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ENHANCED ANTIDIABETIC AND ANTIOXIDANT POTENTIAL OF GREEN-SYNTHESIZED SELENIUM NANOPARTICLES USING *MOMORDICA CYMBALARIA*

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Abstract

This study aimed to synthesize and evaluate the antidiabetic and antioxidant activities of selenium nanoparticles (SeNPs) prepared using Momordica cymbalaria extract, a plant known for its medicinal properties. The extract was employed in a green synthesis approach to produce SeNPs, which were then characterized and assessed for biological activity. UV-Vis spectroscopy confirmed the formation of the nanoparticles through characteristic absorption peaks. The synthesized SeNPs exhibited significant α-amylase and α-glucosidase inhibitory activities, indicating their potential as antidiabetic agents. Additionally, they demonstrated notable antioxidant activity in both DPPH and ABTS assays, suggesting their ability to combat oxidative stress. These findings highlight that selenium nanoparticles synthesized using Momordica cymbalaria possess promising therapeutic potential. The green synthesis method not only offers an eco-friendly approach to nanoparticle production but also enhances the bioactivity of selenium by integrating the plant's bioactive compounds. Further in vivo studies are necessary to validate their efficacy and safety for clinical applications.

Keywords

Antidiabetic activity, Antioxidant activity, Selenium nanoparticles, *Momordica cymbalaria*, Green synthesis, α-Amylase inhibition, α-Glucosidase inhibition, Oxidative stress, Medicinal plants, Nanodrug, Diabetes management, Nanotechnology,.

INTRODUCTION

The coming of nanotechnology over last 30 years.understanding of sedate revelation, advancement, numerous covered up entryways in infection pathophysiology and treatment option (1)(2).The one of a kind properties of nanoparticles (NPs), such as little estimate, expansive surface range, dissolvability, multifunctionality, strikingly interesting. NPs have appeared an awfully solid part as drug carriers, accomplishing colossal victory within the conveyance of helpful atoms.(3)Metal nanoparticles Selenium have a uncommon put within the field of nanotechnology ,it were as restorative operators, but moreover have colossal potential as carriers of chemotherapeutic agents.(4).Selenium may be a normally happening mineral(5) Selenium found as a by-product of sulfuric acid blend. It may be dark shiny and metallic gray in crystalline shape, comparable to sulfur and tellurium (6).Selenium nanoparticles (SeNPs) have indeed garnered attention due to their lower toxicity and enhanced biocompatibility compared to their natural or inorganic forms.(7). the most excellent choice is the green synthesis strategy, which employments plants to bioreduce the compound contained within the nanomaterials.(8). Nanotechnology could be a way to create nanomaterials with perfect properties and nanometer size. Compared to bulk materials, nanoparticles have a gigantic surface area(9).The advancement of bio-inspired nanoparticles could be a quickly developing field in present day innovation. Nanomaterials are normal or man-made materials that contain particles within the free state or totals in which 50% or

more of the particles are within the run of 1 to 100 nm in number, measure, conveyance, or one or more outside sizes(10).The expanding rate of DM places a overwhelming burden on society and person families((11).Conventional Chinese Pharmaceutical (TCM) has gathered important and wealthy encounter within the treatment of DM, obtaining a riches of phytomedicine and observational formulas. Although phytomedicines and their dynamic fixings have less poisonous quality and side effects(12)(13).there is still a need to define them into more reasonable dose shapes to affirm their viability. In later a long time, intrigued within the planning and inquire about of Se-NPs has expanded .(14))SeNPs play a crucial role in antioxidant defense mechanisms and provide significant protection against oxidative stress. (15)(16). This study aimed to prepare *Momordica cymbalaria* aqueous extract and to use it in synthesizing selenium nanoparticles in ecofriendly technique. It also aimed to estimate the biological activity of them as antioxidant, antidiabetic material.

