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Antioxidant and Hypoglycemic Potential of Phytogenic Selenium Nanoparticle- and Light Regime-Mediated *In Vitro Caralluma tuberculata* Callus Culture Extract

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Cite This: *ACS Omega* 2024, 9, 20101−20118

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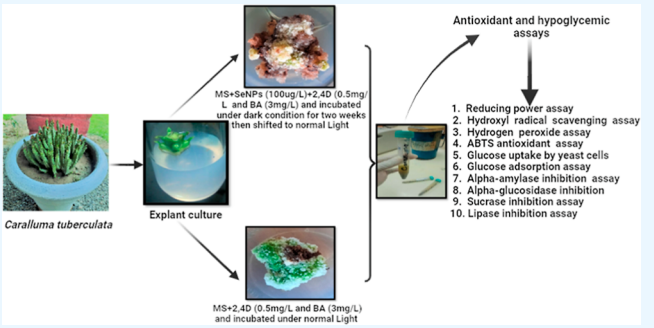
ABSTRACT: *In vitro* plant cultures have emerged as a viable source, holding auspicious reservoirs for medicinal applications. This study aims to delineate the antioxidant and hypoglycemic potential of phytosynthesized selenium nanoparticle (SeNP)- and light stress-mediated *in vitro* callus cultures of *Caralluma tuberculata* extract. The morphophysicochemical characteristics of biogenic SeNPs were assessed through a combination of analytical techniques, including UV−visible spectrophotometry, scanning electron microscopy, energy-dispersive X-rays, Fourier transform infrared spectrometry, and zeta potential spectroscopy. The antioxidative potential of the callus extract 200 and 800 μg/mL concentrations was assessed through various tests and exhibited pronounced scavenging potential in reducing power (26.29%), ABTS + scavenging (42.51%), hydrogen peroxide inhibition (37.26%), hydroxyl radical scavenging (40.23%), and phosphomolybdate (71.66%), respectively. To inspect the hypoglycemic capacity of the callus extract, various assays consistently demonstrated a dosage-dependent relationship, with higher concentrations of the callus extract exerting a potent inhibitory impact on the catalytic sites of the alpha-amylase (78.24%), alpha-glucosidase (71.55%), antisucrase (59.24%), and antilipase (74.26%) enzyme activities, glucose uptake by yeast cells at 5, 10, and 25 mmol/L glucose solution (72.18, 60.58 and 69.33%), and glucose adsorption capacity at 5, 10, and 25 mmol/L glucose solution (74.37, 83.55, and 86.49%), respectively. The findings of this study propose selenium NPs and light-stress-mediated *in vitro* callus cultures of *C. tuberculata* potentially operating as competitive inhibitors. The outcomes of the study were exceptional and hold promising implications for future medicinal applications.



INTRODUCTION

Caralluma tuberculata is a valuable phytotherapeutic herb belonging to the Caralluma genus within the Apocynaceae family. Previously mentioned in the literature as Brussocia tuberculata, *C. tuberculata* is a petite, upright, and succulent perennial herb, reaching a height of 45 cm to 1 m. It is native and critically endangered in Pakistan, while also being present in arid regions across Asia, Africa, and southeastern Europe. This plant is not only utilized as an edible plant but also recognized for antidiabetic properties.[1] Research findings indicate that the succulent stem of *C. tuberculata* contains abundant secondary metabolites, including pregnane, flavonoid, gallic acid, ferulic acid, quercetin, catechin, rutin, reducing sugar, and tannin which exhibit robust antidiabetic potential.[2,3] These secondary metabolites, particularly phenolic compounds, play a pivotal role in counteracting oxidative stress. Beyond their capacity to donate hydrogen or electrons, these compounds serve as stable radical intermediates, effectively functioning as antioxidants.[4] Additionally, phenolic compounds offer protective effects to human consumers when these plants are included in their diet.[5] Generally, the antioxidative prowess of phenolic compounds within plant extracts demonstrates efficacy at low concentrations, bearing significance for the prevention of severe illnesses in humans.[6] An extensive investigation was conducted on the utilization of natural bioactive compounds and revealed promising effects in the treatment of various metabolic disorders.[93] Consequently, exploring the antioxidative potential of extracts derived from different plant species contributes to understanding their potential as sources of novel antioxidant agents.[7] The primary

