



Green Synthesis of Silver Nanoparticles Using Polyherbal Extracts for Antidiabetic Applications

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ABSTRACT

The rising worldwide incidence of diabetes mellitus has created the need to establish new multi-target intervention approaches, which should be effective and biocompatible. This paper will examine how green nanotechnology and traditional polyherbal medicine can be used together to come up with such a plan. It was possible to synthesize silver nanoparticles (AgNPs) using a sustainable and plant-mediated method based on the extracts of two antidiabetic plants *Azadirachta indica* (Neem) seeds and *Syzygiumcumini* (Jammun) seeds. AgNPs A. indica-mediated (AI-AgNPs) and *S. cumini*-mediated (SC-AgNPs) were formed through a specific colour change to reddish-brown and unique surface plasmon resonance (SPR) peaks at 435nm and 425nm respectively, respectively, which were observed under UV-Visible spectroscopy. The presence of plant phytochemicals (phenols, flavonoids) in reducing and capping the nanoparticles was indicated by Fourier Transform Infrared (FTIR) spectroscopy. The crystalline, face-centered cubic structure of the AgNPs was confirmed by the X-ray Diffraction (XRD) analysis with an average crystallite size of 15-25nm. The morphology was identified as spherical to quasi-spherical by the Scanning Electron Microscopy (SEM) and the elemental silver was identified by using the Energy Dispersive X-ray Spectroscopy (EDX).



The nanoparticles showed great *in vitro* antidiabetic properties as evidenced by an increase in the concentration-dependent glucose uptake in yeast cell and a considerable glucose adsorption capacity indicating the possibility of a dual action. These results emphasize the potential of the green-synthesized AgNPs as promising nanotherapeutics. The paper concludes that AgNPs produced by the *A. indica* and *S. cumini* seeds have significant antidiabetic characteristics, which corroborates the idea of the application of medicinal plants in the production of nanomaterials. The given work opens the way to the next *in vivo* studies and the creation of multi-herbal nano-formulations in order to manage diabetes fully.

Keywords: Silver nanoparticles (AgNPs), *Azadirachta indica*, *Syzygium cumini*, Antidiabetic activity, Phytomediated Synthesis.

INTRODUCTION

The branch of engineering and manipulation of matter on atomic and molecular level (usually 1-100 nanometers) called nanotechnology has brought about a paradigm shift in the scientific fields, and medicine is one of the most significant beneficiaries. This field, sometimes called nanomedicine, uses the physics and chemical characteristics of nanomaterials, including a large surface-to-volume ratio, quantum effects, and controllable surface chemistry, to be innovative in diagnostics, drug delivery, imaging, and therapeutic interventions. The behavior of materials at the nanoscale is unlike that of bulk materials, and allows specific interactions with biological systems at the cellular and subcellular scale. Such accuracy enables the creation of advanced systems that can eliminate major shortcomings of traditional treatments such as low bioavailability, non-targeted distribution, systemic toxicity and drug resistance. Intersection of nanotechnology and biology and pharmacology has the potential immeasurably to solve some of the toughest problems in healthcare and lead to more personalized, more effective, and less invasive treatment modalities. Among the vast array of possible agents in this broad discipline, metallic nanoparticles have become notably versatile in the capabilities of their synthesis, versatility, and powerful biological activity¹.

Out of all the various metallic nanoparticles, silver nanoparticles (AgNPs) deserve the unrivaled attention of both independent characteristics and through historical precedent in the application of silver as an antimicrobial agent. AgNPs have the biomedical potential due to their outstanding optical, electrical, catalytic, and above all, biological characteristics. Their good surface plasmon resonance (SPR) in the visible spectrum makes it easy to monitor the synthesis and has applications in biosensing and imaging. They, however, have their therapeutic potential greatly

pegged on their general biocidal activity that is broad spectrum and powerful against bacteria, viruses and fungi. This activity is attributed to various processes, such as the sustained liberation of silver ions (Ag⁺), production of reactive oxygen species (ROS), interference with cell membranes, and interference with important enzymatic and genetic physiological activities in microbial cells. In addition to antimicrobial uses, an emerging research trend has enlightened the potential of AgNPs in their therapeutic uses in other fields such as anti-inflammatory, antioxidant, anti-angiogenic and anticancer uses². Their capability to regulate major cellular signaling pathways and their interaction with biomolecules makes them good potential candidates in the management of complex diseases. Recent studies have also sought to consider their efficacy in non-communicable disease treatment, including diabetes, where their multifaceted effect, which may include enzyme inhibition, antioxidant defense, and anti-inflammatory effects, could provide a new mode of operation to be used, shifting their operation spectrum to external antiseptics to internal regulators of metabolic pathology.

