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Formulation, Characterization, And Assessment Of Nanoparticles Incorporating Lyophilized Amla Pulp For Antidiabetic Efficacy

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Abstract
This study explores the green synthesis of silver nanoparticles (AgNPs) using Phyllanthus emblica (Amla) fruit extract, renowned for its medicinal properties. The synthesis process involved reducing silver ions with phytochemicals present in the extract, resulting in spherical AgNPs confirmed through UV-vis spectroscopy, FTIR, and SEM analyses. Characterization revealed successful formation of nano-sized particles stabilized by bioactive compounds. The antidiabetic potential of these biosynthesized AgNPs was evaluated in vitro by assessing their inhibitory effects on key carbohydrate-digesting enzymes, α-amylase and α-glucosidase. Results demonstrated significant enzyme inhibition, with the synthesized nanoparticles showing comparable or superior activity to standard drugs like Acarbose. These results indicate that environmentally synthesized silver nanoparticles derived from Phyllanthus emblica may have significant potential as therapeutic agents for diabetes treatment, highlighting the need for additional research to understand their mechanisms of action and evaluate their effectiveness in clinical applications.
Keywords: *Silver nanoparticles , Phyllanthus emblica , α-amylase , α-glucosidase , diabetes*

INTRODUCTION

Diabetes is a long-term condition caused by the pancreas not producing enough insulin, which hampers the body's ability to utilize this hormone effectively. Insulin is crucial for controlling blood glucose levels [1]. Diabetes, also known as diabetes mellitus, refers to a collection of hormonal conditions characterized by persistently elevated blood sugar levels [2]. This is a significant health concern in both developed and undeveloped nations [3].Despite numerous treatments for diabetes, they do not completely cure the disease and often cause side effects. Many plants and vegetables have been proven to have antidiabetic activities in animal models, suggesting a search for new, less harmful agents [4].Research has focused on identifying inhibitors for carbohydrate-hydrolyzing enzymes like a-amylase and glucosidase, which is a significant therapeutic approach to reduce high blood sugar levels by hindering glucose uptake [5].Nanotechnology advancements have led to the creation of silver nanoparticles, which effectively inhibit a-glucosidase and a-amylase enzymes, making them a potential treatment for diabetes [6].Indian medicinal plants have been utilized for synthesizing silver nanoparticles, with a limited number being effective as antidiabetic agents, demonstrating the non-toxic, cost-effective, and safe nature of green synthesis [7,8].The experiment uses Phyllanthus Emblica fruit extract as a plant extract, a popular plant in the Indian sub-continent, for its medicinal properties, including antioxidant, hair fall, diabetes, constipation, dental issues, chelating agents, diarrhea, headache, and respiratory problems [9,10]. From the phytochemical analysis, it has been known that Phyllanthus emblica is carrying alkaloid, flavonoids, phenols, tannins, etc [11].These substances are effective in treating various human diseases [12].This experiment utilized Phyllanthus emblica as a reducing agent due to its medicinal effects and

phytochemicals [13].The process of synthesizing silver nanoparticles using plant phytochemicals involves three steps: oxidation of Ag+ by reducing agents, growth of AgNPs due to surface reduction, and electrostatic stabilization to control size. This involves the formation of Agatoms, which are then stabilized by adsorption of excess negatively charged reducing agent ions [14].The bio-fabrication of silver nanoparticles using phyllanthus emblica from the fruit extract has been reported in previous work. These nanoparticles have been used in clinical trials to reduce diabetes, enhance fertility, help the urinary system, act as an anesthetic, reduce cancer risk, support the heart, improve skin, improve eye sight, and boost immunity [15]. The synthesized silver nanoparticles were characterized using UV-vis spectroscopy, FTIR, and SEM, and their in – vitro antidiabetic activity was studied against alpha- glucosidase and alpha-amylase enzymes.