MATERIALS AND METHODS

Preparation of *Momordica cymbalaria* extract:

1 gram of *Momordica cymbalaria* was taken as powdered and make as solution.. then heated for about 20 minutes and boiled at 50-60°C. After boiling, the solution was filtered using muslin cloth to collect the filtrate. The extracted filtrate was then stored for further use.

Preparation of Nanoparticles solution(SeNPs

To prepare the selenium nanoparticle solution we have to take 0.344 grams of sodium selenate and add it to 50 ml of water and plant extract solution(filtrate). Now we have to keep it in an orbital shaker for 48 hours. In Between this process of shaker you have to check any color change using UV reading at a time interval of 3,12,24 & 48. This checking of color change is to find whether the SeNPs is synthesized or not. • The NPs will get ready mostly in the 24 th hour. After the completion of NPs synthetization you have to centrifuge the solution .At last collect the pellet supernatant discarded. This is used for medicinal use. • Antidiabetic activity evaluation of selenium nanodrug was done by using evaluation of activity was done by using Alpha-Amylase Enzyme Inhibition assay. • Antioxidant activity evaluation of selenium nanodrug was done by using evaluation of activity was done by using FRAP assay, ABTS assay,H2O2 assay and Nitric oxide assay.

***In vitro* Antidiabetic assay:**

Determination of α-Amylase Enzyme Inhibition

Preparation of Solutions:Enzyme Solution:Prepare the α-amylase enzyme, starch solution in the buffer.Prepare the DNSA reagent according to standard protocols.Experimental Setup:Add starch solution to the control and to the SeNPs solutions at five different concentrations (10, 20, 30, 40, and 50 µg/ml).Allow the enzyme with the starch solution at 25°C for 3 minutes.Use acarbose as the positive control in the study.Reaction and Detection:The production of maltose was quantified by the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid.Measure the reaction at 540 nm.

Evaluation of α-Glucosidase Enzyme Inhibition

Preparation of Solutions:SeNPs Solutions: Prepare SeNPs solutions at various concentrations.Starch Substrate: Prepare a 2% solution of maltose or sucrose.α-Glucosidase Enzyme: Prepare a solution of α-glucosidase enzyme at 1 U/ml.Experimental Setup:Mix the SeNPs solutions with the starch substrate solution.Incubate the mixture at 37°C for 5 minutes.Add 1 ml of enzyme solution and incubate at 35°C for 40 minutes..Use acarbose as the standard control in the study.Measure the color intensity at 540 nm using an ELISA reader

***In vitro* Antioxidant activity**

DPPH Radical Scavenging Assay

Preparation of Solutions;Stock Solution: Dissolve DPPH in methanol to prepare a 0.1 mM solution.Prepare different concentrations of the SeNPs in methanol.Assay Procedure:In wells of a 96-well microplate, add 1 mL of DPPH solution to 1 mL of the SeNPsolution at various concentrations.Incubate the plate in the dark at room temperature for 10 minutes.Measure the absorbance at 517 nm using a spectrophotometer.

Hydrogen Peroxide Radical Scavenging Assay

Preparation of Solutions:H2O2 Solution: Prepare a 40 mm solution of (H2O2) Assay Procedure:Prepare different concentrations of SeNPs and a standard sample of ascorbic acid.Add 0.6 mL of H2O2 solution to the test samples and standard samples.Incubate the mixtures for 10 minutes.Measure the absorbance at 230 nm using a spectrophotometer.Use vitamin C as a standard for comparison.

FRAP Assay

The FRAP assay was conducted using a working reagent composed of 300 mM acetate buffer (pH 3.6), 20 mM $FeCl_3 \cdot 6H_2O$, and TPTZ dissolved in 40 mM HCl. Standard solutions of $FeSO_4 \cdot 7H_2O$ (0.1–1.5 mM) were prepared in methanol. For the assay, 2.3 mL of FRAP reagent was mixed with 0.7 mL of Momordica cymbalaria extract at various concentrations and incubated at 37°C for 30 minutes in the dark. Absorbance was measured at 593 nm, with increased absorbance indicating higher reducing power. A blank without the sample was used, and ascorbic acid served as the standard. All measurements were performed in triplicate.

