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SYNTHEIS OF SILVER NANOPARTICLES FROM *GYMNEMA SYLVESTRE* LEAF AND EVALUATION OF THEIR *IN VITRO* ANTIDIABETIC ACTIVITY

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ABSTRACT

The environmental friendly synthesis of nanoparticles process is a revolutionary step in the field of nanotechnology. In the is study, the biosynthesis of silver nanoparticaes was carried out using *Gymnema sylvestre* leaf extract as reducing an agent UV-visible spectroscopy was used for identification of silver nanoparticles synthesis. The synthesized silver nanoparticles elctrons were characterized UV-visible spectrophotometry, Scanning Electron Microscopy (SEM) and Fourier Transform Infra red spectrorscopy (FTIR). *In vitro* antidiabetic properties of the bio synthesis silver nanoparticles were found to have high inhibitory activity. The assay result suggest that the presence of bio active compounds, AgNPs exhibit the dose dependent increase in inhibitory on α Amylase enzyme α-gulucosidase and Tyrosinase enzymes. These results indicated that AgNPs from *Gymnema sylvestre* leaf could be used as a source of antidiabetic agents.

KEYWORDS: *Gymnema sylvestre*, Antidiabetic Activity, UV-visible spectra, Silver nanoparticles.

INTRODUCTION

Diabetes mellitus results from the defects in the insulin secretion and action; this may be characterized by chronic hyperglycemia, which is connected with the carbohydrates, protein and lipid metabolism (Geneva, 1999). Globally mortality rate 9% is recorded due to the diabetes. Diabetes mellitus a well known endocrine disorder and it is most common in India now a day. The reason may be life style and genetic factors (Riser us *et al.*, 2009). Diabetes

mellitus (DM) is a group of syndromes characterized by hyperglycemia; altered metabolism of carbohydrate protein and lipids and an increased risk of complications from vascular disease that affects 10% of the population. According to International Diabetic Federation the estimated diabetes prevalence in 2010 has risen to 285 million representing 6.4% of the world's adult population, with a prediction that by 2030, The number of people with diabetes will have risen to 438 million, with this alarming concern, India has been declared as the ''Diabetic capital of world''. Currently 40.9 million people in India suffering from diabetes and by 2030 there would be 79.44 million diabetics in India alone. It is also estimated that by the year 2030, diabetes is likely to be the seventh leading cause of death, accounting 3.3% of total deaths in the world (Singh *et al*., 2011).

Nanotechnology, and alongside nano structured materials, play an ever increasing role in science, research and development as well as also in every days life, as more and more products based on nano structured materials are introduced to the market. The advance and very applicable technology is nanotechnology and it was derived from the term of nano it is the billionth of meter or 10^{-9} m. The Nano come ultimately from the Greek word for dwarf, and is also related to the Spanish word Nino (Taylor 2001). The synthesis of silver nanomaterials or nanoparticles extensively studied by using chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important aspect of nanotechnology. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum. The synthesis of silver nanomaterials or nanoparticles extensively studied by using chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important aspect of nanotechnology. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required. At the same time the biologically synthesized silver nanoparticles has many applications includes catalysts in chemical reactions (kumar *et al*., 2003).

Gymnema Silvestre R.Br. is an imperative remedial woody climber belonging to family Asclepiadaceous- 'The Milk Weed Family'. One special name of this plant species is' Miracle fruit'. The name 'Gymnema' probably derives from the Latin word meaning 'naked' and Sylvester means 'from the forest'. It is native to central and western India and can be also found in tropical Africa and in Australia.

The use of plants as medicine is as old as human civilization. Renewed interest of developing as well as developed countries in the natural resources has opened new horizons for the exploration of natural sources with the perspectives of safety and efficacy. Herbal drugs, in India are also used as household remedy for common ailments since time immemorial. The plant *Gymnema sylvestre*R. Br. (Asclepiadaceae) is a vine which grows in the southern part of China, including the Guangdong, Guangxi and Fujian provinces. *G. sylvestre* occurs mainly in the Deccan peninsula of western India, Tropical Africa, Vietnam, Malaysia, and Srilanka and is widely available in Japan, Germany and the USA as a health food (Mitra *et al*., 1995).

