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In vitro assessment of the efficacy of free-standing silver nanoparticles isolated from *Centella asiatica* against oxidative stress and its antidiabetic activity

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ABSTRACT

The Green synthesis of silver nanoparticles developed into a significant branch of nanotechnology and there is an escalating commercial demand for nanoparticles due to their extensive applications. The present investigation focuses on the green synthesis of silver nanoparticles (CANPs) by utilizing the reducing activity of Centella asiatica and exploring the anti-diabetic property exhibited by CANPs, followed by UV-Visible spectrophotometry, X-Ray Diffraction (XRD) analysis and Scanning Electron Microscope (SEM) for characterization. The shape and size of CANPs were studied using Transmission Electron Microscopy (TEM). UV-Visible spectrophotometry showed the Plasmon resonance peak at 430nm. The antioxidant and antidiabetic property of CANPs was determined. These results indicate that CANPs possess effective anti-oxidant and anti-diabetic properties. In entirety, the silver nanoparticles prepared are safe to be free in the environment and perhaps utilized in industrial and remedial purpose.

Keywords: *Centella asiatica*, Nanoparticles, Diabetes mellitus, Anti-oxidant, UV-Vis

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INTRODUCTION

Diabetes mellitus is a lifelong progressive disease and it is a chronic metabolic disorder due to the relative deficiency of insulin secretion, altering degrees of insulin resistance and it is characterized by high circulating glucose [1]. This disease has reached epidemic proportion among the challenging unresolved health problems of the 21st century. Around 230 million people universal have been affected by diabetes and around 366 million people are expected to get affected by 2030 [2]. China and India together hold the number one spot in the country wise ranking for people affected by diabetes with 40% (approximately 138 million) of the total number living in these two countries [3]. In most cases of Type 2 Diabetes, the condition goes undiagnosed for several years before the patient becomes aware of his/her condition (WHO report). Diabetes is a major life-threatening issue because there are many other complications associated with it like, retinopathy which may result in blindness, renal failure due to nephropathy, neuropathy, a marked up risk of cardiovascular diseases, sexual dysfunction and gangrenes. There have been many researches which claim that lifestyle changes can delay or even prevent the occurrence of diabetes even in people with a high risk of diabetes [4-5]. There are numerous drugs in the market for Diabetes Mellitus like metformin, insulin, glitazones, exenatide and sulfonylureas but there are also many undesirable side effects which arise due to these drugs. Patients may experience nausea, indigestion and diarrhoea in the first few weeks of using metformin, while use of exenatide and sulfonylureas also caused nausea and diarrhoea sometimes to the extent that the drug had to be discontinued [6]. Therefore there has been a revival of interest in using plant medication for treating diabetes in recent times.

Increased oxidative stress and reactive oxygen levels have been noted in case of diabetes in some studies [7]. There have been recent researches which provide evidence that Hyperglycemia produces highly reactive free radicals

which have been known to cause oxidative stresss [8]. This increased free radical generation - especially reactive oxygen - coupled with deteriorated antioxidants might contribute to other diabetic abnormalities [9]. Therefore a plant that exhibits anti-oxidant properties along with anti-diabetic property could delay or prevent diabetic complications more effectively than the available synthetic anti-diabetic drugs. Overall, there have been more than 400 plants documented that shows hypoglycaemic properties [10].

One such plant extract that shows a notable increase in glucose tolerance levels is *Centella asiatica. Centella asiatica*, belonging to the genus *Centella* and family *Apiaceae* is an herbaceous creeper found mainly in moist and swampy areas of India, China, Sri Lanka, Madagascar, South Africa, Australia and Japan [11]. The ethanol and methanol extract from the plant brought the glucose levels back to normal in diabetes induced animals thereby exhibiting an anti-diabetic effect. The presence of substances with free hydroxyls and phenolic constituents like flavonoids and tannis are responsible for the antioxidant activity of ethanol extract of *C. asiatica*. *C. asiatica* also has been known to exhibit anti-epileptic activity, neuro-protective activity, anti-anxiety action, anti-depressant activity, anti-ulcer action, anti-oxidant action, anti-microbial activity, anti-inflammatory action, anti-allergic, anti-pruritic and wound healing [12]. Nanotechnology deals with the application of nanoscience principles to everyday technology. Materials and Manufacturing, Information Technology, Energy, Medicine and Healthcare, Biotechnology, and National Security are just some of the fields that are showing gigantic leaps in the application of nanoscience [13]. Nanotechnology is expected to open up new areas of research, especially in medicine and healthcare mainly because most biomolecules and structures have particle size comparable to nanomaterials. Silver is a competent antimicrobial agent, shows low toxicity and has an assorted array of *in-vitro* and *in-vivo* applications [14]. Silver nanoparticles along with their anti-microbial properties [15-16]also possess anti-oxidant properties[17]. Antioxidants hamper and mend the damages caused by oxidative stress [18]. Silver nanoparticles also exhibit free radical scavenging property [19] which makes it more suitable for this study. There are many methods for synthesis of nanoparticles – chemical methods [20] and physical methods [21] involve using toxic chemical substances that may be a potential environmental hazard. Hence, the biological methods such as using plants and plant extracts [22-24] fungi [25] bacteria [26] yeast [27] are gaining attention.

Therefore, the aim of the present study is to develop a novel method to green synthesize silver nanoparticles using the plant *Centella asiatica* to explore its antioxidant and antidiabetic properties.

MATERIALS AND METHODS

PREPARATION OF PLANT EXTRACT

The powdered plant materials were used for extract preparation. About 10 gram of powder was mixed with 100 ml of double distilled water and boiled in a water bath for 20 mins. The obtained extract was filtered through Whatman no 1 filter paper. Then centrifuged at 6000 rpm for 20 mins. The centrifuged samples were transferred into autoclaved vials and stored at 4°C for further analysis [28].