MATERIALS AND METHODS

Collection and prepration of lyophilization fruit extract

Amla (Phyllanthus emblica) was used as an experimental plant material for synthesizing silver nanoparticles. The amla collected, from local market, minced into small pieces (100g), immersed in distilled water (200ml) for 16 h. Then filtered using Whatmann No-1 filter paper and the filterate was used for lyophilization of amla extract and collect dry amla powder was used.

Synthesis of silver nanoparticles

The silver nanoparticles were synthesized by adding 50ml of 0.1 M silver nitrate solution into 25mg of dry amla powder.The synthesis was carried out in a dark condition to minimize the photo activation of silver nitrate. The color change from yellow to brown indicated the formation of silver nanoparticles. After the synthesis, the reaction mixture was shaken, and centrifuged at 3000rpm for 30min. The silver nanoparticles pellet was dried at room temperature, and the dried powder was used for further analysis.

Characterization of silver nanoparticles

The synthesized silver nanoparticles were characterized using UV-visible spectrophotometry, Fourier Transform Infrared Spectroscopy, and Scanning Electron Microscopy (SEM) were used for the characterization of the synthesized silver nanoparticles. The UV-vis spectrum confirmed the synthesis, and the presence of biomolecules was identified using FTIR spectrophotometer. Scanning Electron Microscopy was used to investigate the morphology and size of the synthesized silver nanoparticles.

Assay of α -Amylase inhibition activity

Sample dilutions of 0-50μg/ml in sodium phosphate buffer (Rankem, Cat no.- S0240)were prepared. The enzyme solution (10 μl) containing 20mg/ml alpha amylase (HIMEDIA GRM638-100g) was placed in defined well of a 96-well plate. The samples (10 μl) were added, and mixture was incubated for 10 minutes. The reaction was then initiated by adding 50 μl substrate (0.1% Soluble Starch-Fisher Scientific –Cat no-20725) and mixture was further incubated for 15 minutes. Finally, after 15 minutes 100μl GOD-POD Reagent (ClinReact GLUCOSE (GOD/POD)-GLU125-033) was added to the mixture and then plate was incubated at room temperature for 10 minutes and absorbance was taken at 490 nm using a micro plate reader (iMark, BioRad). Inhibitor, Acarbose (SRL- Cat no-65457, 50 µg/ml final Concentration) was used as a positive control.$IC_{50}$was calculated using Software Graph Pad Prism 6.

Calculations

% Inhibition = $(A_{Control} - A_{test} / A_{Control}) \times 100$

$A_{Control}$ = Absorbance of control

A_{test} = Absorbance of sample

Assay of α -glucosidase inhibitory activity

The α-glucosidase inhibitory activity of samples was carried out according to the following method of [Telagari & Hullatti, 2015]. In a 96-well plate, reaction mixture containing 50 μl phosphate buffer (100 mM, pH = 6. 8), 10 μl alpha-glucosidase (1 U/ml- SRL Chem- a-Glucosidase (Maltase) ex. Yeast, 75551), and 20 μl of varying concentrations of sample (as mentioned in excel) were preincubated at 37°C for 15 in. Then, 20 μl p-NPG (5 mM- 4-Nitrophenyl a- D-glucopyranoside, Merk-Sigma-487506) was added as a substrate and incubated further at 7°C for 20 min. The reaction was stopped by adding 50 μl Na2 CO3 (0.1 M). The absorbance of the released p-nitrophenol will be measured at 405 nm using ELISA

Microplate (iMark Bio Rad) reader. Acarbose (50 mg/ml) used as Positive Control. IC-50 was calculated using Software Graph Pad Prism 6.

Calculation

% Inhibition = ($A_{Control}$ - A_{test} /$A_{Control}$) ×100

$A_{Control}$ = Absorbance of control

A_{test} = Absorbance of sample

RESULTS AND DISCUSSION

Synthesis of silver nanoparticles

The green synthesis of silver nanoparticles was done by using amla extract as a reducing agent. The process led to the formation of a dark brown precipitate, which shifted from Transparency to brown color.