The plant extracts are also used in folk, Ayurvedic and Homeopathic systems of medicine (Kapoor, 1990). *G. sylvestre* is a traditional medicinal plant, with reported use as a remedy for diabetes mellitus, stomachic and diuretic problems. Traditionally its use has been indicated in adenopathy, cough (Kirtikar and Basu 1975), asthma, biliousness, bronchosis, cardiopathy, conjunctivosis cornea, diabetes, dysuria, fever, furunculosis, glycosuria, hemorrhoid, inflammation, leukoderma, opacities, ophthalmia, and worm (Watt G 1972). The roots of *Gymnema sylvestre* has also been used in snake bite (Watt JM and breyer-brand wijk MG 1962); boil, constipation, and water retention (Bulkill HM 1994); epilepsy, pain (Bone K 1996); high cholesterol, IDDM, NIDDM and obesity. The extract of *G. sylvestre* plays a major role in blood glucose homeostasis through increased serum insulin level and regeneration of the endocrine pancreas (Shanmugasundaram ERB *et al*, 1990).

MATERIALS AND METHODS

Plant Collection

The plant leaves on *Gymnema Silvestres* collected from Mannargudi, Thiruvarur District in Tamil Nadu.

Preparation of plant extracts

The collected plant leaves were washed thrice in sterile distilled water to remove adhering soil particles and salts. The washed samples were shade dried for one week at room temperature. The leaves were cut in to small pieces and grained in to powder. The pure plant extract was prepared by adding 10gm of plant powder in to 100 ml of distilled water and boiled for 5 minutes. The boiled extract was filtered through Watman No.1 filter paper and the supernatant was used.

Biosynthesis of silver nanoparticles

In the typically synthesis process of silver nanoparticles , add 10 ml of pure plant extract sample in to the 90 ml of 1 mm of silver nitrate solution in 250 ml of conical flask. The reaction mixture was kept at room temperature under mechanically stirring. The colour change was noted and the nanoparticles formation was monitored.

UV spectroscopy analysis of AgNPs

Synthesis of silver NPs solution with leaf extract may be easily observed by ultraviolet – visible (UV-Vis) spectroscopy. The reduction of the Ag+ ions in solution was monitored by periodic sampling of aqueous component and measuring the UV- spectra of the solution. UV-spectra of these Aliquots were monitored as a function of time of reaction on a spectrophotometer (UV-1800 series).

FTIR analysis of silver nonoparticales

Possible functional groups involved in the synthesis and stabilization of silver AgNPs was studied by FTIR spectroscopy. The FTIR was recorded in the range of 500-4500 $^{cm-1}$ (Japan polymer lab) the various modes of vibrations were identified and assigned to determine the different functional groups present in the leaf extract of *Gymnema Silvestre*.

SEM analysis of silver nanoparticales

SEM analysis was done using JEOL "JSM- 6610 LV SEM machine. Thin films of the sample was prepared on a carbon coated platinum grid by adjusting the dropping of a very small amount of the sample on the grid, extract solution was removed using a blotting paper. The film on the Sem grid was dried under a mercury lamp for 5 min. The thin film on grid was examined using slanging electron microscope.

α amylase inhibitory activity

The assay mixture containing 200 µl of 0.02 m sodium phosphate buffer, 20 µl of alpha amylase enzyme and the plant fractions (20-100 µg/ml) were incubated for 10 minutes at room temperature followed by addition of 200 µl of starch. The reaction was terminated with the addition of 400 µl DNS reagent and placed in boiling water bath for 5 minutes, cooled and diluted with 15 ml of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any plant fractions. The % inhibition was calculated as follows

Inhibition (%) = Absorbance 540 (control) – Absorbance 540 (extract) X 100

Absorbance 540 (control)

The IC50 values were determined from plots of percent inhibition versus log inhibitor concentration by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha amylase inhibitor. All tests were performed in triplicate.

α glucosidase inhibitory activity

P-Nitrophenyl-α-D-glucopyranoside, Baker's Yeast alpha glucosidase were purchased from Sigma (USA).The yeast alpha glucosidase was dissolved in 100 mm phosphate buffer pH 6.8 and was used as the enzyme extract. P-Nitrophenyl-α-D-glucopyranoside was used as the substrate. Plant fractions were used in the concentration ranging from 20-100 µg/ml. Different concentrations of plant fractions were mixed with 320 µl of 100 mm phosphate buffer pH 6.8 at 30 °C for 5 minutes. 3 ml of 50 mm sodium hydroxide was added to the mixture and the absorbance was read at 410 nm 86. The control samples were prepared without any plant fractions. The % inhibition was calculated according to the formula.