PHYTOCHEMICAL ANALYSIS

The qualitative analysis of *Centella asiatica* was performed to determine the presence of secondary metabolites [29].

PREPARATION OF AQUEOUS SILVER NITRATE

1 mM Silver nitrate solution was prepared and stored in amber coloured bottle.

OPTIMIZATION AND SYNTHESIS OF SILVER NANOPARTICLES

Different concentrations of *Centella asiatica* leaf extracts (2ml, 4ml, 6ml, 8ml and 10ml) were taken separately and to this 1ml of 1mM Siver nitrate solution was added with constant stirring and exposed to direct boiling. The colour change of the solution was checked periodically. The colour change of the leaf extract from yellow to dark brown indicates that the silver nanoparticles were synthesized from the leaves. Surface Plasmon Resonance characteristic of silver nanoparticles was observed while characterizing using UV-Visible absorption spectroscopy.

PRODUCTION AND RECOVERY OF SILVER NANOPARTICLES BY CENTRIFUGATION

1ml of *Centella asiatica* leaf extract shows the presence of nanoparticles. Further, it was chosen for the bulk production. Then, 10ml leaf extract was added to 100ml of 1mM Siver nitrate. After bioreduction, the solution consisting of hydrosols of silver nanoparticles was subjected to centrifugation at 10,000rpm for 30 minutes. The pellet formed was dissolved in 0.1 ml of ethanol and air dried.

CHARACTERIZATION OF SILVER NANOPARTICLES BY UV-VISIBLE SPECTROSCOPY

UV-Visible spectrophotometer can be used to monitor the synthesis of silver nanoparticles. In the phenomenon of Surface Plasmon Resonance, quantized oscillations of surface charge in AgNPs give a strong absorption peak in the 450-480 nm range. Hitachi U-2800 UV-Visible spectrophotometer was used for this purpose. The absorption in this range directly gives a quantitative measure of the concentration of nanoparticles synthesized. Regular aliquots of the sample (1ml) were taken to monitor the bio reduction of Ag+ ions.

CHARACTERIZATION OF SILVER NANOPARTICLES BY SCANNING ELECTRON MICROSCOPY (SEM)

SEM scans was undertaken to know the size and shape of the silver nanoparticles biosynthesized using sample. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very little amount of the sample on the grid, extra solution was removed using a blotting paper. Then the film on the SEM grid was allowed to dry and the images of nanoparticles were taken. The details regarding applied voltage, magnification used and size of the contents of the images were implanted on the images itself.

CHARACTERIZATION OF SILVER NANOPARTICLES BY TRANSMISSION ELECTRON MICROSCOPY (TEM)

TEM is a method of producing image of a sample by illuminating the sample with electronic radiation (under vacuum). Philips CM 200 model was used for the TEM analysis. Dried films of the nanoparticles were made and TEM analysis was done. Here, a beam of electrons were transmitted through the sample and a corresponding image was formed due to the interaction of the sample and electrons. The image was enhanced and fed into the imaging device for further analysis.The specimen image generated by the objective lens was subsequently magnified in one or two more magnification stages by the intermediate and projector lens and projected onto a photographic plate.

CHARACTERIZATION OF SILVER NANOPARTICLES BY X-RAY DIFFRACTION (XRD)

Thoroughly dried powder of AgNPs was prepared by placing in hot air oven and D8 Advanced Bruker X-ray diffractometer with Cu Kα (1.54 Ao) source was used to do diffraction studies. This analytical method is used for phase identification and to determine the unit cell dimensions.

ANTIOXIDANT ACTIVITY

DPPH (2, 2- diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The scavenging effects of CANPs obtained from *Centella asiatica* were determined [30]. 1ml of different concentrations of extracts (1, 10, 100 and 200 lg/mL) prepared in methanol was added to 0.5 mL of a 0.2 Mmol/L DPPH methanolic solution. The mixture was vigorously shaken and left standing at room temperature for 30 min. The absorbance of all the sample solutions was measured at 517nm.The scavenging effect (%) was calculated by using the formulae. Sample blank and control samples were performed according to the method. Scavenging effect of DPPH radical was calculated using the following equation

DPPH radical scavenging activity [%] = [1- (Asample – Asample blank / Acontrol) × 100]

Where A sample is the Absorbance of DPPH solution & test sample, A sample blank is the absorbance of the sample only without DPPH solution. Synthetic antioxidant Ascorbic acid was used as positive controls.

HYDROGEN PEROXIDE (H_2O_2) RADICAL SCAVENGING ACTIVITY

The ability of the CANPs to scavenge H_2O_2 was determined with the slight modification [31]. Briefly, 40mM H_2O_2 was prepared in phosphate buffer (pH-7.4) and the H_2O_2 concentration was determined spectrophotometrically by measuring the absorption. CANPs and ascorbic acid (positive control) was dissolved in distilled water. The Prepared stock solutions were diluted to seven different concentrations of 3.12, 6.25, 12.5, 25, 50, 75,100, 200 µg/ml and added to 0.6 ml of 40mM H_2O_2 solution and the absorbance of H_2O_2 was determined at 230 nm. The percentage of scavenging activity of hydrogen peroxide was calculated using the following formula:

H_2O_2 radical scavenging activity [%] = [(A0 – A1) / A0] × 100

Where A0 – Absorbance of control; A1 – Absorbance of sample.