Diabetes mellitus is an insidious and increasing health crisis in the world, being a chronic hyperglycemia due to insulin secretion, insulin action, or both malfunction. The International Diabetes Federation has estimated that the prevalence of DM will increase exponentially with a huge socioeconomic cost because of the complications involved in DM, including cardiovascular disease, neuropathy, nephropathy, and retinopathy³. Treatment of type 2 diabetes, which is the most common type, is mostly concerned with lifestyle changes and pharmacotherapy, varying with biguanides (e.g., metformin), sulfonylureas, thiazolidinediones, DPP-4 inhibitors, SGLT2 inhibitors, and insulin. Although these agents are effective, they do not lack some major drawbacks. Most of them have negative side effects like low blood sugar levels, weight gain, stomach upsets, and in others, cardiovascular

dangers. Additionally, the existing methods of treatment are mostly aimed at controlling symptoms, in other words reducing blood glucose, but not at the pathophysiology, i.e., oxidative stress, chronic inflammation, and the impaired functioning of β -cells. Secondary failure in which drugs become less effective with time is also a problem. This suggests an urgent and unfulfilled potential of the current therapeutic agents that are both effective in controlling the glycemic but also complex diabetes condition and its other oxidative and inflammatory mediators with reduced side effects. The absence of alternative and complementary to current therapeutic agent(s) has prompted the study of novel and alternative methods, such as the study of bioactive natural products and other sophisticated nanotechnology-based interventions⁴⁻⁶.

The conventional approaches to the production of AgNPs (chemical reduction (through the use of such a reducing agent as sodium borohydride or hydrazine), physical methods (laser ablation or arc-discharge)) are frequently based on harsh conditions, large energy expenditures, and the utilization of toxic solvents and stabilizing agents. These approaches are of great concern on the issue of environmental toxicity, biocompatibility and the likelihood of having hazardous residues that can inhibit biomedical applications. In its turn, the emergence of the concept of green synthesis has become a sustainable, environmentally friendly, and cost-effective paradigm. The green synthesis is based on using biological objects such as plants, algae, fungi, and bacteria as nanofactories to minimize the amount of metal ions and stabilize the formed nanoparticles. Its main concept is to use the natural redox potential and the wide range of biomolecules (e.g., phenols, flavonoids, terpenoids, proteins, alkaloids) these biological extracts contain to use metal salts to produce nanoparticles. And the benefits of this method are immense: it is usually a one-pot synthesis at room temperature and pressure, uses water as a non-toxic solvent, and the biomolecules themselves carry out this role thus producing nanoparticles that are normally greener and biologically active as a result of their non-toxic capping layer. The transformation into a green synthesis will be in line with the concepts of green chemistry, reducing waste and environmental footprint and creating nanoparticles that are more therapeutically useful.

The most popular direction of green nanotechnology has become plant-mediated synthesis, or phyto-synthesis, because it is simple, scalable, and plants provide a comprehensive reservoir of phytochemicals. Its synthesis process is a complicated combination of reduction, nucleation, growth and stabilization. The polyphenols, flavonoids, and terpenoids, which are some phytoconstituents when mixed with a metal salt solution, behave like reducing agents and donate electrons to reduce Ag^+ ions to neutral Ag^0 atoms. A change in colour of the reaction mixture usually signifies this. These atoms then fuse into nuclei, which increase to nanoparticles. At the same time, other biomolecules such as proteins, polysaccharides and organic acids adsorb onto the nascent nanoparticle surfaces, which play the role of capping and stabilizing. This bio-capping coating not only inhibits aggregation, but also produces colloidal stability to the nanoparticles and most importantly, it is known to add biological activity to the nanoparticles. The kinetics, morphology, size, and the properties of the synthesized nanoparticles are determined by the specific phytochemical profile of a plant. An example of this is that plants that contain high amounts of reducing sugars and phenolic acids will produce nanoparticles quickly whereas those containing particular proteins may affect the shape and size distribution. This inherent connection between the chemistry of plants and nanoparticle properties enables the targeted production of AgNPs with a desired set of functionalities to be used in a particular biomedical process, e.g., antidiabetic therapy⁷.