Received: December 30, 2023
Revised: March 27, 2024
Accepted: March 29, 2024
Published: April 24, 2024

step in quantifying the antioxidant activity in plant extracts involves selecting an appropriate method. A range of methodologies exists for determining this parameter, but the wide variability in experimental conditions among these methods complicates the selection and meaningful comparison of outcomes across different studies. The existing methods for quantifying the antioxidant activity can be categorized according to the process through which the administered compounds operate, chain-breaking reactions, with the most commonly employed single electron transfer methods encompassing the ferric reducing assay, reducing power assay, hydrogen peroxide assay, and hydroxyl radical assay.[4]

Glucose stands as the principal bioenergetic source for nearly all biological entities. Glycolysis, a fundamental cellular progression, plays a pivotal role in the generation of ATP (adenosine triphosphate).[8] The etiology of diabetes stems from chronically elevated blood glucose levels and impaired insulin production. Pancreatic cell damage leads to decreased insulin secretion, preventing glucose absorption into cells, and resulting in persistent blood glucose accumulation. This hyperglycemia compromises various bodily functions and establishes diabetes as a prominent global metabolic disorder. A recent investigation carried out by World of Statistics, a group affiliated with the Georgia State University Department of Mathematics and Statistics, indicates that Pakistan has the highest diabetes prevalence rate globally, standing at 30.8%. This comprehensive study collected data from 38 nations, with Pakistan leading the list, followed by Kuwait at 24.9% and Egypt at 20.9% (Dental News report 2023).[9]

Diabetes mellitus type-2 is often attributed to prolonged high glucose intake or the deterioration of pancreatic cells responsible for insulin production. This condition elevates the risk of cardiopathies, junction inflammation, renal ailments, excessive urine excretion, and myocardial infarctions. The majority of diabetic cases, over 95%, fall under type-2 diabetes, with the remainder attributed to insulin-dependent diabetes mellitus arising from genetic anomalies in insulin-producing cells.[10] In the year 2014, the World Health Organization[11] documented a statistical finding, revealing that 85% of adults aged 18 and above were afflicted with diabetes. By 2019, diabetes was responsible for 1.5 million deaths, representing 48% of deaths attributed to diabetes, with an average age of around 70 years. As oxidative stress resulting from diabetes poses a significant threat to the body, the exploration of naturally nutrient-rich herbal sources as alternatives to synthetic antidiabetic drugs, which carry substantial health risks, becomes imperative. Consequently, the identification of novel and safe antidiabetic herbal sources is of paramount importance. Remarkably, *C. tuberculata* has gained substantial traction for diabetes care in both urban and rural residents. Earlier research has extensively investigated the diverse bioactive properties of Caralluma spp., including antioxidant, antihyperglycemic, antirheumatic, and anti-inflammatory effects. The consumption of *C. tuberculata* as a medicinal nutrient-rich food has endowed it with a revered status, and its pharmacological applications continue to expand.[12] Various extracts derived from Caralluma species have exhibited beneficial antihyperglycemic effects, suggesting a promising avenue for diabetes management. An investigation clarified the antioxidant effects of *C. tuberculata* in diabetic rats induced by streptozotocin. Various doses of *C. tuberculata* were administered, revealing that the use of *C. tuberculata* powder had a positive influence on blood glucose levels. Additionally, plant materials from *C. tuberculata* were observed to alleviate the oxidative stress induced by diabetes in rats.[70] Considering the outcomes, it is evident that the optimal dose of *C. tuberculata* exhibits noteworthy antioxidant and antihyperglycemic effects. Consequently, we suggest that *C. tuberculata* could be regarded as a preventive agent for safeguarding tissues against oxidative stress associated with diabetes complications. It examined the impact of the Caralluma fimbriata extract on insulin resistance and oxidative stress. Their research substantiated that the hydroalcoholic extract of *C. fimbriata* effectively enhanced the plasma glucose and insulin levels. Consequently, it can be inferred that the use of *C. fimbriata* extract may prove beneficial in mitigating insulin resistance and alleviating oxidative stress.[71] The antioxidant and antidiabetic potential of *C. fimbriata* was assessed in a diabetic rat model. The study results indicated that the ethanolic extract demonstrated superior antidiabetic activity in lowering blood glucose levels compared to that of the aqueous extract. The findings regarding the antidiabetic efficacy of the ethanolic extract support the traditional medicinal use of *C. fimbriata* as an efficient herbal remedy for managing diabetes in folk medicine.[72] Another examination also explored the antidiabetic properties of the alcoholic extract of *Caralluma sinaica*. The researchers observed a noteworthy decrease in glucose levels at different concentrations of *C. sinaica*, surpassing the effects of clinically available glibenclamide drugs. These favorable outcomes endorse the potential use of this plant as an effective antidiabetic agent in the treatment of this serious medical condition.[73]