Inhibition (%) = Absorbance 410 (control) – Absorbance 410 (extract) X 100

Absorbance 410 **(control)**

The IC 50 values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor. All tests were performed in triplicate.

Tyrosinase inhibitory activity

Test reaction mixtures were prepared by adding 10 µl tyrosinase to10 µl plant fractions and then adding 20 µl 1.5 mm L-tyrosine and 110 µl of 0.1 M sodium phosphate buffer (pH 6.5). The resulting mixture (150 µl) was incubated for 10 minutes at 37°C and absorption at 490 nm was measured 187. The percent inhibition of tyrosinase activity was calculated as follows.

Inhibition (%) = Absorbance 490 (control) – Absorbance 490 (extract) X 100

Absorbance 490 **(control):** The IC 50 values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non linear regression analysis from the mean inhibitory values. L-ascorbic acid was used as the reference tyrosinase inhibitor. All tests were performed in triplicate.

RESULTS AND DISCUSSION

Silver nitrate is used as reducing agent as silver has distinctive properties such as good conductivity, catalytic and chemical stability. The aqueous silver ion when exposed to herbal extracts was reduced in solution, there by leading to the formation of silver hydrosol. The time duration of change in colour varies plant to plant. Silver nanoparticles with their unique chemical and physical properties are proving to be an alternative for the development of new Antidiabetic agents. Silver nanoparticles have also found diverse applications in the form of wound dressings, coatings for medical devices and silver nanoparticle impregnated textile fabrics, etc.

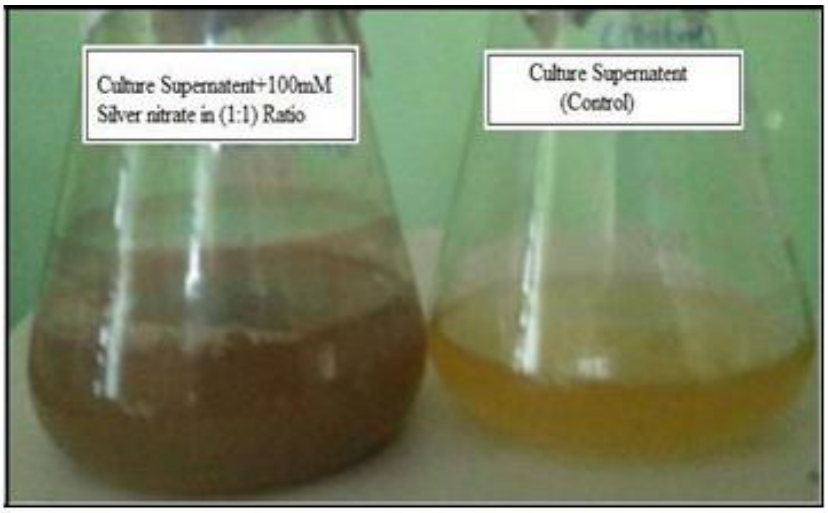
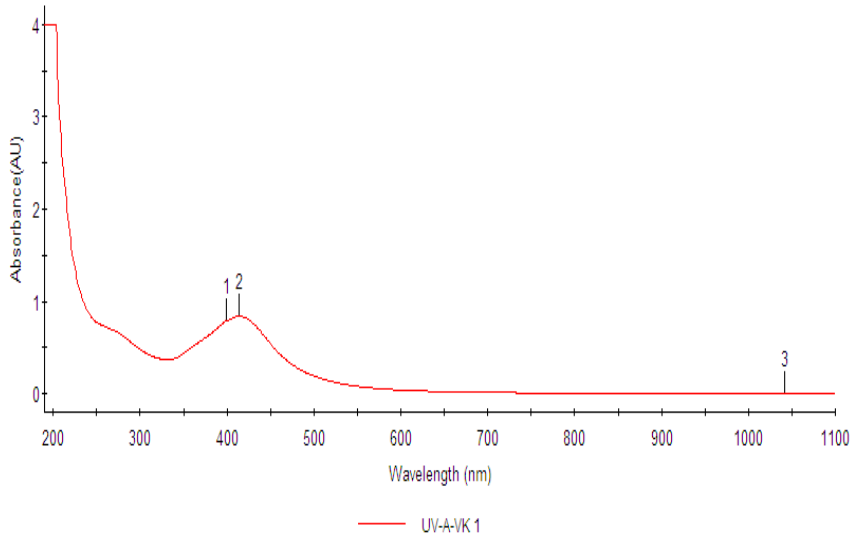


Figure. 1: Visual observation of silver nanoparticles.