Characterization of silver nanoparticles

1) UV-vis spectroscopy

The research illustrates the fabrication of silver nanoparticles (AgNPs) through the use of amla extract as a reducing agent in the presence of silver nitrate. After approximately 30min, the solution truns drak brown, signifying nanoparticle formation. The surface Plasmon resonance (SPR) peak confirm the synthesis, while color changes serve as visual evidence of silver ions reducing to metallic silver. UV- visible spectroscopy reveals absorption bands for the fruit extract, synthesized AgNPs, and slver nitrate solution , with a characteristic resonance peak observed at 449nm.

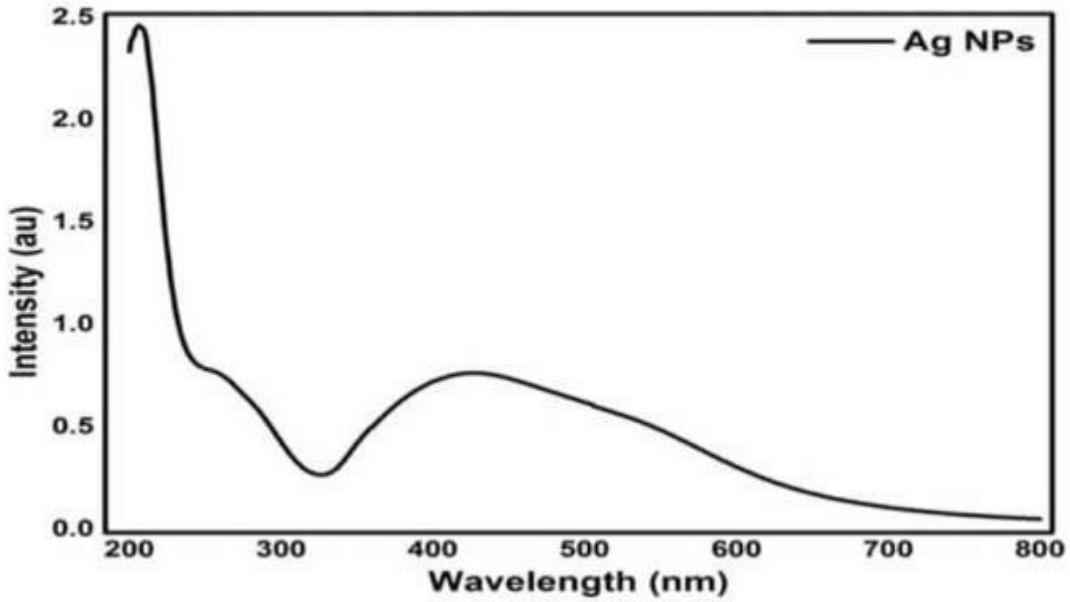

Fig (1): UV- vis spectrum of phyllanthus emblica fruit extract & AgNPs.

2) FTIR analysis

The infrared (IR) spectrum serves as a valuable analytical tool to examine how molecules absob infrared light across different wavelengths. It displays characteristic peaks that correspond to specific chemical bonds or functional groups within a sample, with the position of these peaks indicating their identity and their intensity reflecting their abundance. Key spectral features include O-H or N-H stretching vibrations, which are typical in alcohols, phenols, or amins, C-H stretching in aliphatic compounds, C=O stretching seen in carbonyl groups or aromatic rings, and C-H bending or C-O stretching vibrations. Notable peaks around 3388cm^{-1} and 3738cm^{-1} are indicative of O-H or N-H stretching, while those at 2928cm^{-1} and 2854cm^{-1} associated with C-H stretches. A peak near 1636cm^{-1} may suggest the presence of C=O or C=C stretching within aromatic structures.