Polyherbal formulations are an oral tradition of traditional medicine systems such as Ayurveda, Traditional Chinese Medicine and Unani, where the principle of synergy is applied. The combination effect of the individual effects of the compounds becomes less than the overall effect of the compounds, this effect is called synergy. With a polyherbal extract, the various phytochemicals present in different plants may interact and increase the therapeutic effects, bioavailability, decrease the amount of a single compound required, and alleviate the possible side effects. This whole-person approach aims at various conditions of an illness at the same time, which is especially beneficial in multifactorial diseases, such as diabetes. An extract of one plant might have a dominant active principle but a combination can provide a better range of complementary bioactive

molecules, some of these anti-oxidant support, others anti-carbohydrate enzyme directly⁸. This multi-target, multi-component approach is consistent with the polypharmacology required to manage diabetes and polyherbal extracts have a great potential as a nanosynthesis target, as compared to monobotanical extracts. A combination of extracts of the various plants brings more complexity and possibly richer cocktail of reducing and capping agents into the medium of reaction. The diversity may result in faster and more efficient silver ions reduction since different types of reductants with different redox potentials are available. More to the point, the layer that gets created on the surface of the nanoparticles is most likely to be more diverse and stronger, and a broader range of functional groups of various classes of phytochemicals should be incorporated into it. Such a complex bio-corona has the potential to stabilize the colloidal stability of the nanoparticles within physiological conditions and can confer better or new biological functions. The synergistic phytochemical responses that are advantageous to herbal therapy could be so transferred onto the nano-particle surface to form a so-called synergistic nano-therapeutic of the AgNP core and the physiologically-mixed phytochemical corona acting in synergy to generate a potentiated antidiabetic effect. This is a new approach of combining the age-old herbal knowledge and the new nanotechnology⁹.

Phytosynthesized AgNPs are postulated to fight diabetes by a combination of synergistic actions, which is why they are better than most single-target drugs. To start with, the phytochemical corona, which is frequently enriched with enzyme-inhibiting compounds, can create the strong inhibitory effect on the major carbohydrate-digesting enzymes such as α -amylase and α -glucosidase in the intestine. This suppression reduces the degradation of complex carbohydrates to absorb glucose leading to an attenuated postprandial glycemic spike. Secondly, the AgNP core, as well as the antioxidant phytochemicals in the capping layer, can react with, and eliminate free radicals, and up-regulate endogenous antioxidant responses (e.g. glutathione, SOD, CAT). This prevents oxidative stress which is a major contributor to insulin resistance and β -cell apoptosis. Third, the anti-inflammatory qualities of AgNPs and its phytochemical ligands may be beneficial in the suppression of chronic low-grade

inflammation that is linked with diabetes, which enhances the insulin signaling in peripheral tissues. Lastly, there is some new evidence that specific biocompatible AgNPs can increase the growth and survival of pancreatic β -cells, which can cure the underlying cause of insulin deficiency. Therefore, one polyherbal-AgNP particle has the potential to theoretically regulate dietary glucose uptake, alleviate systemic oxidative stress and inflammation, and safeguard insulin-producing cells as a panacean treatment approach¹⁰.

MATERIALS AND METHODS

Plant Material Collection and Authentication

The foundation of any phytochemical research rests upon the accurate identification and procurement of plant material. For this study, two medicinally significant plant species, renowned in traditional systems for managing hyperglycemia, were selected for the green synthesis of silver nanoparticles (AgNPs). To ensure taxonomic accuracy, the collected plant materials (*A. indica* seeds and *S. cumini* fruits with seeds).