UV-visible spectroscopy analysis

Silver ions were reduced to silver nanoparticle when added to *Gymnema sylvestre* plant powders. It was observed that colour of the solution turned to dark brown after 24 hours of the reaction which indicated the formation of silver nanoparticles.

The synthesized silver nanoparticles in the colloidal solution were monitored by UV–visible spectrophotometer analysis. Figure3 shows that the absorption spectra of silver nanoparticles formed in the action media has an absorbance peak at 398nm. The increased absobance at 24 hrs and the peak at 398 nm correspond to the surface Plasmon resonance of silver nanoparticles. It is reported earlier that absorbance at around 402 nm for silver is a characteristic of these metal particles .Thus, the study suggests that leaf extract of *G.sylvestre* might reduce Ag^{+} to Ag^{o}.



Fourier Transform Infrared Spectroscopy (FTIR) Analysis

FTIR analysis was used for the characterization of silver nanoparticles. FTIR absorption spectra of synthesis of silver nanoparticles are shown in (fig.-2). The absorption bands in are observed in the region of 4000-500 cm-1 is showed peaks at of 3437.04, 2075.17, 1637.43, and 668.24 cm^{-1} .The FTIR analysis spectrum showed sharp absorbance between 500 and 4000 cms^{-1}.

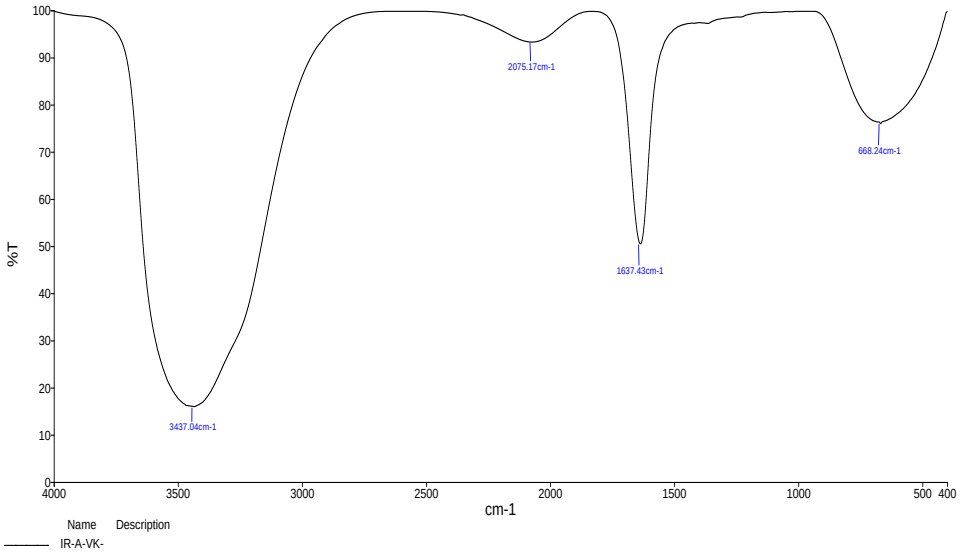
This represents the different functional groups of absorbed biomolecules on the surface of nanoparticles. It indicates the influence of organic moieties on the formation of silver nano particles and its stabilisation.

The absorption peak at around 668.24 cm^{-1}can be assigned as alkene =C-H stretching, likewise the peak at 1637.43 cm^{-1} as amide C=O stretching, 2075.17cm^{-1} as alkyne -ↄ≡ↄ-, 3437.04cm^{-1} as alcohol O-H stretching.