ANTIDIABETIC ACTIVITY

Glucose Uptake by Yeast Cells

Commercial baker's yeast was thoroughly washed by repeated centrifugation at 3000rpm for 5mins. The supernatant was collected and 10ml of 10% (v/v) yeast cell suspension was prepared. Different concentrations CANPs of (250, 500, 750 and 1000 µg/ml) 1ml of glucose solution at concentrations (5mM and 10mM) were added and incubated at

37°C for 10mins. Reaction was started by adding 100µl of 10% yeast suspension to the above mixture and incubated at 37°C for 1hr. After incubation, the mixture was centrifuged at 2500rpm for 5mins. Glucose estimation was performed with the supernatant [32]. Percentage inhibition was calculated by using the formula:

Increase in glucose uptake (%) = Abs sample – Abs control × 100/Abs sample

Abscontrol = Absorbance of the control; Abssample = Absorbance of the sample

Non-enzymatic glycosylation of haemoglobin

Antidiabetic activity of CANPs of *Centella asiatica* was investigated by estimating the degree of non-enzymatic hemoglobin glycosylation and it is measured colorimetrically at 520nm (Trease et al.,1989). Glucose (2%), hemoglobin (0.06%) and Gentamycin (0.02%) solutions were prepared in phosphate buffer 0.01 M, pH 7.4.1 ml each of above solution was mixed. CANPs was weighed and dissolved in DMSO to obtain stock solution and then 1-5 µg/ml solutions were prepared. 1 ml of each concentration was added to above mixture. Mixture was incubated in dark at room temperature for 72hrs. The degree of glycosylation of hemoglobin was measured colorimetrically at 520nm. α –tocopherol was used as a standard drug for assay. Percentage inhibition was calculated by using the formula:

Inhibition % = Abs sample−Abs controlAbs sample ×100

Where, Abs control is the absorbance of the control reaction containing all reagents without the test sample, Abs sample is the absorbance of the test sample [33].

α- Amylase Inhibition Assay

Alpha amylase inhibitory activity was based on the starch iodine method that was originally developed by (Fuwa 1954). CANPs sample was dissolved in 0.1M Sodium acetate buffer to get a stock solution of extract 2mg/ml. CANPs of stock solution were taken in various concentrations via, (250-1000µg/ml) then take 1ml of 1% w/v of soluble starch solution, 1ml of α-amylase enzyme & 2ml of 0.1M Sodium phosphate buffer (pH 7.4) was added. Then this mixture solution was incubated for 1hr at 37°C. After incubation, 0.1ml of Iodine-iodide indicator was added. The intensity of the colour absorbance was measured at 565nm using UV Visible spectrophotometer.

Inhibition of enzyme activity was calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Abs sample} - \text{Abs control} \times 100}{\text{Abs sample}}$$

Where, Abs control is the absorbance of the control reaction containing all reagents without the test sample ,Abs sample is the absorbance of the test sample and 0.1M Sodium acetate buffer was used as a blank [34].

RESULTS AND DISCUSSION

In the present study, we tend to examine the *Centella asiatica* to evaluate the amount of phytochemicals to determine the standard procedure for qualitative analysis. It showed the high phenolic and flavonoids content. Synthesized nanoparticles show higher activity on antioxidant and antidiabetic. Diabetes mellitus is a group of metabolic disorder characterized by highly raised blood glucose levels [35]. Herbal treatments have been used throughout the world for the therapy of diabetes mellitus. In some medications and other alternative medicines, herbal drugs have been known to cure and control diabetes [36]. In diabetic patients, a sustained reduction of hyperglycaemia is shown to reduce the risk of developing microvascular and macrovascular diseases and their associated complication [37]. The present study creates an interest in the use of eco-friendly and economical resources which propels the use of highly acclaimed medicinal plants to direct the green synthesis of metal Nanoparticles. Stable spherical colloidal AgNps were synthesized by *Centella asiatica.* It showed a good potent on antioxidant and antidiabetic. Inert, spherical Nanoparticles with a size range were characterized by UV–Vis spectroscopy, TEM, SEM and XRD stability. Bioreduction of silver solution was monitored by periodic sampling of the reaction mixture at regular intervals by using UV-Vis spectroscopy. strong characteristic absorbance peak at around 430nm was observed. [38].

Phytochemical Analysis

The qualitative analysis of *Centella asiatica* illustrates the presence of bioactive compounds like alkaloids, carbohydrates, saponins, steroids, proteins and glycosides.

Uv-Visible Absorption Spectroscopy

SEM is the most widely used technique for characterizing the nanoparticles in terms of physical morphology of the particles. SEM micrographs suggest that the biosynthesized silver nanoparticles are almost spherical in structure. The initial development of CANPs was implied by the colour change in plant sample on addition of 2:1 ratio of $AgNO_3$. The solution turned brownish yellow and remained stable indicating that the nanoparticles did not aggregate and remained dispersed in solution. Due to the Surface Plasmon Resonance, the oscillation of the electrons cause a peak at 430nm throughout the bioreaction confirming success of silver nanoparticle synthesis Fig. 1. investigation by spectrophotometer was ended up to 8hrs.

