Anti-Diabetic Activity of *Bougainvillea glabra* mediated Silver Nanoparticles

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Abstract

The present study concentrates on the green synthesis of silver nanoparticles (AgNPs) from methanol bark extract of Bougainvillea glabra. The phytocomponents' presence causes the metal ions to be reduced and the silver nanoparticles to become stabilized. Green synthesis of silver nanoparticles was characterized by UV-Visible, DLS TEM and X-ray diffraction analysis. TEM and XRD study showed that the particles are crystalline in nature with face centred cubic geometry. EDX analysis shows the presence of silver element. In the present study, Bougainvillea glabra mediated silver nanoparticles were studied using an in vitro model for alpha amylase and alpha glucosidase inhibition.

Antioxidant activity was also examined by using free radical scavenging method while compared to standard acarbose. The BG-AgNps exhibits considerable inhibitory effects against 70.85% α-amylase and 78.04% α-glucosidase at a 100 µg/ml concentration respectively. Therefore, BG-AgNps have enriched herbal antidiabetic and antioxidant compounds and will have capacity for controlling diabetes mellitus.

Keywords: Anti-oxidant, Anti-diabetic, *Bougainvillea glabra* bark, DPPH.

Introduction

Diabetes mellitus is an autoimmune condition which impacts the metabolism of protein, carbs and lipids. It is explained for hyperglycemia, a condition where blood sugar levels are raised because the pancreas generates inadequate insulin or cells in the body resist to react frequently to the insulin produced[5]. Type 1 diabetes is caused by insufficient insulin synthesis via the pancreatic β-cells and resistance in insulin which will cause type II diabetes (a state where peripheral cells are less responsive to insulin)[4]. This illness is treated by reducing insulin requirement, increasing secretion of insulin, improving insulin's transport mechanism at target tissues and preventing oligo-and disaccharides breakdown[3,9]. Alpha glucosidase enzymes are in charge for the conversion of oligo and disaccharides to monosaccharides.

As a result of such inhibitory effect of enzyme, blood glucose levels fall. The activity of alpha amylase enzymes breaks down starch into simpler sugars like glucose, maltotriose and maltose. In hyperglycemic people, the alpha amylase inhibitors maintain serum blood glucose levels, prolonging the glucose absorption[1]. The carbohydrates digestion is delayed in the small intestine and the blood glucose level after a meal is reduced by alpha amylase and alpha glucosidase inhibitors[7]. Ascorbic acid, carotenoids and phenolic substances are examples of extremely potent antioxidants with the ability to interrupt the free radical chain reaction[2]. They are recognized to suppress lipid peroxidation, free radicals scavenging and prolonging a reaction cycle via reactive species like oxygen and heavy metal ions binding[14].

In recent years, natural medications have gained greater significance in the management of diabetes because of lack of adverse effects and more cost-effectiveness in comparison to synthetic hypoglycemic treatments. There are 18 species in *Bougainvillea* genus, some of them are used as traditional medicine. *Bougainvillea glabra* is noted for its extensive therapeutic characteristics including antiviral, antidiabetic, antifertility, anti-inflammatory and antimicrobial effects. Thus, using *in vitro* assay techniques, the antidiabetic and antioxidant properties of synthesised BG-AgNps were assessed in the current work.

Material and Methods

***Bougainvillea glabra* Bark Collection**: The *Bougainvillea glabra* bark were collected in and around Tiruchirapalli district.

Preparation of Methanol Extract of *Bougainvillea glabra* Bark: The bark of *Bougainvillea glabra* was cleaned with water, chopped into small pieces and then allowed to dry in the shade for a week before being ground into a fine powder. The methanol extract of sample was prepared by soaking 25g of sample in 100ml of methanol for about 48 hours and filtered through Whatmann filter paper no. 42 and it was refrigerated. Using the standard protocol, the filtrate was utilized as a sample for further qualitative and quantitative phytochemical screening.

Anti-oxidant Activity (DPPH Free Radical Scavenging Activity) Determination: DPPH free radical activity has been employed to examine the scavenging effect of the BG-AgNps antioxidant activity. Taking different concentrations (20–100 µg/ml), ethanol solution of 0.05 mM DPPH in 300 µl was mixed with 40 µl of the BG-AgNps. Freshly prepared DPPH is kept in the dark at 4°C. Mixture was shaken forcefully by adding ethanol 96% (2.7 ml). Letting the mixture to stand for 5 minutes, absorbance was measured

spectrophotometrically at 517 nm. Absorbance zero was adjusted using ethanol. Methodically, a blank sample with the same concentration of DPPH and ethanol was kept. Every measurement was carried out three times. From the following equation[10], percentage of inhibition was measured to tested the novel scavenging activities of the BG-AgNps.