The functional groups of compounds adsorbed on the AgNPs were identified using FTIR studies. The peak near in FTIR 3437,2075 and 1637 cm^{-1} assigned to alcohol O-H stretching, alkye stretching and alkene C=C stretching respectively (Jain *et.al.*, 2009 and S.Sulochana, Palaniyandi Krishnamoorthy *et.al.*, 2012). The total disappeance of this band after the bioreduction may be due to the fact that the polyols are mainly responsible for the reduction of Ag ions. Thus result suggests that AgNPs might be capped by water soluble secondary plat metabolites.

Table. 1: FTIR peak value of water plant extract *Gymnema sylvestre.*

S. No.	Group of Frequency cm-1 of The Sample	Functional Group	Interpretation
1	3437.04 cm^{-1}	Stretch,H-bonded O-H	Alcohol
2	2075.17 cm^{-1}	Stretch –C=C-	Alkyne
3	1637.43 cm^{-1}	Stretch C=O	Amide
4	668.24 cm^{-1}	Bending =C-H	Alkene



Scanning Electron Microscopy (SEM) Analysis

Fig.3 shows representative SEM images recorded from drop-coated films of the silver nanoparticles synthesized by treating silver nitrate solution with *Gymnema sylvestre* leaf extract. The silver nanoparticles formed were predominantly tubeular and cubical with uniform shape the similar phenomenon was reported by (Chandran *et al.*, 2006). It is known that the shape of metal nanoparticles considerably change their optical and electronic properties (Kall *et al.*, 2002).

The synthesised silver nanoparticles using *G.sylvestre* were well distributed as aggregates in solution. The synthesis of silver nanoparticle using *G.sylvestre* leads to the formation of crystalline nanoparticles with variety of shapes and sizes of ranging from 1-100nm. Interestingly, the size of *G. sylvestre* reduced AgNPs were found to range from 24-90 nm under SEM observation.

A SEM employed to analyze the morphology and size details of the silver nanoparticles that were formed from Figure 5 It was showed that the silver nanoparticles formed were spherical in shape, with an average size of around 100nm and uniformly distributed silver

nanoparticles on the surface of the cell was observed. Thus study suggests that SEM image showed the high density silver nanoparticles synthesised by the *Gymnema sylvestre* development of silver nano structures.

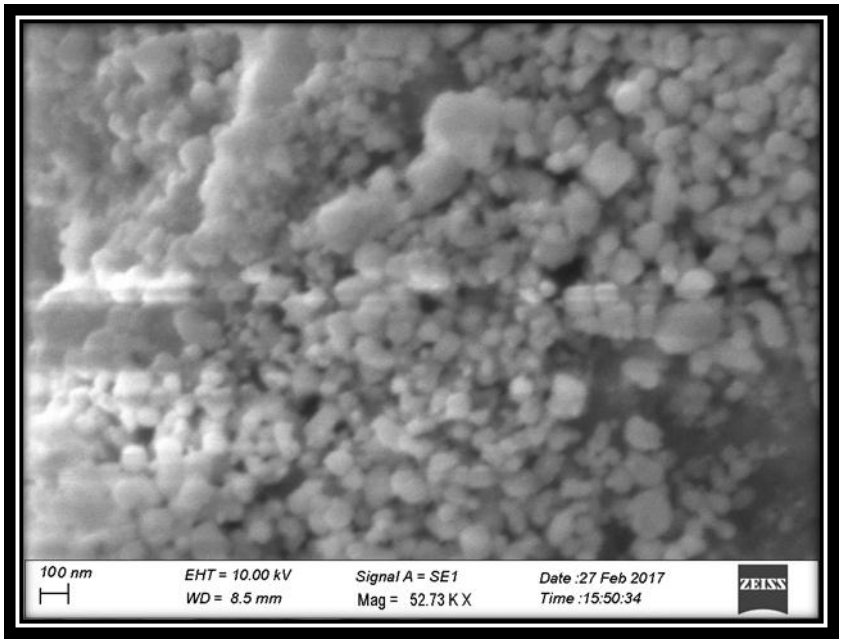


Figure. 3: The SEM image showed the high density silver nanoparticles synthesised in *Gymnema sylvestre.*