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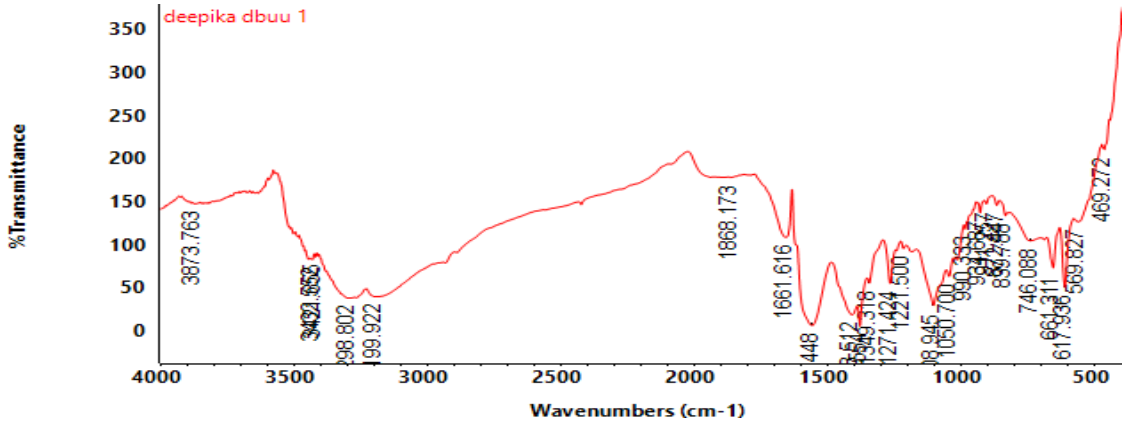

Fig (2): FTIR Spectrum of amla fruit extract and silver nanoparticles

3) Scanning electron microscopic (SEM) and (EDX) analysis

The morphology of the synthesized silver nanoparticles was analyzed using Scanning Electron Microscopy (SEM). As illustrated in (fig 3), the particles mainly display a spherical morphology. Elemental composition was examined through Energy Dispersive X-ray Spectroscopy (EDX), shown in (fig 4). The dominant peak near 3 keV confirms the presence of elemental silver, indicating the successful formation of nanocrytals. Fuethermore, a signal around 0.5 keV, potentially arising from chloride ions, may be associated with components derived from the fruit extract of Phyllanthus emblica.