Percentage (%) inhibition of DPPH activity= [(A-B)/A] X 100

where B is absorbance value of the test sample and A is absorbance value of the blank sample. Plotting the graph using inhibition percentage against concentration was executed. The concentration of BG-AgNps needed to achieve 50% inhibition for each test solution is represented by the IC50 value.

Alpha-Amylase Inhibitory Assay: 0.5 mg/mL of α-amylase in 0.02M sodium phosphate buffer having a pH 6.9 was combined with 250µL of BG-AgNps (20–100 µg/ml) in a tube. Pre-incubating this mixture for 10 minutes at 25 °C was carried out by a timely addition of 1% starch solution in 250 µL of 0.02M sodium phosphate buffer having pH 6.9 which was further incubated for additional 10 minutes at 25 °C. The DNS reagent (500 µL) was incorporated to carry out the chemical process. After five minutes of boiling in water, tubes undergo a cooling process to the ambient temperature.

With the reaction mixture that was diluted with 5 mL of DD water, an ultraviolet spectrophotometer was used to determine the absorbance at 540 nm. The distilled water was utilized instead of the BG-AgNps for the control, which was prepared using the same method[10]. Plotting α-amylase inhibiting capacity against percentage inhibition was carried out:

% inhibition= [(Abs control-Abs extract)/Abs control]x100

α-Glucosidase Inhibition Assay: The technique was implemented in order to assess the α-glucosidase activity using Saccharomyces cerevisiae BG-AgNps α-glucosidase. The substrate solution was made by incorporating 20 mM phosphate buffer, pH 6.9 and p-nitrophenyl glucopyranoside (p-NPG). With different concentrations (20–100 µg/ml), pre-incubate the solution containing 100 µL of alpha-glucosidase mixed with 50 microliter of BG-AgNps for 10min. To initiate the reaction, 50 microliter of 3.0 millimolar pNPG liquefied in phosphate buffer of having pH 6.9 was added to the substrate. After that, add 2 milliliters of 0.1M Na_2CO_3 to the reaction mixture which was kept for 20-minute incubation period at 37°C. The paranitrophenol discharged from pNPG, which emits paranitrophenol at 405 nm, was examined to determine the α-glucosidase activity. The outcomes provide a percentage of the reference blank[15]. The percentage inhibition of α-glucosidase inhibition assay was determined:

% Inhibitive activity = [(Absorbance control – Absorbance sample) / Absorbance control] x 100.

Graphically BG-AgNps strength occurring in 50 percentage inhibition with IC50 enzyme activity was determined.

Statistical Analysis: Every assay was run three times. Duncan's test was run after an Analysis of Variance (ANOVA) using SPSS 16.0 for analysis using statistics. P-values were considered significant differences if they were less than 0.05.

Table 1
Qualitative results of *Bougainvillea glabra* bark

Test for	Observation	Result
Tannin	Brownish green	+++
Phlobatannin	Red precipitate	A
Saponins	Blue colour	+++
Flavonoids	Yellow colour	+++
Steroids	Blue colour	+
Terpenoids	Reddish Brown	+++
Cardiac glycosides	Brown ring	+++
Leucoanthocyanin	Red layer	A
Anthocyanin	Bluish Violet	A
Anthraquinone	Red	A
Protein	White precipitate	A
Coumarin	Yellow colour	+++
Glycosides	Green colour	+
Phenol	Blue Black	+
Xanthoprotein	Reddish orange precipitate	A
Alkaloids	Yellow colour	+++
Emodin	Red colour	A
Carbohydrate	Reddish violet colour	+

+ - Trace ++ - Moderate +++ - Strong A- Absence

Results and Discussion

Qualitative Phytochemical Screening of *B. glabra* Bark: The phytochemical screening of *B. glabra* bark shows the existence of tannin, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, coumarin and alkaloids (Table 1) and its qualitative performance was shown in fig. 1.

Quantitative Phytochemical Estimation of *B. glabra* Bark: The quantitative phytochemical estimation of B. glabra bark was tabulated in table 2 and depicted in fig. 2. The *B. glabra* bark contains 0.003 mg/g of flavonoids, 0.019 mg/g of saponins, 0.015 mg/g of alkaloids, 0.005 mg/g of phenol, 0.001 mg/g of tannin and 0.001 mg/g of terpenoids.