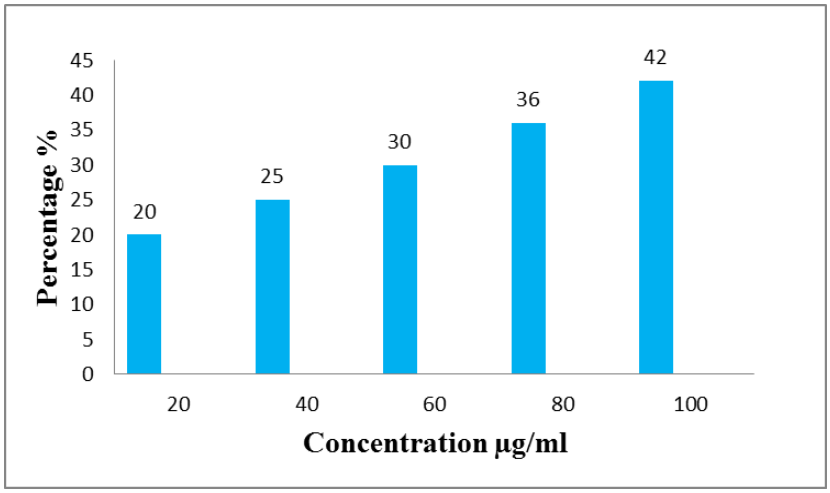
α -Amylase inhibition activity

Inhibition of α-amylase activity by the SNPs was found to be dose dependent from 20 to 100 mg/ml concentrations (fig 4). A maximum of 42% inhibition of α-amylase activity was observed at 20 to 100 mg/ml concentration for the AgNPs respectively.

Therefore, we suggest that inhibition activities against α-amylase and could be part of the possible mechanisms of *Gymnema sylvestr*e variety in therapeutic/dietary management of diabetes, by retardation of starch hydrolysis in the gastrointestinal tract α-amylase inhibitory potential of AgNPs like acarbose would delay the degradation of starch and oligo saccharides, which would in turn cause a decrease in the absorption of glucose and consequently inhibit the increase in postprandial blood glucose (Krentz and Bailey 2005). This inhibitory potential against the target enzymes might be due to the presence of specific secondary metabolites.

Table. 2: Inhibition of the α Amylase activity of silver nanoparticles from *Gymnema sylvestre*

S. No.	Concentration µG/ML	Inhibition (%)
1	20	20%
2	40	25%
3	60	30%
4	80	36%
5	100	42%



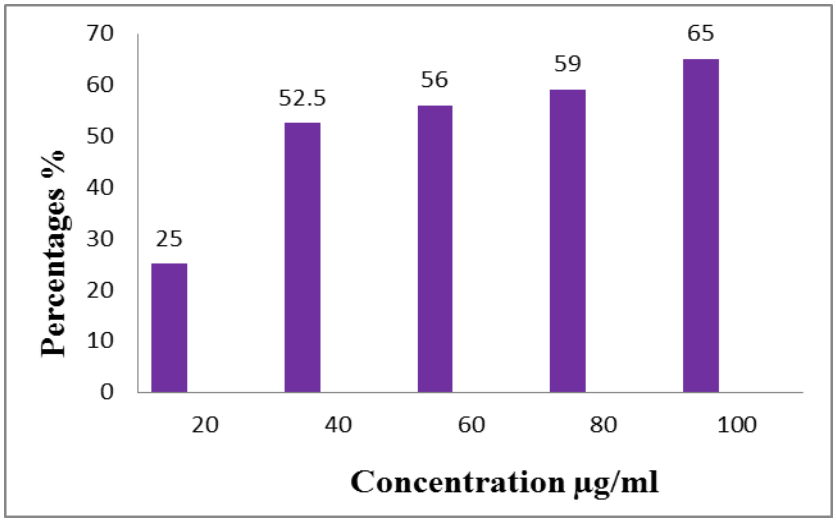
α- Glycosidase inhibitory activity

To explore the underlying mechanism of blood glucose-lowering effects of the SNPs, the inhibitory effects of SNPs from *Gymnema sylvestre* leaf on α-glycosidase activity were assayed *in vitro*. SNPs induced a concentration-dependent suppression on α-glycosidase activity 25% to 65% for SNPs at the concentration of 20mg/ml to 40mg/ml compared to the *α*-glycosidase inhibitor, agarbase (fig.5).