Preparation of Crude Plant Extracts

The process of extraction will proceed to extract the bioactive phytoconstituents in the plant matrices, and this will be used as reducing, stabilizing and capping agent during nanoparticle production. Neem seeds and Jammun seeds were taken through the same primary processing. They were initially washed under the running tap water to remove dust and dirty particles then a final wash was done with the help of the distilled water. The sanitized seeds were subsequently placed evenly on sterile trays and air-dried in the shade at ambient temperature (25 \pm 3degC) during two weeks to avoid thermal degradation of heat labile compounds. Brittle seeds were a good indication of complete dryness. The dried seeds were ground into a fine homogenous powder in an electrical stainless-steel grinder (Panasonic Model MX-AC210, Osaka, Japan). The sieving was done using a 60-mesh sieve to produce a homogenous size of the particles that improve the surface area of the particles to penetrate the solvent during the extraction process.

Characterization of Biosynthesized Silver Nanoparticles

A multi-technique approach was adopted to

confirm the formation, determine the physicochemical properties, and understand the surface chemistry of the synthesized AgNPs.

Visual Observation and UV-Visible Spectral Analysis

The color change was the initial indication of the AgNP formation. In the case of quantitative analysis, the SPR absorption of the nanoparticles was captured. Sonication of the aqueous dispersions of the lyophilized Al-AgNPs, SC-AgNPs, and PH-AgNPs (80 µg/mL) was used to prepare them. A UV-Vis spectrophotometer (Multiskan GO; Thermo Scientific, USA) was used to scan their absorption spectra in the wavelength range of 300-800nm at 2nm. The typical characteristic SPR peak of the AgNPs in its spherical form is that of 400-450nm. To determine the progress of the reactions, spectra were taken at period intervals when synthesis was taking place¹¹.

Fourier Transform Infrared (FTIR) Spectroscopy

The identification of biomolecule functional groups responsible of the reduction and capping was done via FTIR analysis. The pure plant extracts (AIE, SCE) and the lyophilized AgNPs (Al-AgNPs, SC-AgNPs, PH-AgNPs) were combined with dry potassium bromide (KBr) and pellets were formed. A spectrometer (IRTracer-100, Shimadzu, Japan) at 4000-500 cm⁻¹ was used to obtain the FTIR spectra. The changes in the spectra before and after the synthesis of nanoparticles show alteration or disappearance of peaks, which proves the participation of the certain functional groups (e.g., -OH, -C=O, -NH) in the synthesis¹².

X-ray diffraction (XRD) analysis

The XRD was used to determine the crystalline nature and phase purity of the biosynthesized AgNPs. Each type of AgNPs was lyophilized and a uniform coating of the powder applied on a glass slide. Patterns were obtained by XRD in an X-ray diffractometer (Model D8 Advance, Bruker, Germany) with the Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$) with a current of 40 kV and 40 mA. Samples were sampled in the 2 θ range 20° to 80° in steps of 0.02deg. The peaks in the observed diffraction were compared with standard Joint Committee on Powder Diffraction Standards (JCPDS) file of face-centered cubic (fcc) silver. Debye-Scherrer equation was used to estimate the average size of the crystallites.

Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy

SEM with EDX was used to determine the surface morphology, size, and elemental composition of the AgNPs. On one of the carbon stubs, a gold-sputtered layer of the nanoparticle powder was put to increase the conductivity of the product and make it more conductive. The imaging was carried out by use of a high-resolution SEM (Hitachi, S-4300SE, Japan) at an accelerating voltage of 15kV. The same instrument was analyzed using EDX analysis to determine the presence of elemental silver and other related elements of the capping agents^{13,14}.

Dynamic Light Scattering and Zeta Potential Analysis

The hydrodynamic diameter (size distribution in solution), polydispersity index (PDI) and surface charge (zeta potential) of the nanoparticles are essential in determining the stability and biological interaction of the nanoparticles. The AgNPs (100 µg/mL) were prepared in water and sonicated with a Zetasizer Nano ZS (Malvern Instruments, UK) at 25°C. The zeta potential is used to determine the electrostatic stability of the colloid; a value above ± 30 mV is an indication of good physical stability¹⁵⁻¹⁷.