Characterization of Silver Nanoparticles

UV-Visible Spectroscopy: In this work, the incorporation of methanolic bark extract of *Bougainvillea glabra* into 5 mM silver nitrate solution results in colour exchange from colourless to brown which shows the presence of silver nanoparticles. In addition, the peak discovered at 447nm showed the silver nanoparticles boom of height because of SPR of electrons in reaction mixture indicating that the particles have been polydispersed (Fig. 3)

Fig. 1: Qualitative analysis of bark of *Bougainvillea glabra*

Table 2
Quantitative results of *Bougainvillea glabra*

Phyto constituents	*Bougainvillea Glabra* (mg/g)
Flavonoids	0.003
Saponins	0.019
Alkaloids	0.015
Phenol	0.005
Tannin	0.001
Terpenoids	0.001

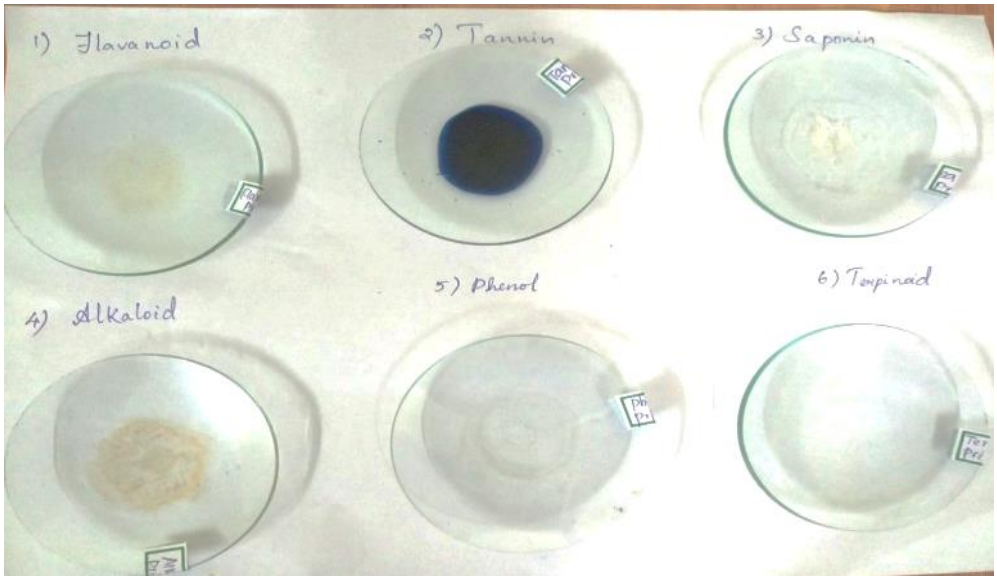

Fig. 2: Quantitative analysis of *Bougainvillea glabra*

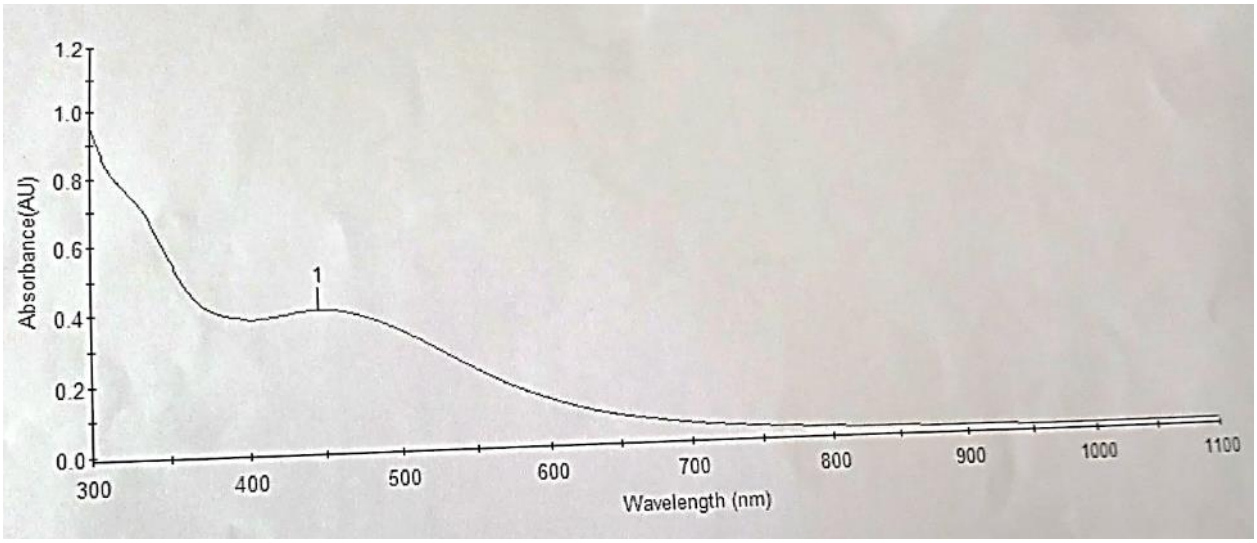

Fig. 3: Silver nanoparticles characterization by UV-Vis spectroscopy

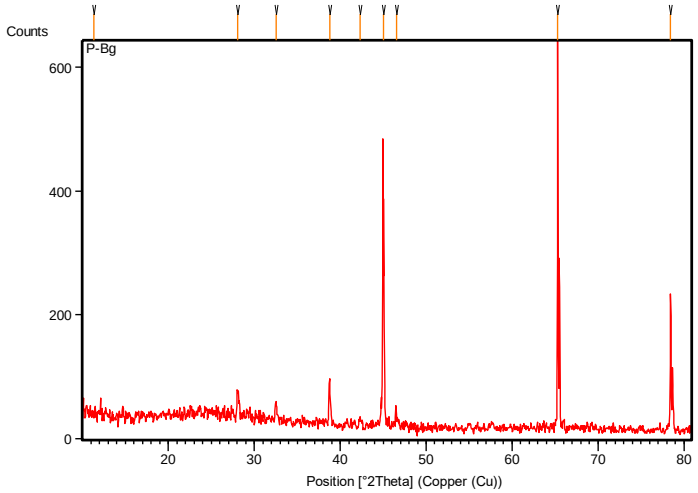

Fig. 4: The X-RD patterns of synthesized silver nanoparticles using a bark *Bougainvillea glabra* extract

Table 3
XRD results of synthesised silver nanoparticles using *Bougainvillea glabra* bark extract

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
11.3590	7.67	2.3616	7.79013	2.09
28.0719	41.41	0.1968	3.17872	11.26
32.5628	27.68	0.2952	2.74986	7.53
38.7738	74.60	0.1968	2.32248	20.28
42.3514	9.82	0.2952	2.13421	2.67
45.0069	272.48	0.1968	2.01426	74.08
46.5974	14.69	0.3936	1.94915	3.99
65.2878	367.81	0.1476	1.42921	100.00
78.4270	223.86	0.1200	1.21842	60.86