ABTS Assay

The ABTS assay was performed by mixing 7.0 mM ABTS with 2.45 mM potassium persulfate (1:1) and incubating the mixture at 4°C for 24 hours to generate ABTS•+ radicals. Before use, the solution was diluted with 50% ethanol to an absorbance of 1.0 ± 0.02 at 734 nm. Various concentrations of selenium nanoparticles (SeNPs) were prepared in distilled water, with ascorbic acid as the standard and ethanol as the blank. In a 96-well plate, 250 µL of ABTS•+ solution and 20 µL of sample were added, incubated for 10 minutes at room temperature, and absorbance was measured at 734 nm.

Nitric Oxide Radical Inhibition Assay

The nitric oxide radical inhibition assay was performed using a modified Griess Illosvoy reaction. A reaction mixture containing 2 mL of 10 mM sodium nitroprusside, 0.5 mL of PBS, and 0.5 mL of Momordica cymbalaria extract at various concentrations was incubated at 25°C for 150 minutes. After incubation, 0.5 mL of the mixture was combined with 1 mL of 0.33% sulfanilic acid reagent and allowed to stand for 5 minutes for diazotization. Then, 1 mL of 0.1% naphthylethylenediamine dihydrochloride was added, and the mixture was incubated for 30 minutes in diffused light to develop a pink chromophore. Absorbance was measured at 540 nm using a spectrophotometer, with ascorbic acid as the standard and blanks prepared accordingly.

RESULTS AND DISCUSSION

The results indicate that Momordica cymbalaria-derived selenium nanoparticles (SeNPs) exhibit dose-dependent antidiabetic and antioxidant activities. SeNPs showed strong α-amylase and β-glucosidase inhibitory effects, closely matching the standard inhibitors at higher concentrations (≥20 µg/mL), with slightly reduced activity at lower concentrations. Antioxidant assays (DPPH, ABTS, H_2O_2 scavenging, and FRAP) revealed that SeNPs demonstrated comparable or slightly lower activity than the standard antioxidants, with marginal differences that diminished at higher doses. Overall, SeNPs displayed consistent, reliable, and concentration-dependent inhibition, highlighting their potential as natural antidiabetic and antioxidant agents (Figure 1- 7). The study demonstrates the successful synthesis of SeNPs using an extract of *Momordica cymbalaria*, employing a green synthesis approach. The spherical

morphology and narrow size distribution of the SeNPs suggest that the *Momordica cymbalaria* extract acts effectively as both a reducing and stabilizing agent.The SeNPs exhibited significant inhibitory effects on both enzymes, indicating potential to reduce postprandial blood glucose levels. This inhibition is crucial for diabetes management, as it helps in controlling the glycemic index and reducing the risk of hyperglycemia(7). The bioactive compounds present in *Momordica cymbalaria*, known for their hypoglycemic effects, likely contribute to the enhanced activity of the SeNPs.In addition to their antidiabetic properties, the SeNPs showed remarkable antioxidant activity. The antioxidant capacity of the SeNPs suggests they can neutralize free radicals and mitigate oxidative damage, thereby providing a dual therapeutic benefit.(17).The green synthesis method offers several advantages, including eco-friendliness, cost-effectiveness, and the avoidance of toxic chemicals typically used in nanoparticle synthesis. This method leverages the natural phytochemicals in *Momordica cymbalaria* to produce SeNPs, making it a sustainable alternative for nanoparticle synthesis.