In vitro evaluation of antidiabetic potential Glucose uptake assay by yeast cells

This test determines the capacity of AgNPs to increase the intake of glucose in periphery cells as an imitation of insulin-like participation. The yeast of a commercial baker (*Saccharomyces cerevisiae*) was employed. Washing and the preparation of yeast cells were done in a fashion of distilled water suspension of 10% (v/v). Incubation of different concentrations (10-80 µg/mL) of Al-AgNPs, SC-AgNPs and PH-AgNPs with 1 mL 5 mM glucose solution and 1 mL of yeast suspension was done at 37°C¹⁸⁻²⁰. The mixture was centrifuged and the amount of glucose in the supernatant was estimated by using glucose oxidase-peroxidase (GOD-POD) kit by measuring the absorbance at 520nm. The standard was metformin (500 µg/mL). The percentage glucose uptake was calculated as:

$$\% \text{Glucose Uptake} = \frac{[(\text{Abs_Control} - \text{Abs_Sample}) / \text{Abs_Control}] \times 100}$$

Glucose Adsorption Assay

The physical adsorption of glucose by the nanoparticles, which might slow the intestinal absorption of glucose, was determined by this assay that incubated 1 g of each sample of the AgNPs in glucose solutions of different concentrations (5, 10, 15, 20, 25, 30 mM) at 37°C in a shaking water bath. Aliquots were collected after 6 h and centrifuged and glucose level in the supernatant (G6) was determined. The first concentration was G1. The amount of glucose adsorbed per gram of nanoparticles was calculated as:

$$\text{Bound Glucose (mmol/g)} = \frac{[(G1 - G6) / \text{Sample weight (g)}] \times \text{Volume of sample (L)}}$$

RESULTS AND DISCUSSION

Synthesis and preliminary characterization of AgNPs

The initial evidence of successful green synthesis of silver nanoparticles (AgNPs) using *A. indica* seed extract (AI-AgNPs) and *S. cumini* seed extract (SC-AgNPs) was a visible color change of the reaction mixtures of a pale yellow color to reddish-brown in color, which indicated the decrease of the Ag^+ ions to Ag^0 . The quantitative validation of this was done using UV-Visible spectroscopy which displayed high levels of Surface Plasmon Resonance (SPR). The intensity of these peaks rose as observed with time (Fig. 4), which confirms the gradual development of nanoparticles. The sharp SPR bands of the AI-AgNPs and SC-AgNPs at about 435 nm and 425 nm are related to spherical or more or less spherical silver nanoparticles. The discrete peaks were one and symmetric, indicating a relatively monodisperse distribution, with no major aggregation when the analysis was done.

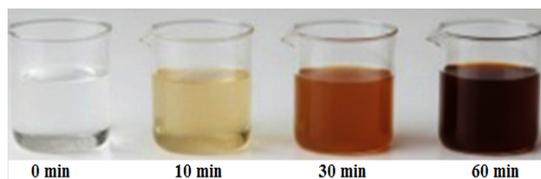


Fig. 1. Visual Color Change of reaction Mixture indicate the Ag^+ ions

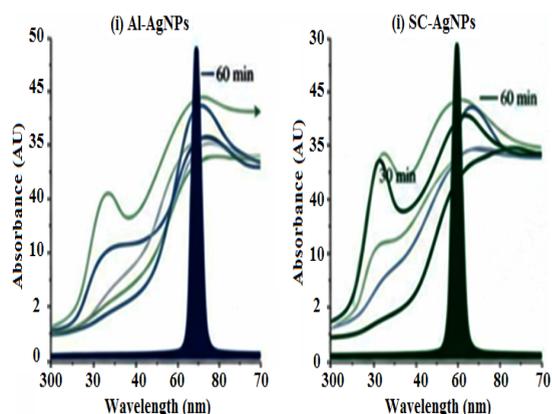


Fig. 2. Time dependent UV-Vis (i) AI-AgNPs (ii) SC-AgNPs
Phytochemical capping and functional group analysis