XRD: The XRD spectrum was recorded to verify the crystallinity of synthesized silver nanoparticles using bark extract of *Bougainvillea glabra* and the pattern shown in the fig. 4. The diffraction peaks were observed at 11.3590, 28.0719, 32.5628, 38.7738, 42.3514, 45.0069, 46.5974, 65.2878 and 78.4270 in the 2Ɵ range. Braggs diffraction of silver nanoparticles was measured at two theta values of 38.7738 and 46.5974 corresponding to the lattice planes (111), (200 which included indexing for silver. Some of these unassigned peaks are attributable to the existence of phytochemical extracts that might be capping onto the surface of nanoparticles[11,13].

TEM: Silver nanoparticles derived from *Bougainvillea glabra* bark extract are depicted in fig. 5 with a TEM picture which reveals the particles' polydisperse nature. Altogether,

the synthesized silver nanoparticles are spherical in nature. Depending upon the phytochemical's presence, nanoparticles will be in small, large and undefined shapes.

DLS: The DLS uses light as medium to find the particle size in solution. PDI explains the distribution of silver nanoparticles and confirms that the particles are not aggregated and ranges vary from 0.0000 to 0.5. PDI greater than 0.5 indicates that the particles are aggregated. The particle size distribution graph obtained from the DLS silver nanoparticles synthesized by *Bougainvillea glabra* is shown in fig. 6. The result indicates that obtained size of silver nanoparticles synthesized from *Bougainvillea Glabra* was 293 nm. For the conformation of monodispersity, DLS result indicates that 0.336PDI shows that nanoparticles are well dispersed in the methanol solvent.