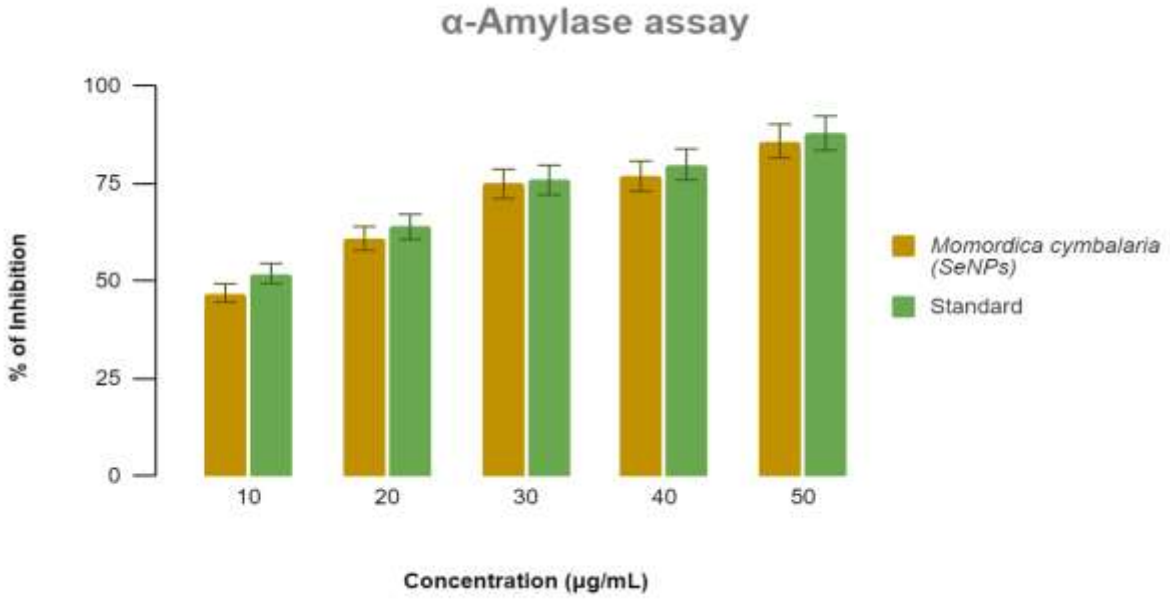

Figure 1 :The inhibition capacity of SeNPs increases with concentration and closely matches that of the standard inhibitor at higher concentrations (20 µg/mL and above).At lower concentrations (10 µg/mL), SeNPs are slightly less effective than the standard, but this difference diminishes as concentration increases.*Momordica cymbalaria* SeNPs demonstrate significant potential as α-Amylase inhibitors, comparable to the standard inhibitor at higher concentrations.