The utilization of a plant *in vitro* culture for the fabrication of bioactive compounds has emerged as a promising avenue for advancing drug development and clinical research within pharmacology and medicine. Extracts derived from plant cell cultures, in the form of fractions and isolated compounds, have garnered significant attention as potential bioactive agents.[13,14] The induction of callus through *in vitro* cultivation has been observed to yield a diverse array of active compounds, including ferulic acid, catechin, rutin, coumarins, quercetin, caffeic acid, and gallic acid. The modulation of secondary metabolite production is affected by external stimuli such as nanoelicitors and light stress.[15] The scientific community has recently employed nanoparticles (NPs) in a variety of research fields because of their nano size, which ranges from 1 to 100 nm.[74] Biosynthesis has emerged as a viable approach for producing nanoparticles beneficial for both human health and the environment. Utilizing biological systems provides a rapid, efficient, and eco-friendly means of nanoparticle synthesis.[75] Moreover, the toxicity and size characteristics of nanoparticles (NPs) can be tailored to suit specific applications. Various microorganisms, including bacteria, actinobacteria, fungi, yeast, microalgae, and viruses, have been extensively investigated for their ability to synthesize nanoparticles.[76] Researchers have explored green synthesis, employing plant extracts as a safe and environmentally friendly strategy to address concerns related to nanotoxicity.[77] To mitigate health and environmental risks, biosynthesis of nanoparticles is preferred, as they are produced and capped by reducing agents. Many studies have employed the biological technique, using different plant parts such as leaves, stems, roots, and fruits, to synthesize metal or metal oxide nanoparticles. Plants contain diverse biomolecules, including proteins, coenzymes, and carbohydrates, which aid in the reduction of metal salts into nanoparticles. Several plant species have been explored for their ability to stabilize and

https://doi.org/10.1021/acsomega.3c10222
ACS Omega 2024, 9, 20101−20118

reduce selenium nanoparticles (SeNPs).[78] Plant extracts, rich in various compounds, such as proteins, lignin, flavonoids, phenolic compounds, vitamins, organic acids, and sterols, have been utilized for the green synthesis and stabilization of SeNPs. *Aloe vera* leaf extract, for instance, has been demonstrated to contain natural reductants and stabilizers, playing a crucial role in reducing Se salt and synthesizing SeNPs.[79] Additionally, *Vitis vinifera* (raisin) garlic clove extract (*Allium sativum*) has been employed as a reducing and stabilizing agent in the green synthesis of SeNPs.[80] Selenium (Se) plays a crucial role in plant growth, enhancing root development, glucose metabolism, and chloroplast ultrastructure. It facilitates chlorophyll biosynthesis, prevents chlorophyll degradation, and boosts antioxidant enzyme activity.[81,82] Recent investigations[83,84] indicate that the application of Se nanoparticles (Se-NPs) encourages higher production of secondary metabolites in plants. In line with this, a study by ref 85 demonstrated that Se-induced phenol and flavonoid accumulation in lettuce resulted in improved antioxidant efficacy and stress adaptation. Notably, the application of Se-NPs significantly elevated polyphenol levels and enhanced the antioxidant system in wheat plants, ultimately mitigating reactive oxygen species (ROS) damage to cellular organelles. SeNPs can act as a bioactive form of selenium and provide beneficial effects on plant cell growth. SeNPs possess strong antioxidant properties, which can help plants mitigate oxidative stress caused by ROS.[86] *In vitro* cultures, especially callus cultures, are often subjected to stress conditions, and the presence of SeNPs can protect the callus cells from damage caused by ROS. They can promote cell division and improve the overall biomass production, which is desirable in *in vitro* callus cultures aiming for regeneration. SeNPs at optimal levels are generally considered biocompatible and nontoxic to living organisms. This characteristic makes them suitable for applications in plant tissue culture systems, where they should not negatively affect the viability and development of callus cells.[2]