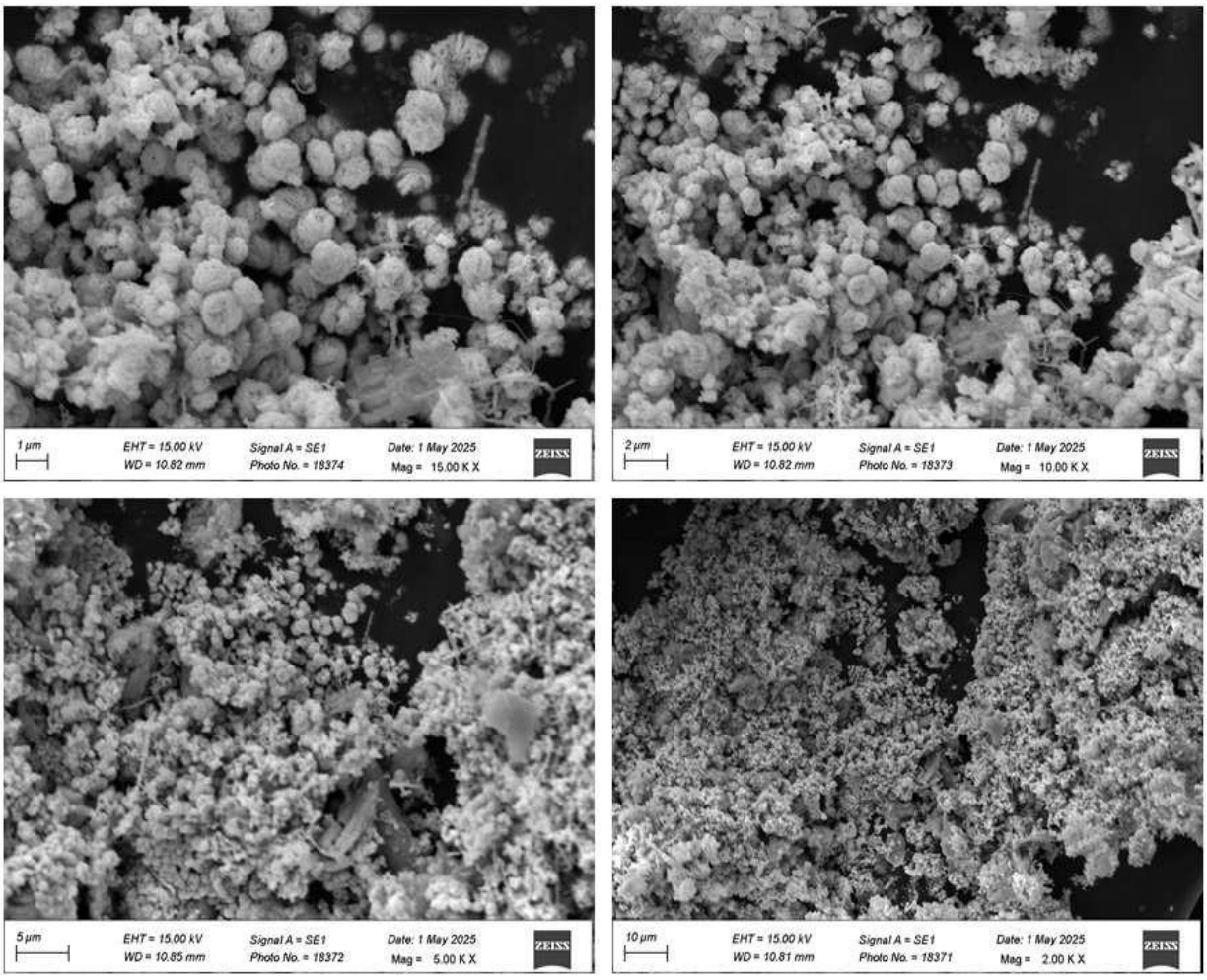

Fig (3): SEM synthesized silver nanoparticles of amla fruit extract

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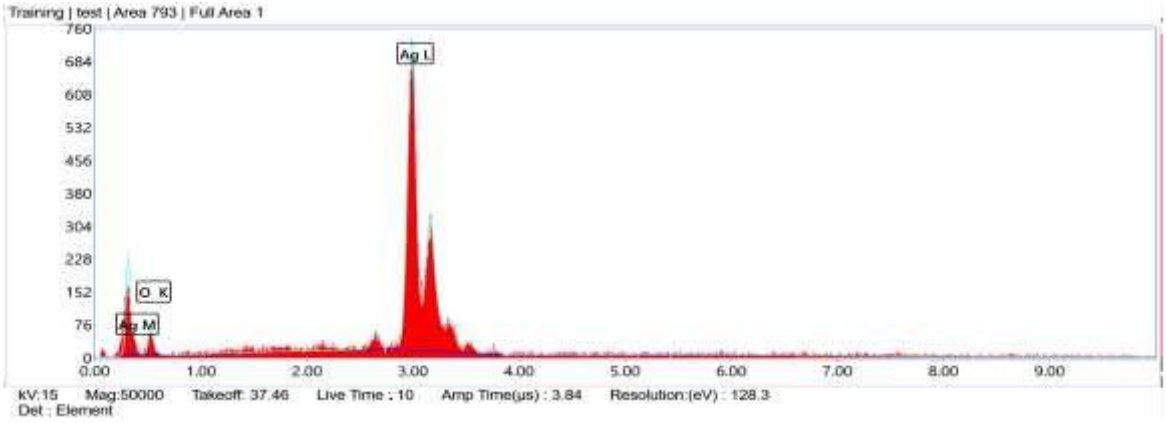