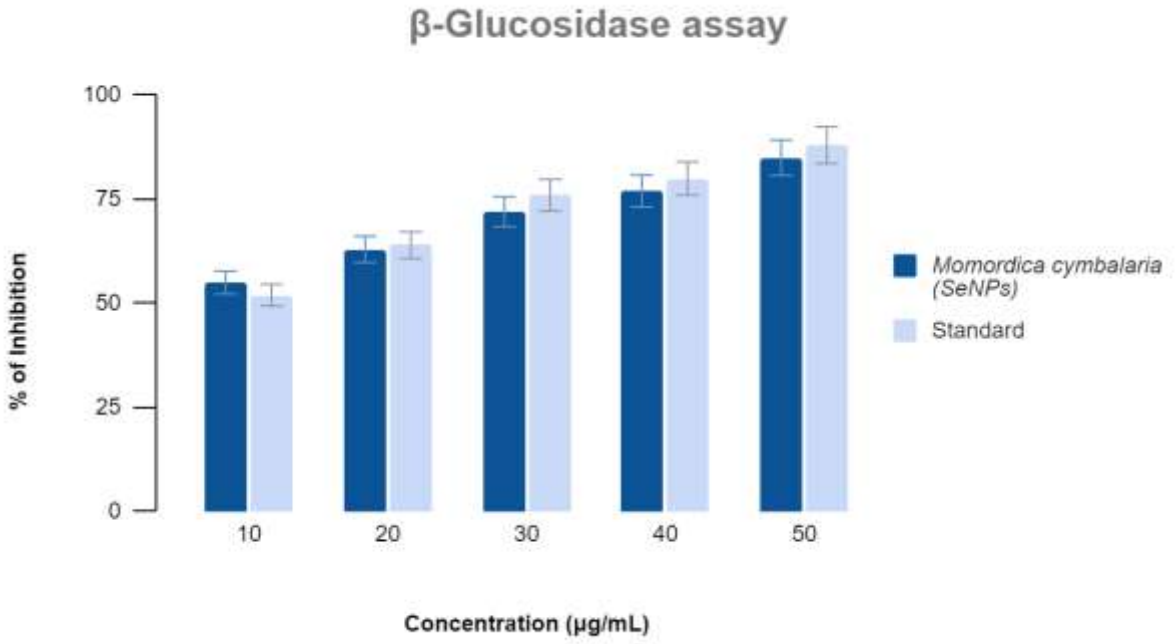

Figure 2 :*Momordica cymbalaria* SeNPs exhibit a dose-dependent inhibition of β-Glucosidase activity.The inhibition capacity of SeNPs increases with concentration, closely matching the standard inhibitor at higher concentrations.At lower concentrations (10 µg/mL), SeNPs are slightly less effective than the standard, but this difference diminishes as the concentration increases.*Momordica cymbalaria* SeNPs demonstrate significant potential as β-Glucosidase inhibitors, with performance comparable to the standard inhibitor at higher concentrations.

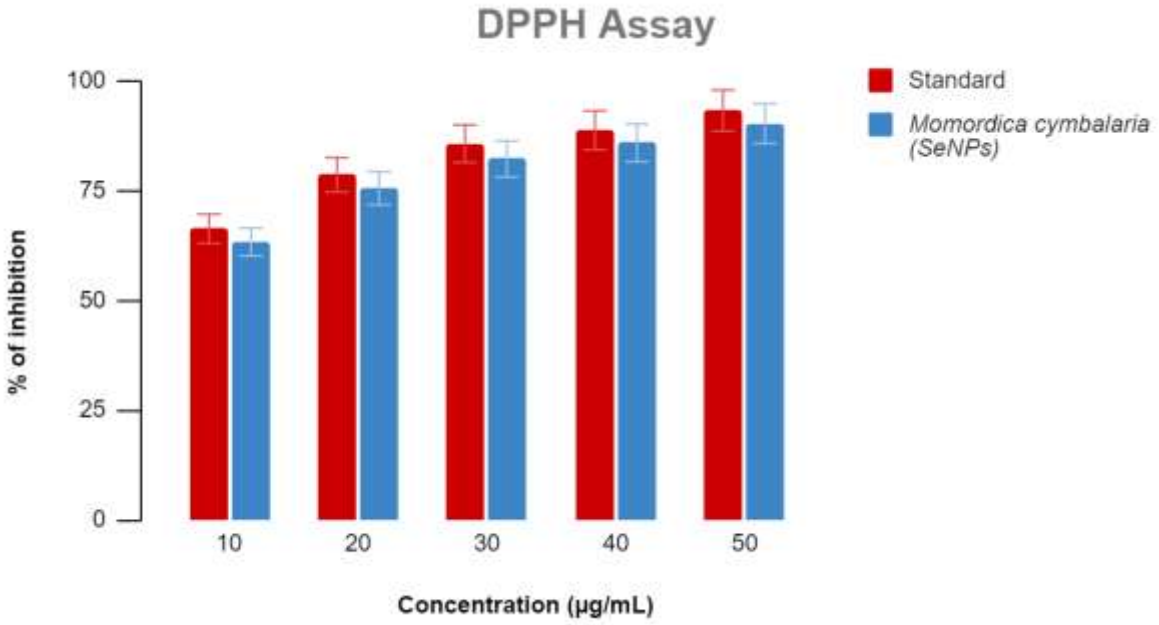

Figure 3: *Momordica cymbalaria* (SeNPs) demonstrates comparable antioxidant activity to the standard antioxidant, especially at higher concentrations. This suggests that Momordica cymbalaria (SeNPs) could be an effective natural antioxidant, potentially comparable to the standard used in this assay.