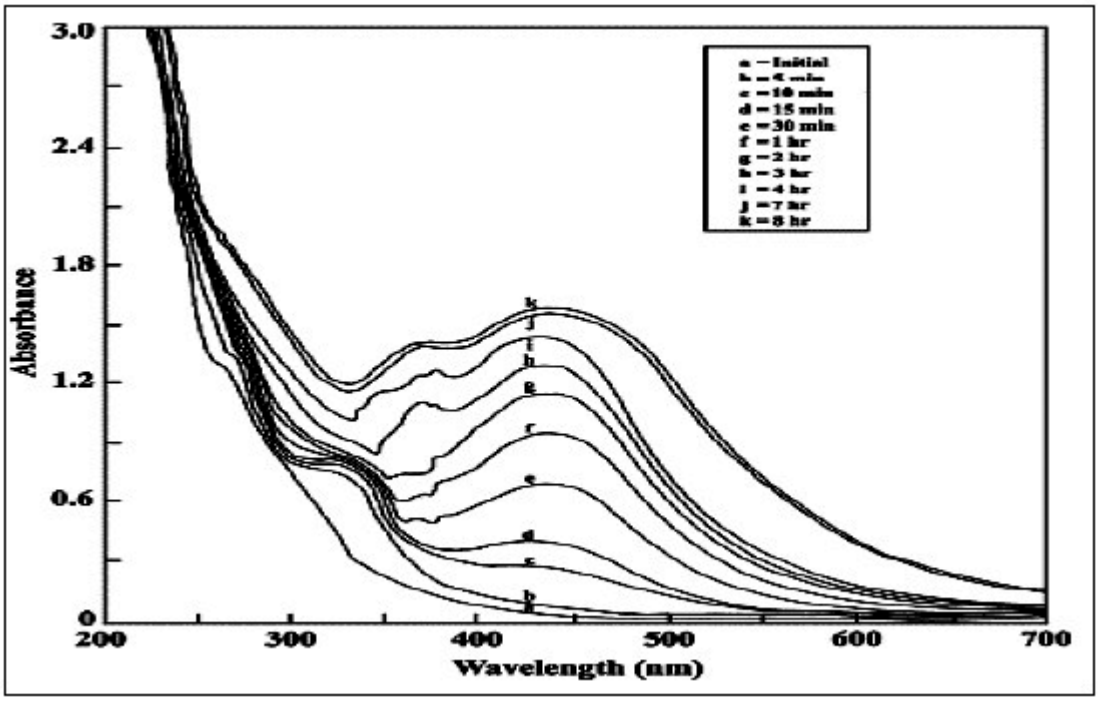

Figure 1. UV-Vis spectroscopy of silver nanoparticles

SEM

SEM analysis was done for the CANPs and the resulting images clearly show that they are spherical in shape at the room temperature in which it was synthesized. The size range varied from 30 to 50nm (diameter) Fig.2.

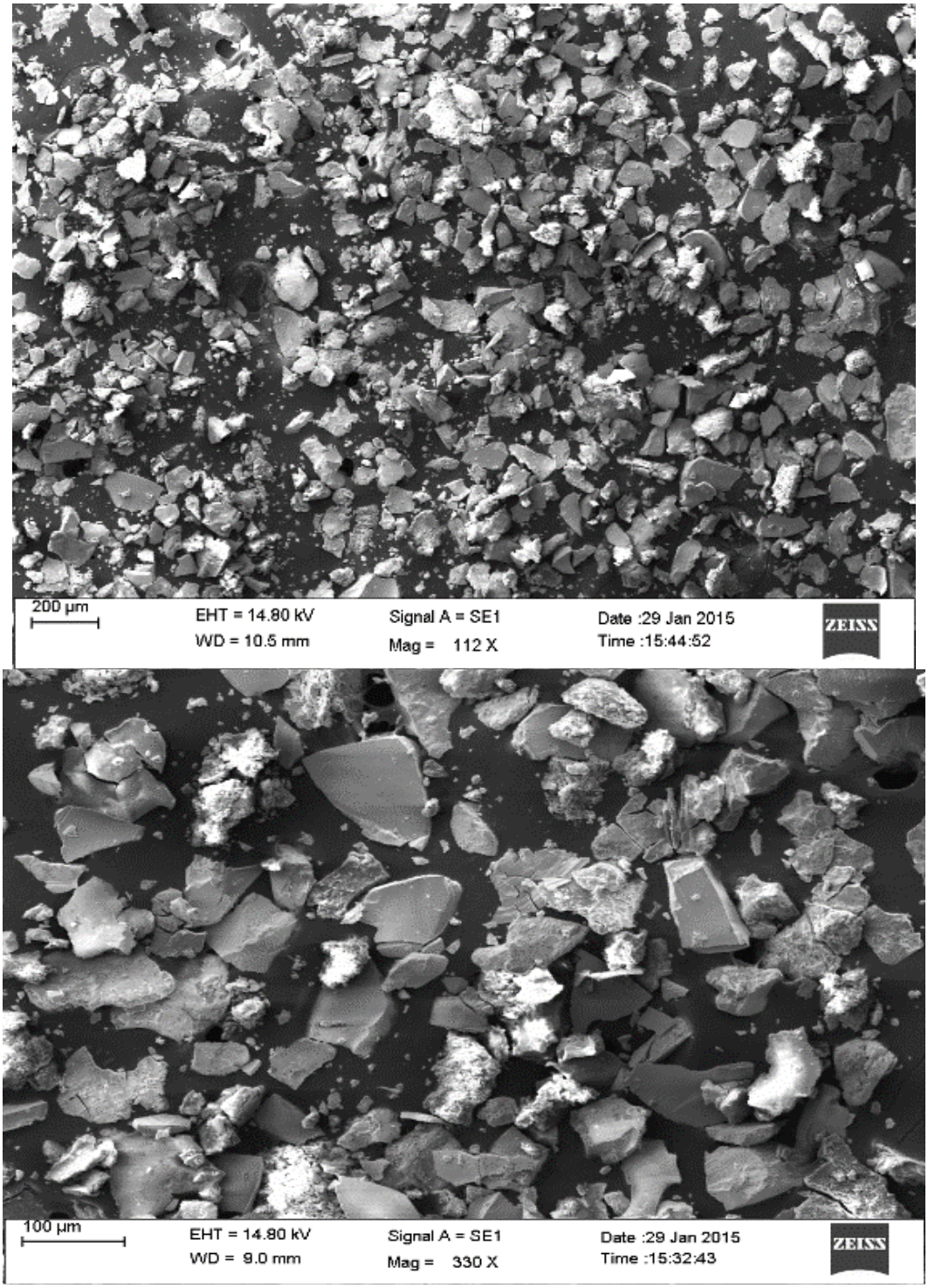

Figure 2. SEM analysis of (a) silver nanoparticle in 200µm and (b) silver nanoparticle in 100µm

TEM

TEM provided insight into the morphology and particle size distribution profile of the silver nanoparticles. TEM analysis confirmed the synthesis of spherical silver nanoparticles in the response mixture. The small-sized nanoparticles were able to easily penetrate across the membrane [39]. TEM analysis for the CANPs was done and the images showed varied size distribution between 8-15nm and 26-35nm prevalently. When the nanoparticles appeared tapered, the dimensions were found to be between 4.2-8.8nm (radial diameter). Fig.3.