In vitro antidiabetic activities

1) *α*-Amylase inhibition activity

Amylase is a crucial enzyme involved in carbohydrate metabolism. Inhibiting amylase is an effective strategy to lower blood sugar levels. Amylase inhibitors, or starch blockers, work by preventing the body from absorbing dietary starches. As a result, they help to diminish the typical increase in blood sugar that occurs after consuming carbohydrate-rich foods.It has been reported that green biosynthesized silver nanoparticles can function as amylase inhibitors, potentially aiding in the reduction of blood sugar levels. Based on the results obtained from the study, Enzyme Inhibition Activity (Alpha Amylase) was estimated in the sample. 50% inhibition at this concentration i.e. $IC_{50}$of sample- D-1was estimated as 17.63 µg/mlin comparison to standard Acarbose (IC_{50} = 11.9 ± 0.024µg/ml).17.63 µg of the sample D-1 was found equivalent to11.9 µg of the standard Acarbose. Sample D-1was found to be highly active.Lower is the IC_{50}, higher be inhibition activity.In the same way, the silver nanoparticles synthesized from other medicinal plants were also showed a-amylase inhibition activity

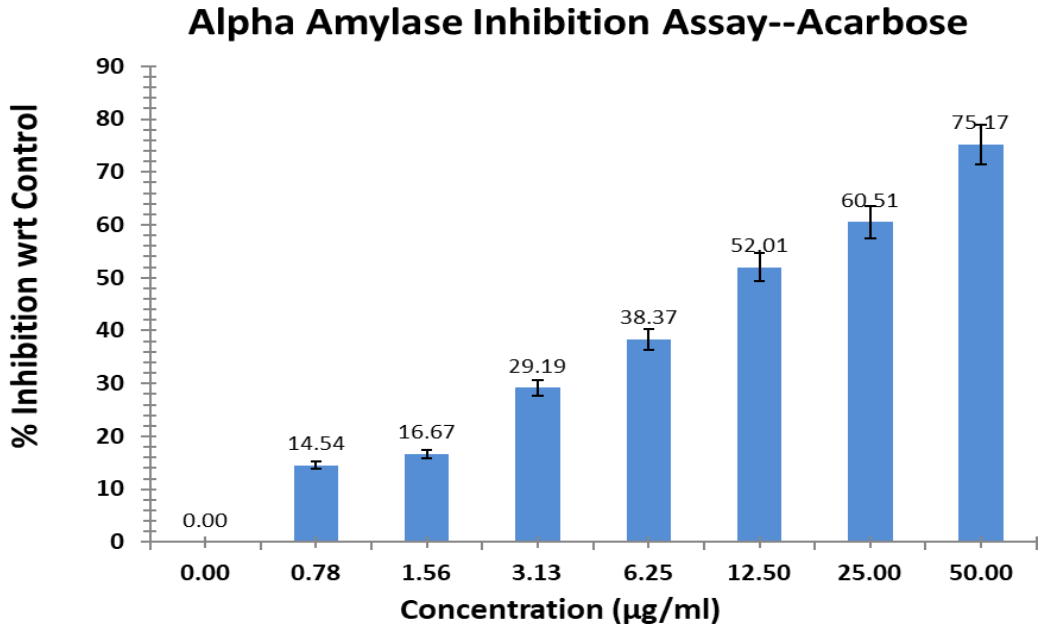

Fig.5. *α*-Amylase inhibition activity of silver nanoparticles from Phyllanthus emblica (Silver nanoparticle was compared with acarbose

2) *α* -Glucosidase inhibitory activity

α-Glucosidase is a key enzyme in carbohydrate metabolism that facilitates the breakdown of oligosaccharides and disaccharides into monosaccharides through hydrolysis. Research indicates that

inhibiting α -glucosidase can slow down the digestion and absorption of carbohydrates, leading to a decrease in blood glucose levels. Based on the results obtained from the study, the Enzyme Inhibition Activity (α-glucosidase) was observed in sample and 50% Inhibitory concentration is mentioned in table 1. sample- D-1 was found to be highly active as compared to standard - Acarbose. 4.035 µg of the sample-D-1 was found equivalent to 647.6 µg of the standard- Acarbose. So it can be used as an α -glucosidase inhibitory agent for the treatment of diabetes.