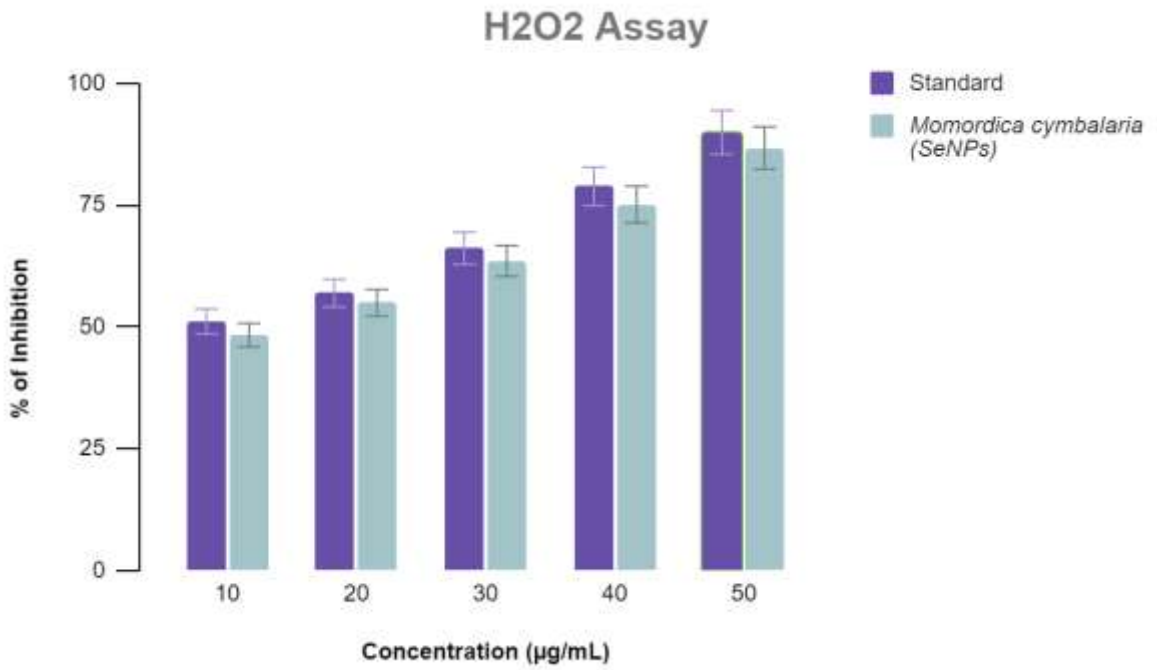

Figure 4 : *Momordica cymbalaria* (SeNPs) exhibits slightly better antioxidant activity compared to the standard antioxidant, especially noticeable at lower concentrations. This suggests that Momordica cymbalaria (SeNPs) could be a potent natural antioxidant with a capability to effectively neutralize the oxidative effects of hydrogen peroxide.