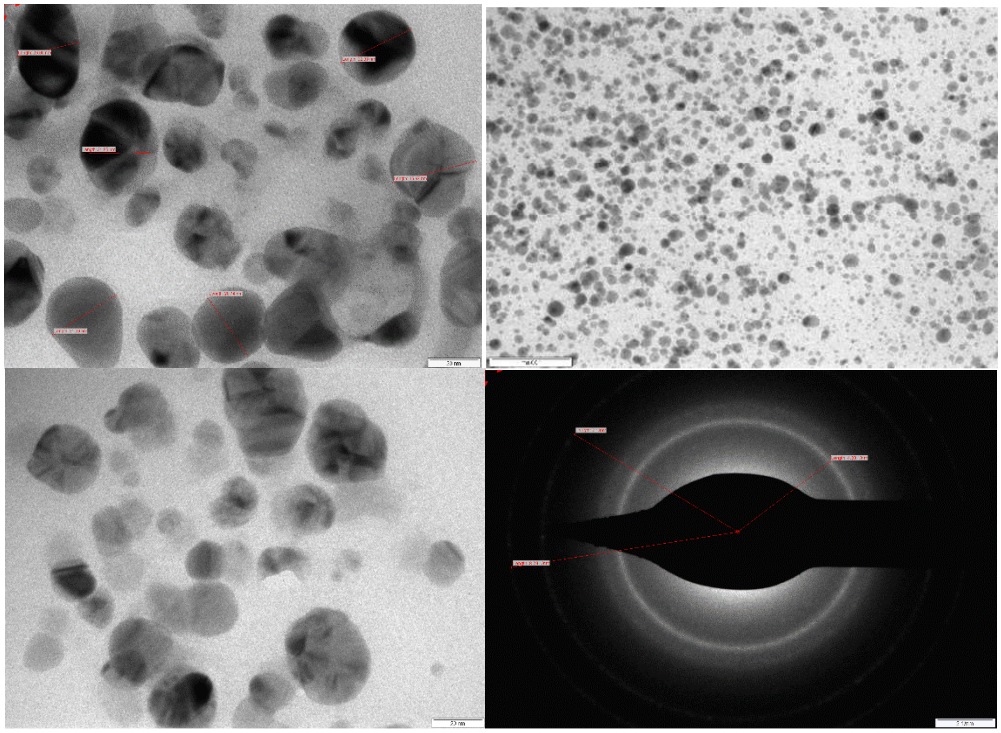

Figure 3. Transmission electron micrograph (TEM) of silver nanoparticles obtained by reduction of silver nitrate with the leaves extract of *Centella asiatica*

XRD

This analysis revealed the orthorhombic crystals of silver nanoparticles. Crystal behavior of the purified solid CAgNPs was determined using Powder XRD. Fig. 4. Shows the XRD pattern of CAgNPs prepared using *C.asiatica* extracts. A number of strong diffraction peaks are seen at 2θ values of 27.84, 32.25, 46.26, 54.8 , 57.52 ,67.50 ,74.51 and 76.80 which correspond to the (111), (200), (220), (311) , (322),(400),(313) and (402) inter planar reflections of cubical structure respectively . The lattice constants were in conformity with the database of Joint Committee on Powder Diffraction Standards (JCPDS. No. 96-901-1667), whereas broadening in the diffraction peaks point to that small crystallite size is obtained. The average particle size has been estimated by using Debye–Scherer formula [D = kλ/βcos (θ)] where D is the average crystal size, k is the Scherer coefficient (0.891), k is the X-ray wave length (k = 1.5406 Å), θ is Bragg's angle (2θ), b is the full width at half maximum intensity (FWHM) in radians. From the Scherer equation the average particle size of CAgNPs is found to be around 31 nm which supports the above results obtained from TEM study.

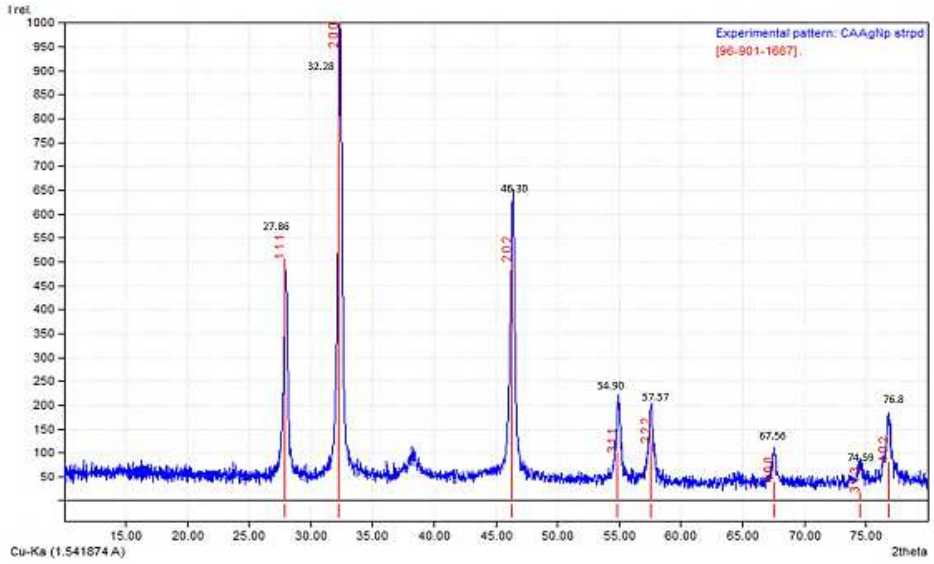

Figure 4. X-ray diffraction (XRD) pattern of silver nanoparticles synthesized using the leaf extract of *Centella asiatica*