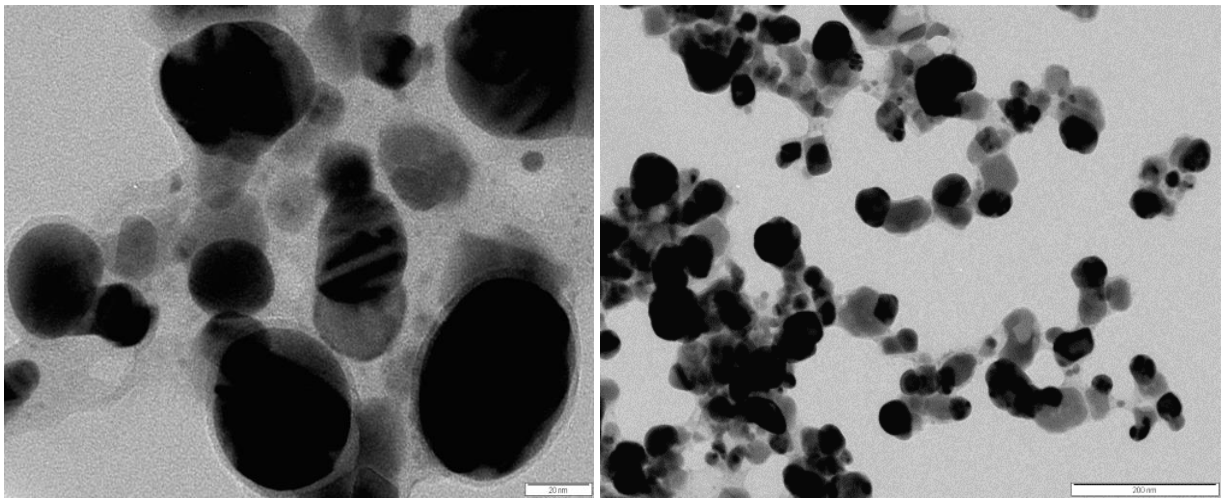

Fig. 5: TEM image for synthesized silver nanoparticles

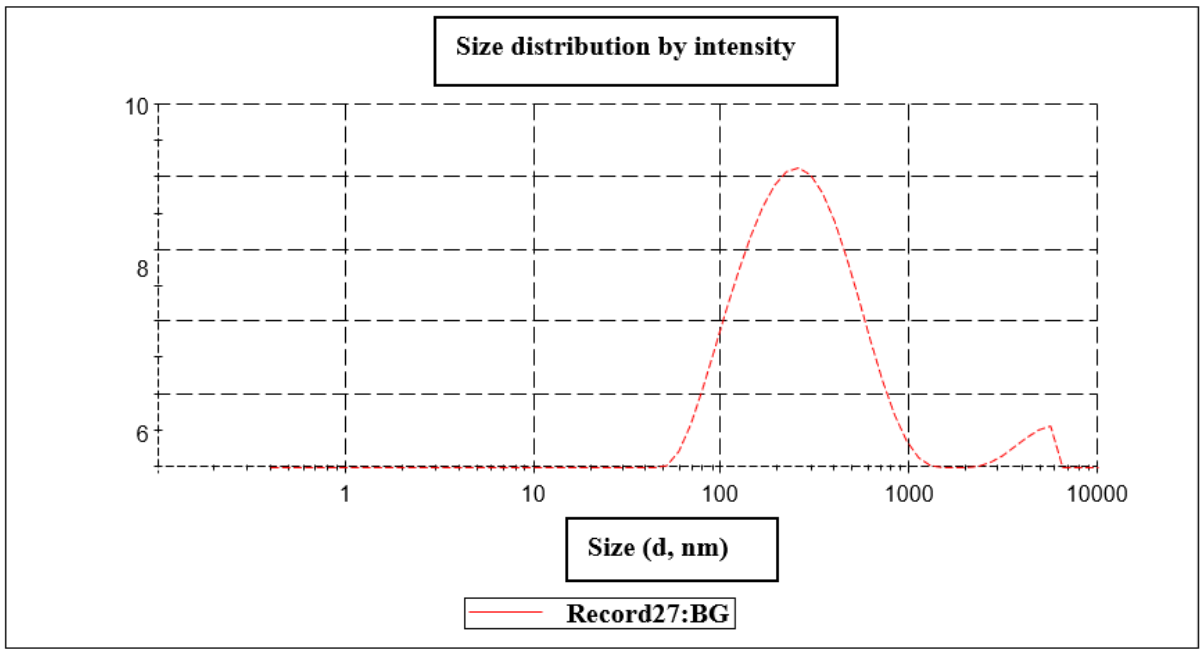

Fig. 6: DLS curve of synthesized silver nanoparticles

Table 4
Particle size analysis result of synthesized silver nanoparticles

			Size (d.nm)	%Intensity	Standard deviation
Z-Average(d.nm):	221.0	Peak1:	292.8	95.9	185.6
Poly dispersity index	0.336	Peak2:	4476	4.1	893.7
Intercept: Result Quality Good	0.935	Peak3:	0.000	0.0	0.000

BG-AgNps Antioxidant Activity as Determined by the DPPH Method: The findings show that when compared to ascorbic acid, the BG-AgNps exhibited higher percentage antioxidant activity at high concentrations (Table 5). The antioxidant result of BG-AgNps is mainly unpaid to radicals scavenging activity of phenolic compound such as alkaloids, flavanoids, phenol and tannin. The compound showed 80.70% activity at 100µg/ml concentration while at the same concentration, ascorbic acid gives 94.69% (Fig. 7).

***In vitro* Alpha Amylase Inhibitory Assay**: The present investigation examined the *in vitro* α-amylase inhibitory activity of BG-AgNps. The significance confirmed that there has been a dose-reliant on upward push in percentage inhibitory interest towards α -amylase enzyme. The BG-AgNps confirmed powerful α-amylase inhibitory interest in a dose reliant on manner.