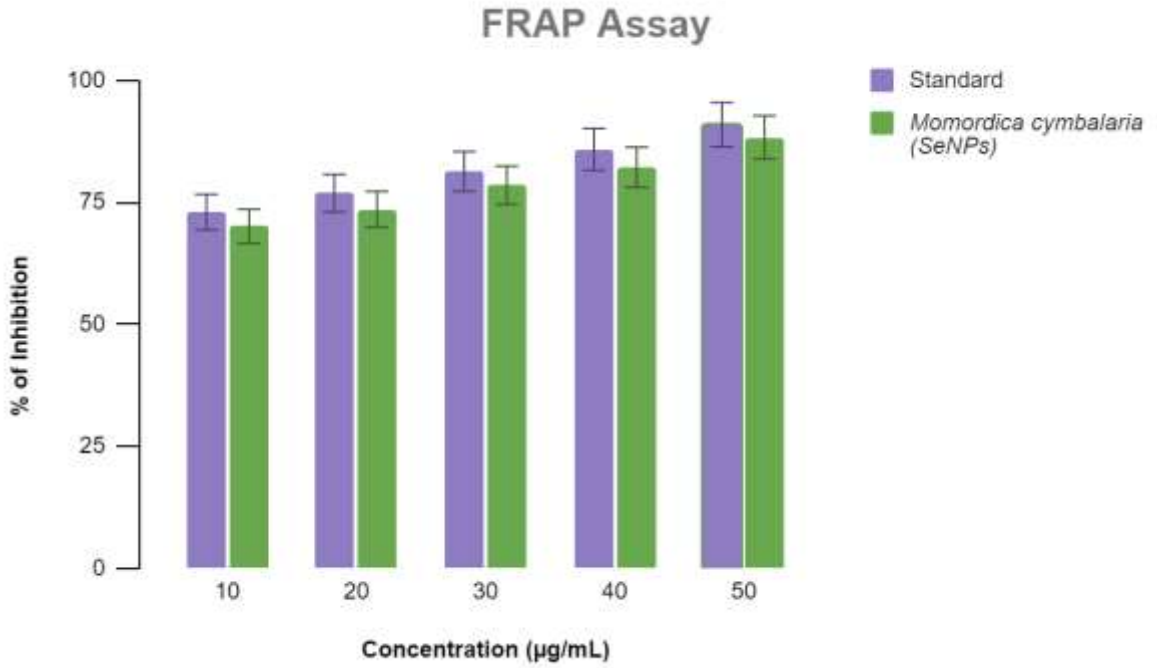

Figure 5: Both the standard and *Momordica cymbalaria* (SeNPs) exhibit strong antioxidant activity as measured by the FRAP assay. While the standard antioxidant shows marginally higher inhibition percentages across most concentrations, *Momordica cymbalaria* (SeNPs) is very close in performance. This suggests that *Momordica cymbalaria* (SeNPs) is an effective natural antioxidant, comparable to the standard used in this assay.