Antioxidant Activity

DPPH Free Radical Scavenging Assay

DPPH reduction was investigated against ascorbic acid is a positive control. The DPPH free radical is a sensitive way to determine the antioxidant activity [40]. The radical scavenging activity of antioxidant compounds is usually measured by DPPH. The ensuing decolorization is stoichiometric with esteem to the number of electrons captured. The more antioxidants occurred in the sample, the more DPPH reduction occurs. DPPH free radical gives a strong absorption band at 517 nm. This results is a visually noticeable as it shows discoloration from purple to yellow. It loses this absorption when accepting an electron or a free radical species. The colour turns from purple to yellow when the odd electron of DPPH radical can be paired with hydrogen as of free radical scavenging antioxidant to form the reduced DPPH [41]. The scavenging activity of *C. asiatica* shows that at 25% to 80%, as the concentration increased from 3.12 to 200 µg/mL the CANPs synthesized showed more scavenging activity compared to the ascorbic acid ranging from 32% to 95%, slightly lesser than the scavenging activity of the standard Ascorbic acid Fig.5.

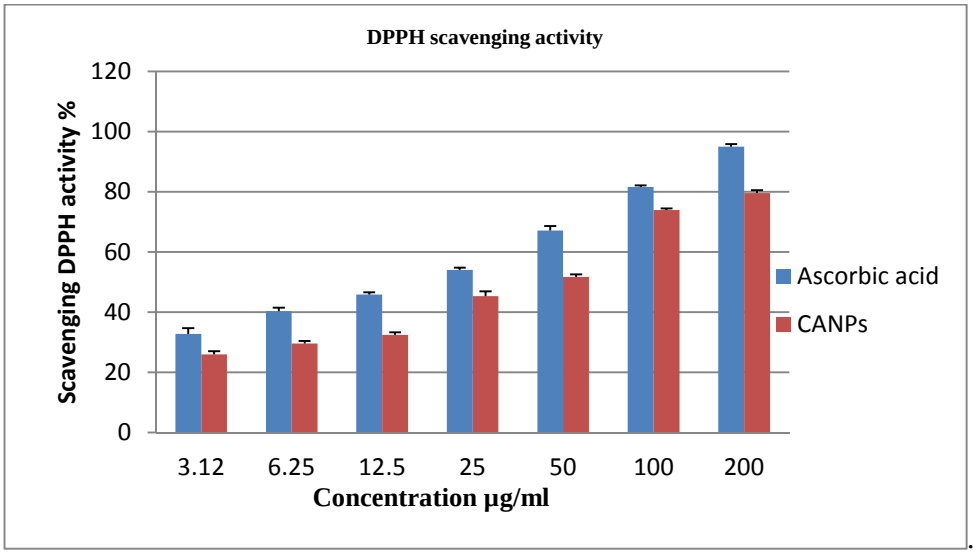

Figure 5. DPPH radical scavenging activity of CANPs

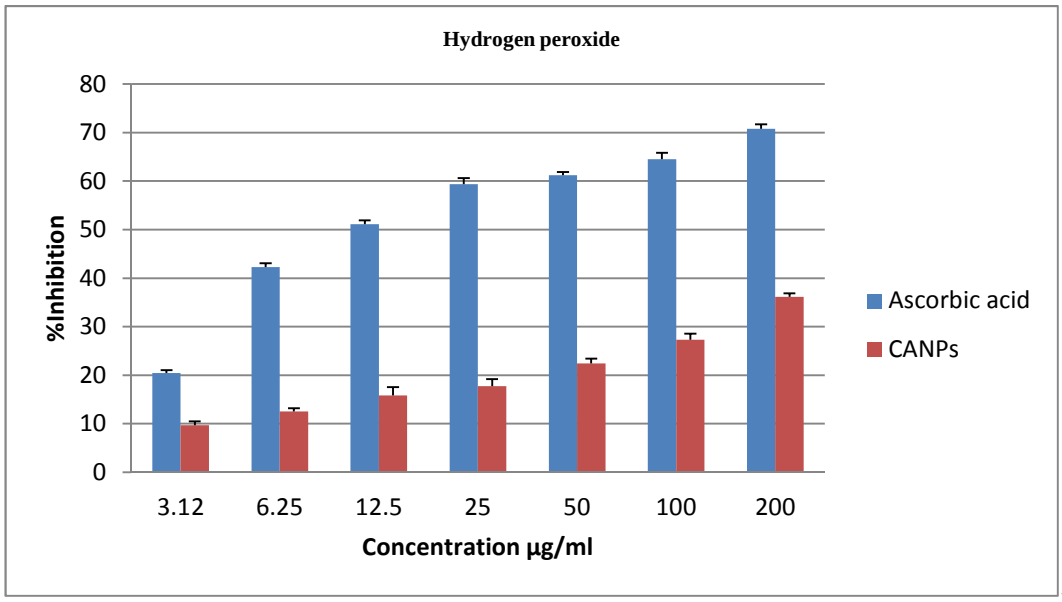

Fig.6. H_2O_2 radical scavenging activity of CANPs

Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly. Free radicals especially OH group are unstable, highly reactive, and energized molecules generated from superoxide anion and hydrogen peroxide in the catabolic system. It can cross cell membranes rapidly; once inside the cell, it can probably react with

$Fe2^{+}$possibly , $Cu2^{+}$ ions to form hydroxyl radicals and this may be the origin of many of its oxide effects [42]. From the results, it shows that the H_2O_2 scavenging activity of the sample is significant compared to that of the ascorbic acid. Fig.6. shows the hydrogen peroxide scavenging activity of CANPs in compared with Ascorbic acid standard.