For this purpose, we have previously established a protocol for the interactive role of phytomediated selenium NPs and varying light treatments on the growth and biologically active compound production in *in vitro C tuberculata* callus cultures.[2] However, no report in the previous report exists on the investigations of antioxidant and hypoglycemic activities of *in vitro* calli of *C. tuberculata* treated with SeNPs and light regimes. Therefore, the current study explored for the first time the antioxidant capabilities of SeNP- and light stress-mediated *in vitro* cultures of *C. tuberculata* in the presence of diverse radical species. This assessment confirms the potential utility of callus extracts for biological systems. Moreover, this study also scrutinized the blood sugar-lowering capability of the callus extract by suppressing the enzyme's catalytic functions which are responsible for disaccharide and polysaccharide digestion, thereby reducing glucose release within the body. The callus extract from *C. tuberculata* acts as a competitive inhibitor in enzymatic reactions, assuming an antienzyme role. The comprehensive outcomes of various *in vitro* assays underscore that selenium nanoparticle- and light stress-mediated *in vitro* cultures of *C. tuberculata* exhibit considerable antioxidant capabilities and possess remarkable antienzyme potential. The findings from this investigation support the viability of employing SeNP- and light stress-mediated *in vitro* callus extracts from *C. tuberculata* for *in vivo* studies and subsequent pharmacological trials.

MATERIALS AND METHODOLOGY

Green Synthesis of Selenium Nanoparticles (SeNPs). For the green formulation of selenium NPs, about 5 g of weighed garlic cloves were meticulously collected and thoroughly rinsed with both tap and distilled water. Subsequently, the washed cloves were finely chopped and then introduced into distilled water (400 mL) under constant stirring. The resultant garlic extract was meticulously filtered *via* filter paper Whatman no. 1. The procedure for the formulation of SeNPs commenced with the preparation of a 10 mM salt solution of sodium selenite that was purchased from Sigma-Aldrich, constituting 20 mL of volume. To this solution was cautiously added 10 mL of the previously prepared garlic extract dropwise, employing constant magnetic agitation. After the mixing process, the solution was subjected to pH 6, incubation period of 4 to 6 days, maintained at 36 °C, and agitated at 120 rpm using an orbital shaker. These incubation conditions were upheld in a light-free environment. The occurrence of SeNP formation was visually confirmed as the solution transitioned from its original colorless state to a distinctive brick-red hue. The ensuing mixture underwent a centrifugation step, characterized by a 15 min spin at 10,000 rpm and room temperature. The resulting pellet containing the SeNPs was subjected to two rounds of resuspension and centrifugation. The pellet was then delicately treated with 2 mL of methanol, and the purified SeNP-enriched pellet was meticulously dried and stored, awaiting its application in experimental procedures.[16]

Characterization of SeNPs. The initial verification of the existence of plant-based SeNPs was substantiated by using UV−visible spectrometry (UV-2300 TECHCOMP). The absorbance spectrum was then recorded across the wavelength range of 200−700 nm using a spectrophotometer. To discern the chemical moiety present in the SeNPs, Fourier transform infrared (FT-IR Model NICOLET 6700, Thermo, Waltham, MA, USA) spectrometry was employed. For structural analysis of the SeNPs, a scanning electron microscope (JSM5910 JEOL, Tokyo, Japan) was employed. Elemental composition analysis of the phytosynthesized SeNPs was determined by using an energy-dispersive X-ray model (EDX SIGMA).[17] The patterns obtained from the X-ray diffraction (XRD) study of recently synthesized SeNPs were documented by using a Siefert X-ray diffractometer. To analyze the surface charge and particle size of the phytomediated SeNPs, the dynamic light scattering technique was performed by utilizing a Malvern Zetasizer nanosizer. The size analyzed ranged from 0.1 to 10,000 nm.