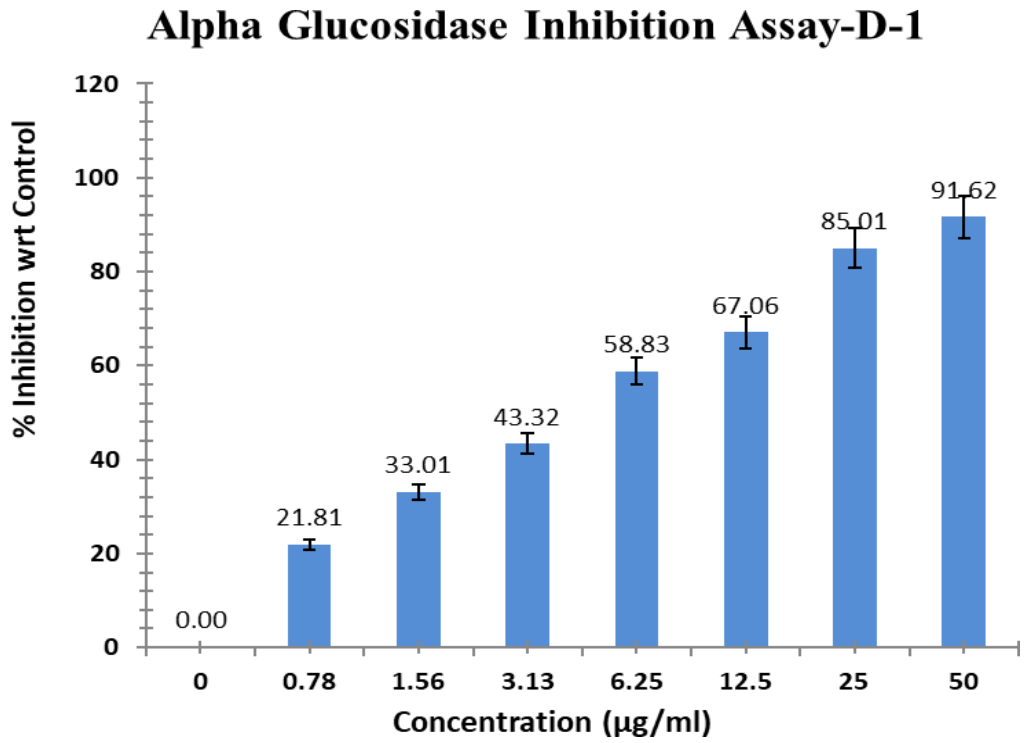

Fig.6. α -Glucosidase inhibition activity of silver nanoparticles fromPhyllanthus emblica(Silver nanoparticle was compared with acarbose

CONCLUSION

This study focused on the eco-friendly production of silver nanoparticles utilizing Phyllanthus emblica, along with assessing their potential to fighting diabetes through the inhibition of carbohydrate-digesting enzymes. The analyses conducted using UV–vis Spectrophotometry, FT-IR, and SEM demonstrated that the synthesized particles were nano-sized, spherical in morphology, and coated with functional groups originating from the metabolites present in the Phyllanthus emblicaextract. The synthesized silver nanoparticles demonstrated enhanced antidiabetic activity by inhibiting carbohydrate-metabolizing enzymes like α-amylase and α-glucosidase. The synthesized nanoparticles have the potential to serve as effective therapeutic agents for managing diabetes by inhibiting carbohydrate-hydrolyzing enzymes. Additional research is necessary to establish its exact mechanism of action in both animal and human models before recommending this product as a therapeutic treatment for diabetes.

CONSENT FOR PUBLICATION

All authors final approval of the version to be published.

ETHICAL APPROVAL

NO animal was harmed during this research study.

FUNDING

Not applicable

CONFLICT OF INTEREST

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

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