Antidiabetic activity

Glucose Uptake by Yeast Cells

Inhibition of glucose uptake in 5mMand 10mM glucose concentration:

The movement of glucose across the membrane of the yeast cells was a behaviour in an *in vitro* system, which involves the yeast cells suspended in the varying concentrations. The rate of of glucose uptake into yeast cells are linear in the (5mM and 10mM) of glucose solution including the extracts. After incubation the glucose uptake of the yeast cells was determined by the amount of glucose which is present in the solution. The effect of CANPs has a enhanced efficiency in increasing the glucose uptake by the yeast cells while compared to standards in both 5mM & 10mM glucose concentrations. This uses specific carriers that transport solutes according to the concentration gradients. For effective transport to occur there has to be removal of intracellular glucose [43]. Results obtained for the glucose uptake in 5mM concentration assay are represented below. The rate of glucose transport across cell membrane in yeast cells system was explored and the results are given in Fig.7. Five different concentrations 40, 80,120, 160 & 200 µg/ml of CANPs and acarbose showed a concentration-dependent reduction. The highest concentration 200µg/ml of CANPs and Acarbose showed a maximum inhibition of 65.12±0.64 and 76.34±0.29 while the lowest concentration 200µg/ml of CANPs and Acarbose showed a minimum inhibition of 25.41±0.23 & 57.17±0.36. and in 10mM concentration of glucose while compared to 5mM it shows less parentage increase inhibition (19.73±0.34 to 63.27±0.57)

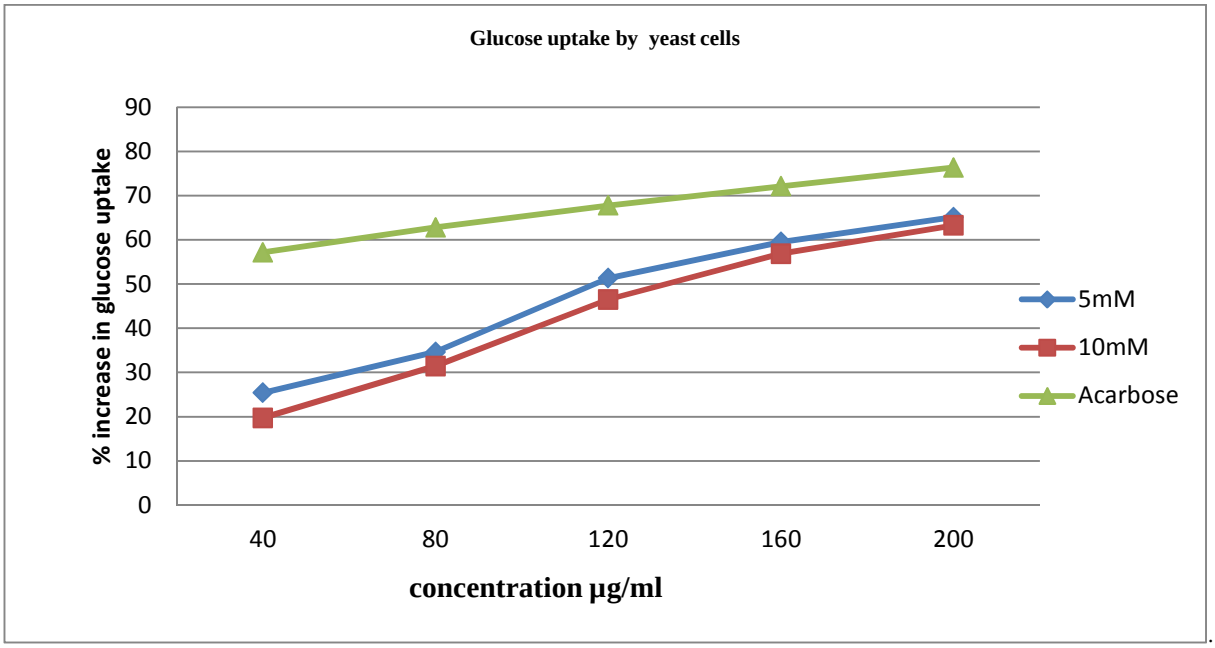

Fig.7. Inhibition of glucose uptake at 5mM and 10mM concentration

Non Enzymatic Glycosylation of Hemoglobin Assay

With regard to Diabetes Mellitus, amounts of Glycated Haemoglobin more than the baseline value have been associated with nephropathy, retinopathy and cardiovascular diseases. CANPs showed a good antidiabetic activity. The percentage inhibition of glycosylation is dependent to dose. Fig.8. Because as the concentration of drug increases the formation of glucose-haemoglobin complex decreases and free hemoglobin increases and shows the inhibition of glycosylated hemoglobin. Hence this concentration is a dependent reaction and it is compared to the standard α- Tocopherol. The percentage of inhibition at the concentrations of 200, 400, 600, 800 & 1000 µg/ml by the CANPs showed a concentration-dependent reduction. The highest concentration 1000 µg/ml of CANPs and α-tocopherol showed a maximum inhibition 52.91.± 0.421 & 75.13 ± 0.314 while the lowest concentration 200µg/ml of CANPs and α-tocopherol showed a minimum inhibition of 19.87 ± 0.633 & 24.19 ± 0.215 respectively.