The BG-AgNps offered inhibitory pastime from 34.04 to 86.85 % at concentration 100µg/ml. Acarbose is a common drug used as an α-amylase inhibitor. Acarbose exhibited an α-amylase inhibition pastime between 39.85±0.24 and 85.87±0.37% at equal dosages of 100 µg/ml when used at concentrations ranging from 20 to 100 µg/ml. A contrast of α–amylase inhibition pastime among the same old pills have shown in fig. 9.

***In vitro* Alpha Glucosidase Inhibitory Assay**: Table 7 displays the anti-diabetic activity results of BG-AgNps utilizing the alpha glucosidase inhibitory assay. The alpha glucosidase enzyme's significant inhibitory activity has been proven by the BG-AgNps. The percentage inhibition at BG-AgNps concentrations ranging from 20 to 100 µg/ml revealed a dose dependence on an increase in percentage inhibition. From the highest concentration to the lowest concentration, the % inhibition changed. Therefore, the BG-AgNps ability to inhibit alpha glucosidase would slowdown the breakdown of carbohydrates, which would then encourage a reduction in glucose absorption and consequent decrease in the rise in postprandial blood glucose levels.

Table 5
Antioxidant activity of BG-AgNps

Concentrations	Scavenging Effect (%)	
	Bark of *Bougainvillea Glabra*	Ascorbic acid
20 (µg/ml)	38.69±1.56	41.60±1.33
40 (µg/ml)	58.479±1.24	66.85±1.37
60 (µg/ml)	60.23±1.35	76.74±1.42
80 (µg/ml)	73.09±1.42	82.34±1.47
100 (µg/ml)	80.70±1.20	94.69±1.50

The data are presented as mean± SEM, with each value acquired by averaging three experiments.

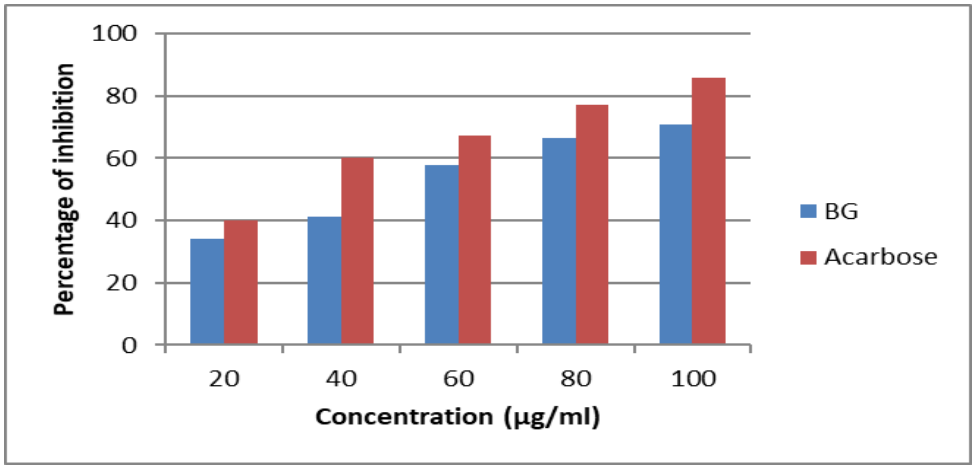

Fig. 7: Graphical representation of antioxidant activity of BG-AgNps

Table 6
***In vitro* anti-diabetic activity of the BG-AgNps using alpha amylase method**

Concentrations	Alpha amylase (%)	
	BG-AgNps	Acarbose
20 (µg/ml)	34.04±0.68	39.85±0.24
40 (µg/ml)	41.13±0.61	60.21±1.37
60 (µg/ml)	57.92±0.58	67.20±1.42
80 (µg/ml)	66.43±0.54	77.25±1.47
100 (µg/ml)	70.85±0.49	85.87±0.37