α- Glucosidase is an intestinal enzyme that digests carbohydrates to release glucose (Whiting *et al*., 1993). Inhibition of α-glucosidase may suppress the rate of digestion of carbohydrates and block the absorption of glucose from food. In diabetic patients, α-glucosidase inhibitors may decrease blood glycated hemoglobin A1C level. Inhibitors of α-glycosidase, such as acarbose, have been identified as effective antidiabetic drugs for type 2 diabetes and prediabetes. AgNPs potentialy inhibite α-glucosidase. This result suggesting that inhibiton of α-glucosidase may improve lipid disorder in diabetic condition.

Table. 3: Inhibition of α Glycosidase activity of silver nanoparticles from *Gymnema sylvestre.*

S. No.	Concentration (µg/ml)	Inhibition (%)
1	20	25%
2	40	52.5%
3	60	56%
4	80	59%
5	100	65%

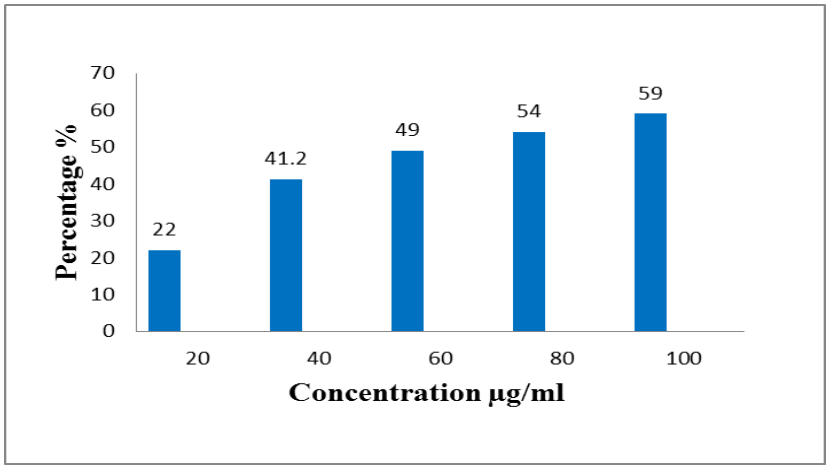


Tyrosines inhibitory activity: Varies skin disease are commonly observed in diabetic patients. Typical biophysical properties of diabetic skin such as lower skin elasticity decreased water content in stratum corneum, increased itching and sweating disturbances are reported. The melanine content is related to glycemic control of diabetic and obesity. The lower melanin contents the higher possibility of microangipathy. Tyrosinase is a polyphenol oxidase with a dinuclear copper active site and is involved in the formation of mammalian melanin pigments. Over-activity of this enzyme leads to hyperpigmentation of the skin. Chemical agents that demonstrated tyrosinase inhibitory activity have been used suppress melanogenesis and can be clinically useful for the some dermatological disassociated with melanin hyperpigmentation. (Sasaki *et al.*, 2002).

The tyrosinase inhibition activities of *Gymnema sylvestre* are shown in Fig. 6. AgNPs inhibit tyrosinase activity ranged between 22 to 59 % and 20 to 100 concentrations. Previously, tyrosinase inhibitory potential of flavonoids such as naringin, hesperidin and nobiletin from citrus leaf were reported. AgNPs from *Gymnema sylvestre* leaf can be used as a preventive agent against enzymatic oxidation in food and living systems, especially in the treatment of hyper pigmentation associated with the high production of melanocytes in human cells.

Table. 4: Inhibition of Tyrosines activity of silver Nanoparticles from *Gymnema sylvestre.*

S. No.	Concentration µg/ml	Inhibition (%)
1	20	22%
2	40	41.2%
3	60	49%
4	80	54%
5	100	59%



CONCLUSION

Silver nanoparticles (AgNPs) were successfully obtained from bioreduction of silver nitrate solution using *Gymnema sylvestre* leaf extract and evaluation antidiabetic activity. In summary UV-visible, FTIR and SEM, spectroscopy techniques confirmed the formation of silver nanoparticles by Gymnema sylvestre leaf. The work indicates that *Gymnema sylvestre* had a good valuable potential in the future for production of silver nanoparticles. Hence, due to their benign and stable nature these silver nanoparticles (AgNPs) may be well utilized in industrial and remedial purpose. However, plant uptake and utilization of silver nanoparticles (AgNPs) require more detailed research on many issues like uptake potential of varies species. AgNPs has potent antidiabetic activity, So that it can be used as treatment of medicine.

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