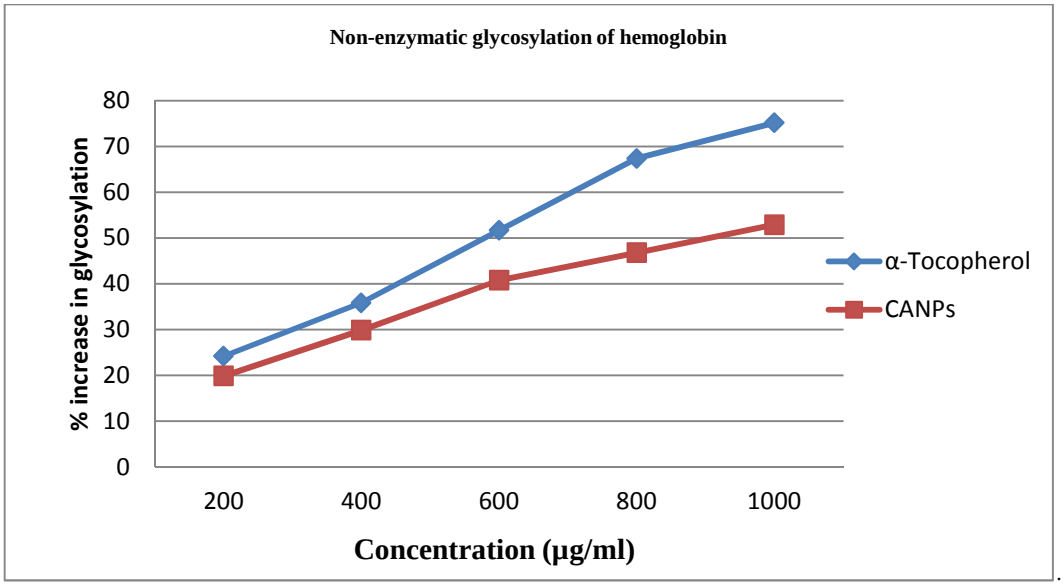

Fig. 8. Non - enzymatic glycosylation of hemoglobin assay

Inhibition of Alpha Amylase Enzyme Assay

α-amylase is an enzyme that hydrolyses alpha-bonds of large linked polysaccharide such as glycogen and starch to yield glucose and maltose [44]. In the experimental study it was observed that CANPs demonstrated significant α-amylase inhibition activity. Pancreatic α-amylase catalyses the first step in the hydrolysis of starch. This enzyme results in the delay of carbohydrate digestion thus decreasing the rate of glucose absorption and therefore plummeting the post prandial plasma glucose. The inference of this test was that CANPs possess good anti-diabetic activity. The primary action of α-amylase is the hydrolysis of alpha-bonds of large alpha linked polysaccharide like glycogen and starch giving glucose and maltose. The medicinal plant based on α amylase inhibitors offers a potential therapeutic approach for the management of diabetes. CANPs on alpha amylase was examined in this study and the results were shown in Fig.9. Results of *in vitro* α-amylase study against CANPs showed that the percentage inhibition at the α-amylase concentrations of 200, 400, 600, 800 & 1000 µg/ml by the CANPs and acarbose shows the concentration dependent reduction. The highest concentration 1000µg/ml of CANPs and Acarbose showed a maximum inhibition of 43.96±0.91 and 55.75±0.71 while the lowest concentration 200µg/ml of CANPs and Acarbose showed a minimum inhibition of 31.59±1.02 & 39.07±.071. The CANPs showed good α-amylase inhibition as compared with standard Acarbose.

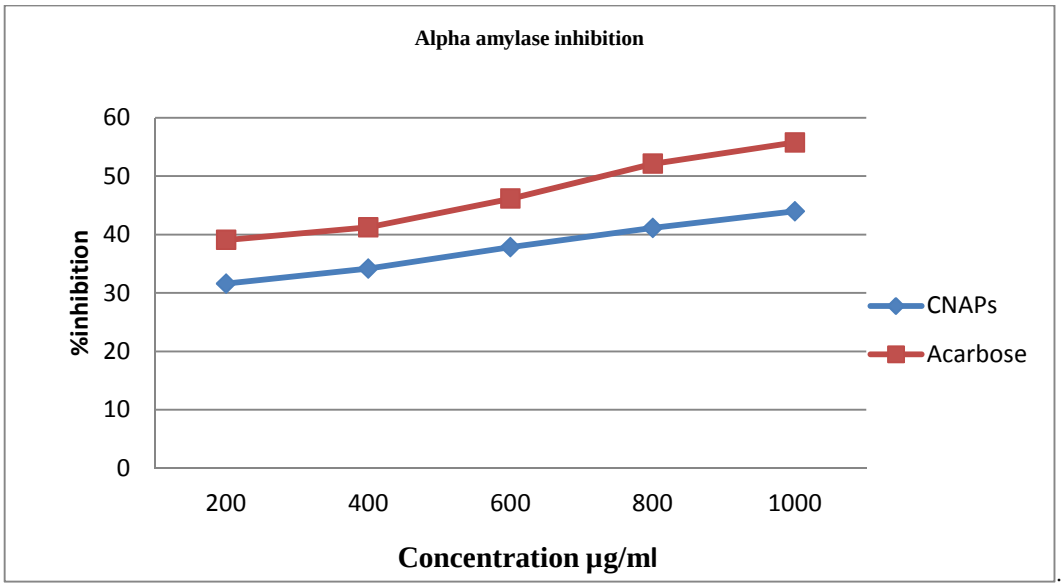

Fig.9. Inhibition of alpha amylase enzyme assay

CONCLUSION

The leaves of *Centella asiatica*, can be a good source for the synthesis of silver nanoparticles through a green chemistry approach with several advantages. Silver nanoparticles (AgNPs) have been successfully attained from bioreduction of silver nitrate solutions using *Centella asiatica* leaf extract. CANPs shows the strong antioxidant and anti diabetic activity. Silver nanoparticles have been characterized using UV-vis spectroscopy, SEM, TEM and XRD analysis. Results denoted *Centella asiatica* leaf extract to be a better reducing agent and revealed the efficient capping and stabilization properties of these AgNPs. The important outcome of the study will be the development of value added product from *Centella asiatica* for biomedical and nanotechnology based industries. Further, *in vivo* studies are essential for providing scientific information on the medicinal plant.

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