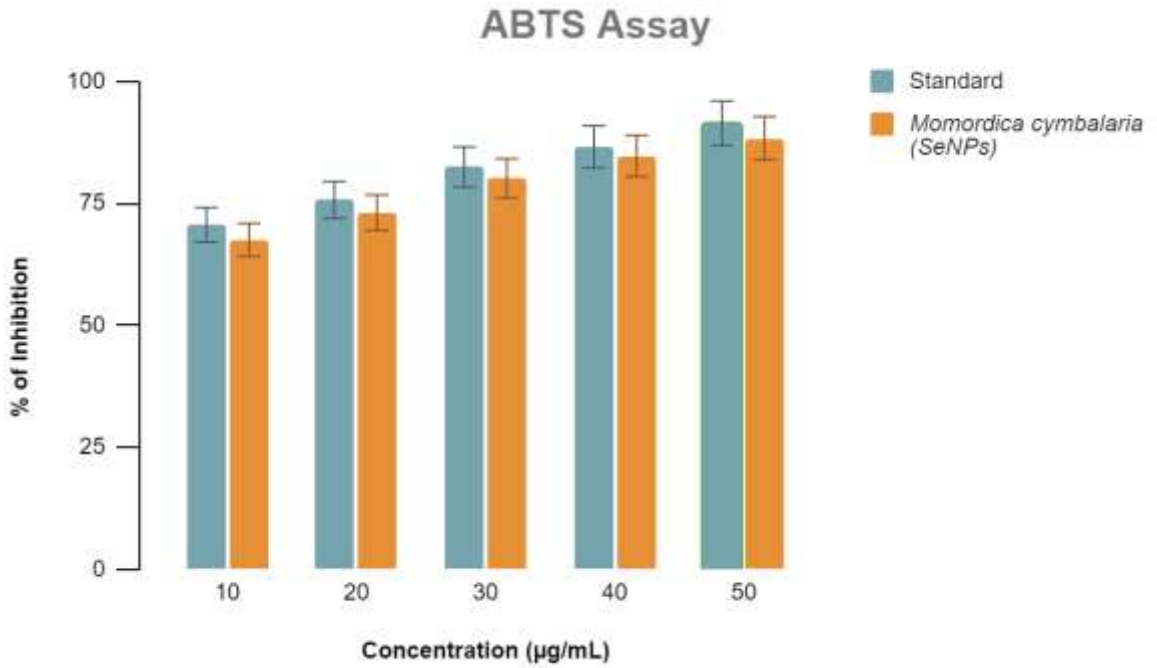

Figure 6: Both the standard and *Momordica cymbalaria* (SeNPs) show an increase in the percentage of inhibition as the concentration increases from 10 to 50 µg/mL.At each concentration level, the standard shows a slightly higher percentage of inhibition compared to *Momordica cymbalaria* (SeNPs). Both the standard and *Momordica cymbalaria* (SeNPs) have relatively small error bars, suggesting consistent and reliable results.The trend indicates that both the standard and *Momordica cymbalaria* (SeNPs) are effective in inhibiting the ABTS radical, with the standard being marginally more effective across all tested concentrations.

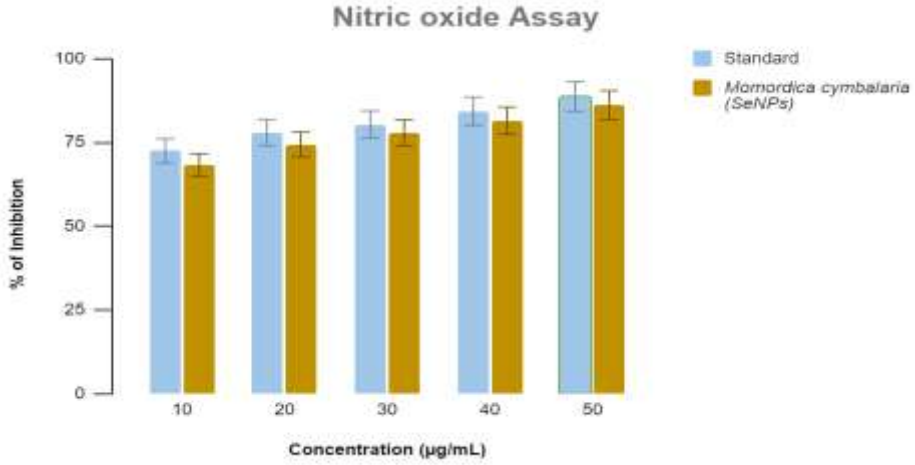

Figure 7: Both the standard and *Momordica cymbalaria* (SeNPs) show an increase in the percentage of inhibition as the concentration increases from 10 to 50 µg/mL.At each concentration level, both the standard and *Momordica cymbalaria* (SeNPs) show very similar percentages of inhibitionThe error bars, representing the variability or standard

error of the measurements, are relatively small for both the standard and *Momordica cymbalaria* (SeNPs), suggesting consistent and reliable results.

CONCLUSION

In conclusion, selenium nanoparticles synthesized using *Momordica cymbalaria* exhibit potent antidiabetic and antioxidant activities, making them promising candidates for diabetes management and oxidative stress-related conditions. The green synthesis approach not only provides a sustainable production method but also enhances the therapeutic potential of selenium by incorporating the bioactive compounds of *Momordica cymbalaria*. The significant inhibition of α-amylase and α-glucosidase, along with robust antioxidant activity, underscores the potential of SeNPs as a multifunctional nanodrug. Further in vivo studies are needed to validate these findings and explore the clinical applications of SeNPs. The integration of nanotechnology with herbal medicine holds great promise for developing innovative treatments for chronic diseases like diabetes.

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