The FTIR spectroscopy technique was used to determine the biomolecules used to reduce and stabilize the AgNPs. The spectra of the pure *A. indica* and *S. cumini* extracts contained broad peaks in the $3200\text{--}3400\text{ cm}^{-1}$ range that is indicative of O-H stretching phenols and alcohols. Peaks associated with amide I ($\text{C}=\text{O}$ stretch), aromatic $\text{C}=\text{C}$ and $\text{C}-\text{O}$ stretches were also common. An important finding was made regarding the FTIR spectra of the synthesized AgNPs (AI-AgNPs, SC-AgNPs): The observed change in the shape or the decrease in the intensity of these peaks, especially $-\text{OH}$ and $-\text{C}=\text{O}$ groups. This change affirms the participation of these functional groups in the depletion of silver ions and the resultant capping of the nanoparticles, which gives it stability and prevents coalescence.

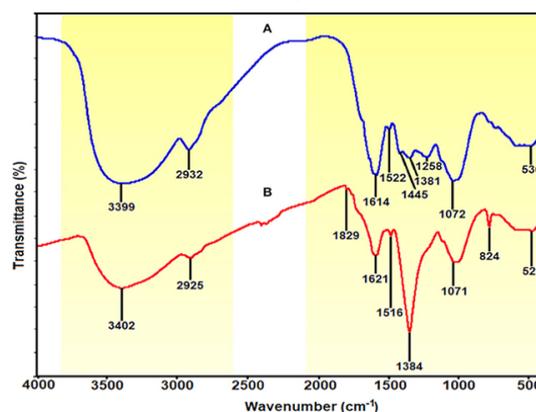


Fig. 3. FTIR spectra of (a) *A. indica* (AIE) and *S. cumini* (SCE) extracts dosage and (b) *A. indica* (AIE) and *S. cumini* (SCE) Ag-NPs at best parameters of the biosynthesis process

Crystalline structure and phase purity

The XRD analysis showed beyond any doubt that the biosynthesized nanoparticles were

crystalline. The two AI-AgNPs and SC-AgNPs have shown different diffraction peaks at 2th values of about 38.1°, 44.3°, 64.5°, and 77.4° corresponding to the (111), (200), (220) and lattice planes of face-centered cubic silver, respectively. The failure to have extraneous peaks verified that the synthesized AgNPs had high purity in the form of the phase. Based on the Debye-Scherrer equation to the strongest (111) peak, the average size of crystallite was estimated to be between 15-25 nm on both types of nanoparticles.

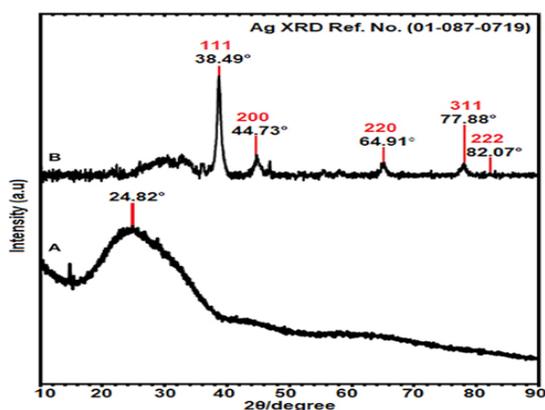


Fig. 4. The XRD patterns of (a) *A. indica* (AIE) and *S. cumini* (SCE) and (b) *A. indica* (AIE) and *S. cumini* (SCE)Ag-NPs at the best parameters to synthesize Ag-NPs

Morphology, Elemental Composition, and Hydrodynamic Properties

The SEM analysis has demonstrated that the shape of the AI-AgNPs and SC-AgNPs were mainly spherical and quasi-spherical, and to some extent, aggregation can be observed in the dry form. The distribution of the particles was good and their size as seen under the SEM supported the crystallite size estimated with XRD. The elemental silver was conclusively determined through the use of EDX spectroscopy which indicated that there was a strong optical absorption peak at about 3 keV. The carbon and oxygen indications were also observed further with the phytochemical capping compounds which were wrapped around the nanoparticles.