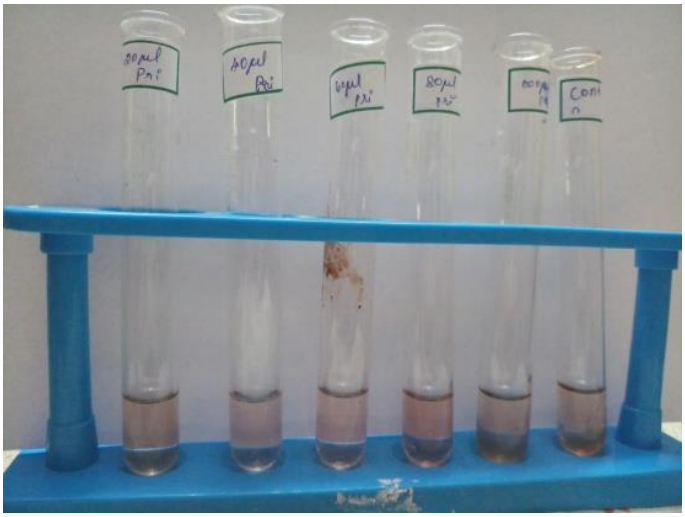

Fig. 8: Anti-oxidant activity of BG-AgNps

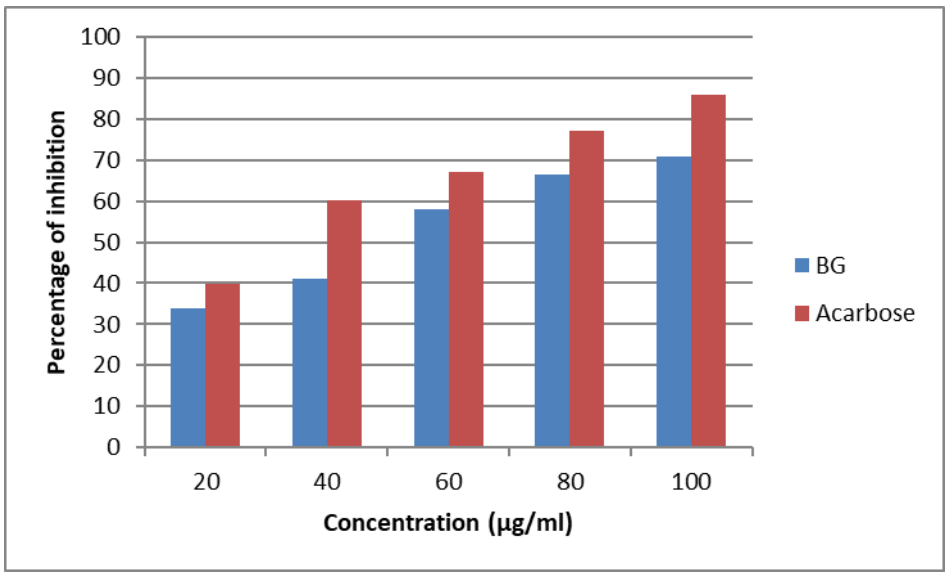

Fig. 9: Graphical representation of *in vitro* anti-diabetic activity of the sample using alpha amylase synthesized using BG-AgNps

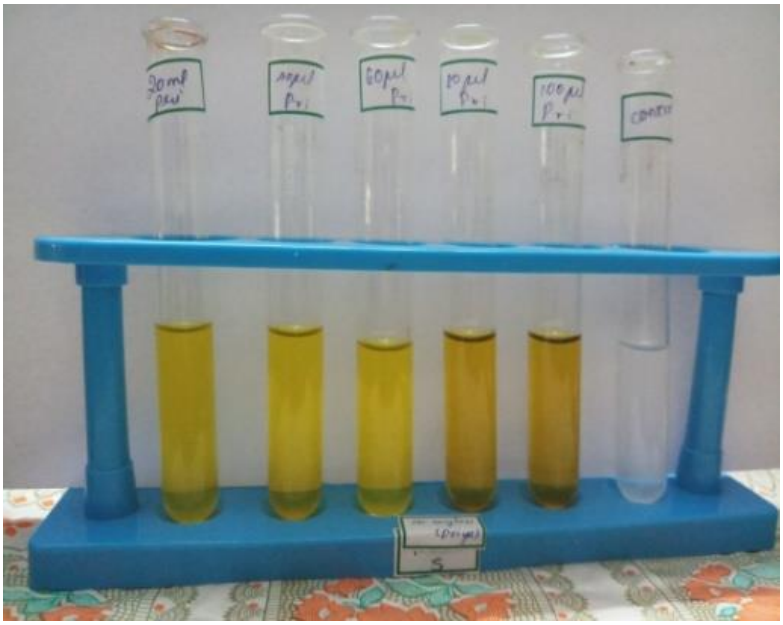

Fig. 10: Anti-diabetic activity of the BG-AgNps using alpha amylase method

Table 7
In vitro antidiabetics activity of the BG-AgNps using alpha glucosidase method

Concentrations	Alpha glucosidase (%)	
	BG-AgNps	Acarbose
20 (µg/ml)	38.21±0.74	72.70 ±1.40
40 (µg/ml)	47.96±0.62	62.34±1.37
60 (µg/ml)	52.03±0.56	75.48±1.42
80 (µg/ml)	68.29±0.54	84.54±1.47
100 (µg/ml)	78.04±0.53	95.68±1.38

