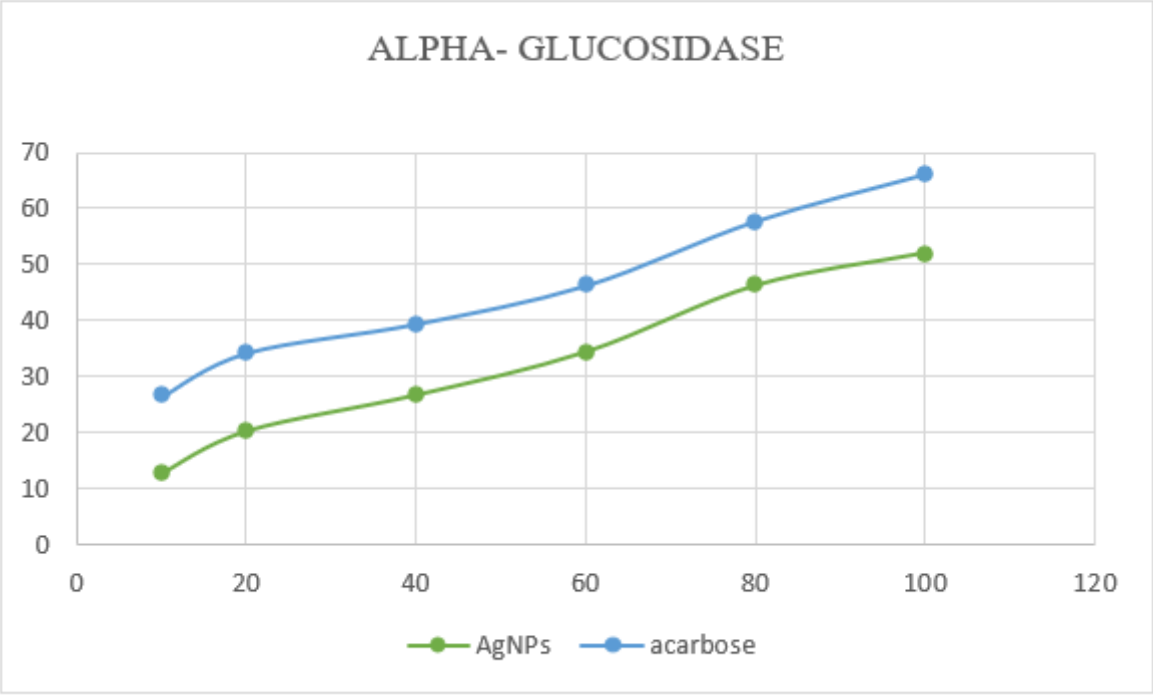

Figure 3

Inhibition of alpha- glucosidase

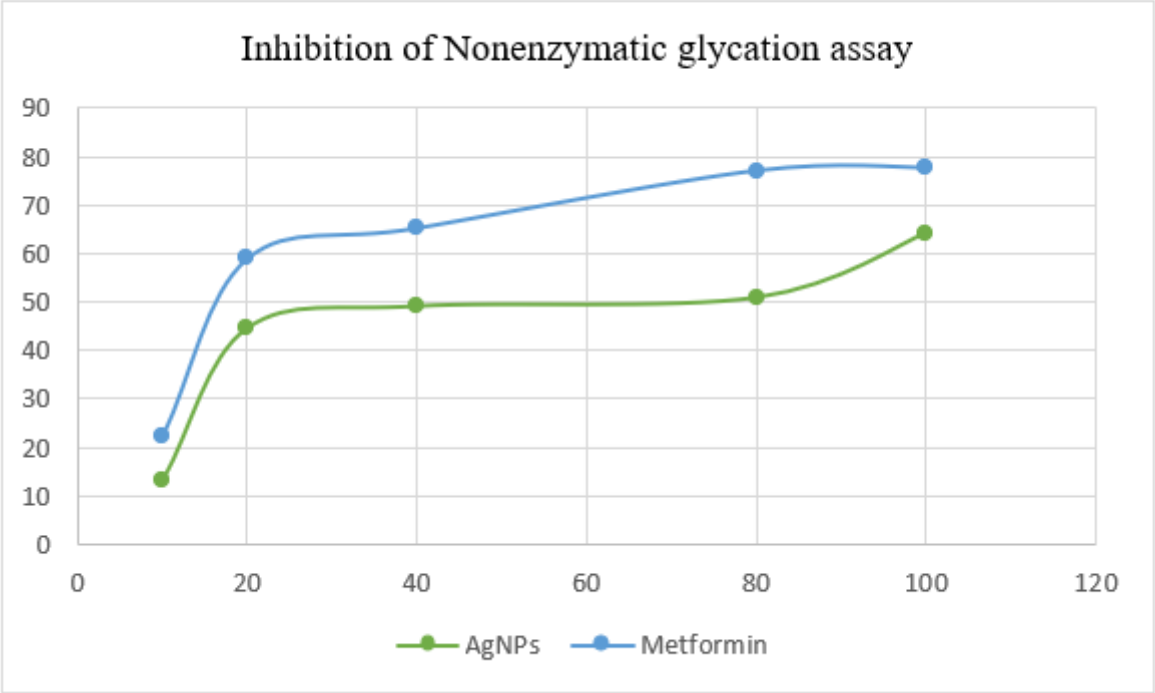

Figure 4

Inhibition of Nonenzymatic glycation assay

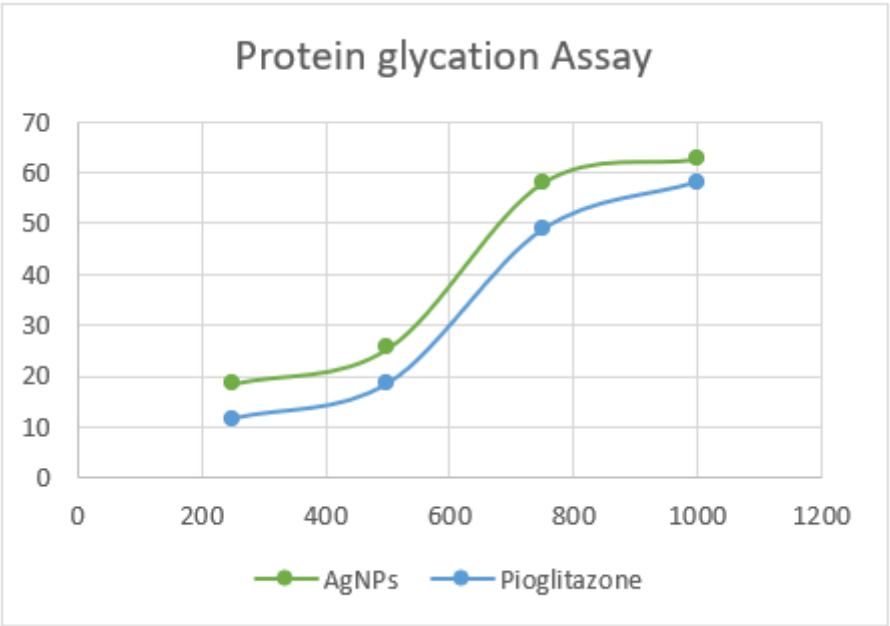

Figure 5

Inhibition of protein glycation