In vitro Antidiabetic Potential

The antidiabetic potential of the biosynthesized AgNPs was evaluated through two complementary mechanisms: cellular glucose uptake and direct glucose adsorption.

Enhancement of cellular glucose uptake

The glucose uptake assay of the yeast

cell showed an increase in glucose utilisation with a concentration in both AI-AgNPs and SC-AgNPs. The nanoparticles at the highest concentration tested (80 µg/mL) supported the uptake of glucose with the likes of the standard drug metformin. This has been explained by the nanoparticles being insulin-mimetic and therefore able to interact with cellular membranes or signaling pathways and increases glucose permeability of yeast cells. The bioactivity is probably due to synergistic effect of the silver core and phytochemicals that are adsorbed on the surface that may have natural antidiabetic activity.

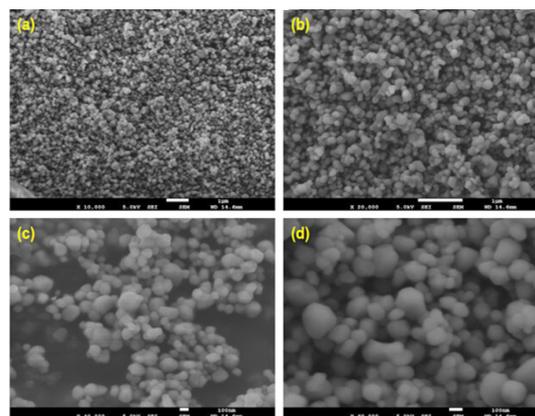


Fig. 5. SEM Image of Herbal Extract

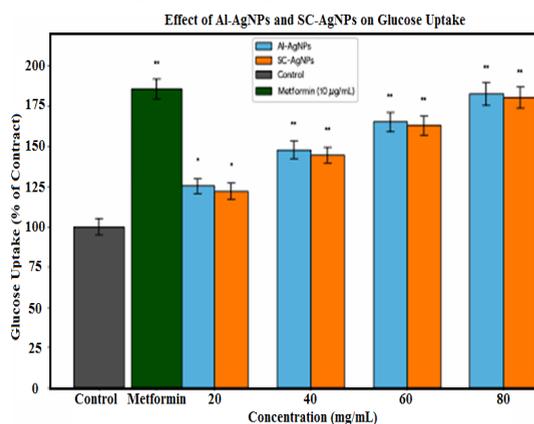


Fig. 6. Concentration-dependent effect of AI-AgNPs and SC-AgNPs on glucose uptake in yeast cells

CONCLUSION

This study has been able to illustrate the green synthesis of silver nanoparticles using seed extracts of *Azadirachtaindica* and *Syzygiumcumini*, two plants that have proven ethnopharmacology in the treatment of diabetes. Its synthesis protocol was an easy, environmentally friendly and efficient alternative to the traditional chemical processes. The

formation of spherical, crystalline AgNPs having a layer of bioactive phytoconstituents was successfully characterized by comprehensive characterization, including UV-Vis, FTIR, XRD, SEM, and EDX. The *in vitro* assessment demonstrated the considerable antidiabetic potential of the biosynthesized Al-AgNPs and SC-AgNPs, mainly because of the increase of the peripheral glucose uptake and adsorption of dietary glucose. The mechanisms are the reflections of the desired effects in the management of diabetes, including the enhancement of insulin sensitivity and the decrease of postprandial hyperglycemia. The hypothesis that the phytochemical corona around the nanoparticles plays a very important role in the biological activity is confirmed by the results. To sum up, the combination of green nanotechnology and therapeutic concept of medicinal plants provide the strong and prospective avenue in the creation of

new antidiabetic agents. The biosynthesized AgNPs are an attractive, multi-mechanistic candidate of interest, and they should be investigated further, their *in vivo* toxicity and efficacy studies, polyherbal combinations with synergetic effects, and the mechanistic understanding of their pathway-specific mechanisms should all be explored to bring a better understanding of their actions.

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Conflict of interest

The author declare that we have no conflict of interest.

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