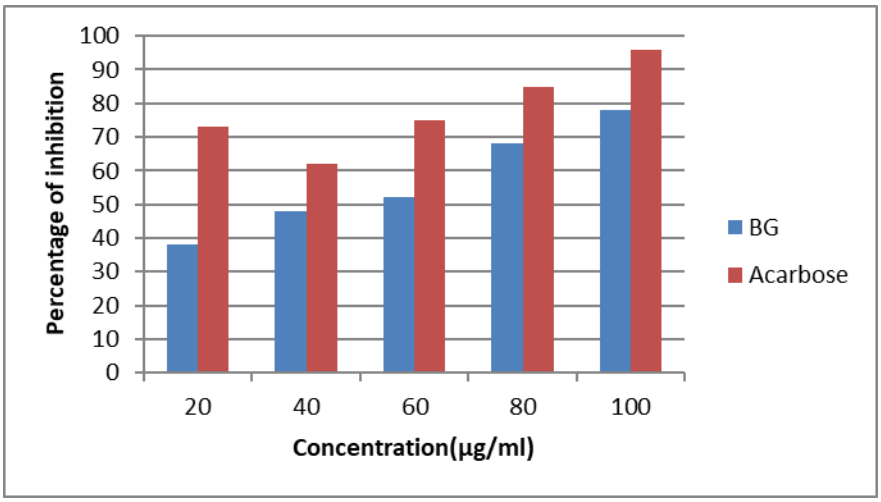

Fig. 11: Graphical representation of alpha glucosidase inhibitory activity of BG-AgNps

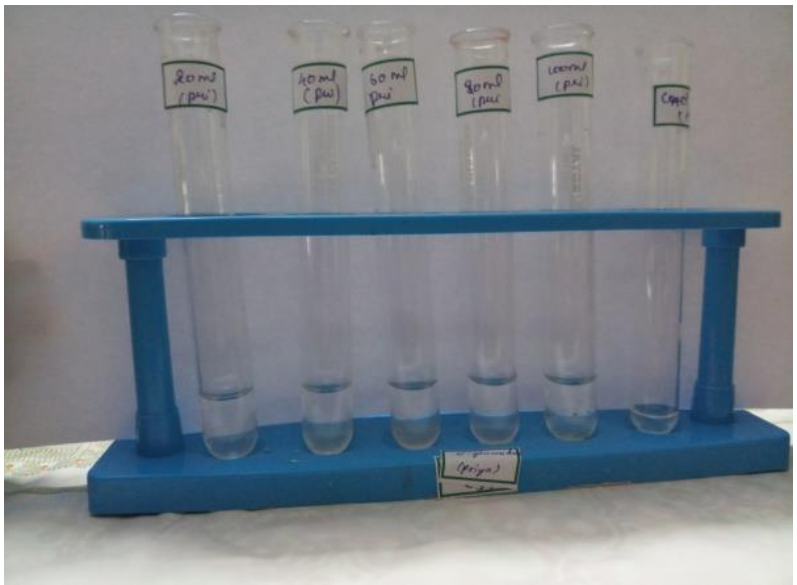

Fig. 12: Alpha-glucosidase inhibitory activity of BG-AgNps

Acarbose is a typical medication for alpha glucosidase inhibitors. Acarbose demonstrated alpha glucosidase inhibition activity ranging between 72.70±1.40 and 95.68±1.38 % at a concentration of 20–100µg/ml. BG-AgNps is actual powerful alpha amylase and alpha glucosidase inhibitor in comparison to the standard[8]. The ability to inhibit alpha amylase and alpha glucosidase enzymes allowed BG-AgNps from hypoglycemic activity and isolated components (linoleic acid, lupeol acetate, lupeol, trans-p-coumaric acid, beta sitosterol and cis-p-coumaric acid) to be readily available[16].

Conclusion

The main aim of green synthesis of silver nanoparticles is eco-friendly and cost effective and provides alternative way for diminishing the harmful effects created by chemical and physical methods. The phytochemical qualitative result shows the presence of following phytoconstiuents like tannin, saponins, flavonoids, steroids, terpenoids, cardiac glycosides, coumarin and alkaloids. The phytochemical quantitative analysis has been done for phytoconstiuents like flavanoids, saponins, alkaloids, phenol, tannin, terpenoids

and weighed. The synthesized silver nanoparticles were examined by UV- Visible spectrophotometer and the peak intensity appears at 447nm. The TEM studies show that synthesized silver nanoparticles are spherical in nature.

The DLS was performed to know the size distribution. The XRD analysis was done and the crystalline nature of the BG-AgNps was found out to be face centered cubic. BG-AgNps, have the ability to scavenge free radicals and inhibit the activity of α-amylase and α-glucosidase. The BG-AgNps exhibit considerable inhibitory effects against 70.85 % α-amylase and 78.04 % α-glucosidase at 100 µg/ml concentration. This healing property can be utilized in conjunction with the treatment of type 2 diabetes mellitus to control postprandial hyperglycemia.

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