Collaborative Impact of Selenium NPs along Light Regimes on Callus Growth Kinetics and Proliferation. All callus cultures, which were initiated and had reached the fourth week of growth, were placed on culture media enriched with plant growth regulators (PGRs) (2,4-D: 0.5 mg/L and BA: 2 mg/L), as well as SeNPs at a 100 μg/L concentration. These cultures were then incubated under conditions of complete darkness for 2 weeks. After that, cultures were transferred to a standard light environment to facilitate callus proliferation.[2] The growth chamber conditions were carefully set to maintain a light cycle of 16 h, followed by an 8 h dark photoperiod. The temperature within the chamber was held at 25 ± 1 °C and 70% relative humidity. The proliferated calli were collected after 56 days and further used for antioxidant and antidiabetic activities.

https://doi.org/10.1021/acsomega.3c10222
ACS Omega 2024, 9, 20101−20118

Preparation of Callus Culture Extract. Approximately 300 mg calli samples were employed for further phytochemical constituent analysis. The powdered sample was crushed and then blended with 50% methanol. This mixture was kept on a shaker operating for 1 day at 24 rpm under 25−30 °C. Afterward, 30 min of sonication was followed by 30 min of vortexing and an additional 15 min of sonication.[18] The resulting sample mixtures were centrifuged for 10 min at 6500 rpm. After centrifugation, the supernatant was collected, syringe-filtered, and then transferred into Eppendorf tubes. Samples were stored at 4 °C for phytochemical analysis.

Antioxidant Potential Evaluation of SeNP-Mediated Callus Culture of *C. tuberuclata*. *ABTS Antioxidant Assay.* The ABTS antioxidant assay was conducted to evaluate the antioxidant activity of *in vitro-derived* calli extract, followed by the protocol of ref 19. The preparation of the mixture under reaction involved combining the callus extract (1 mL) of various concentrations (50, 100, 200, 400, and 800 μg/mL) with 1 mL of ABTS + solution containing K2S2O8 as an oxidizing agent. 1 mL of methanol was employed as a negative control, whereas ascorbic acid functioned as the positive control. Following a 6 min incubation period, the absorbance of the reaction mixture was assessed at 734 nm to calculate the percentage of inhibition.

Assay for Hydrogen Peroxide Antioxidant Activity. The hydrogen peroxide activity assay was performed by following the protocol documented by Yakoob *et al.*[19] Various levels of callus extract (50, 100, 200, 400, and 800 μg/mL) were equipped and each concentration was mixed with 300 μL of phosphate buffer (50 mM, pH 7.4), and 600 μL of hydrogen peroxide was strongly agitated for 10 min; then, the absorbance was analyzed by using a spectrophotometer at 230 nm. As a standard, $C_6H_8O_6$ was utilized, while the blank solution consisted of a phosphate buffer.

Hydroxyl Radical Antioxidant Assay. The antioxidant potential of calli extract against the hydroxyl radical was conducted following the established procedure outlined in ref 10. The resulting reaction mixture has 750 μL of various concentrations of callus extract (50, 100, 200, 400, and 800 μg/mL), sodium phosphate buffer at 45, 15 μL of deoxyribose, 150 μL of a solution containing both FeSO4 (10 mM) and 10 mM of EDTA, 15 μL of hydrogen peroxide (H2O2:10 mM), and of deionized water (525 μL). Furthermore, the solution was incubated for a duration of 4 h. To stop the reaction, 75 μL of trichloroacetic acid (2.8%) and 75 μL of TBA (1% in a 50 mM NaOH solution) were introduced. The sample was then subjected to a 10 min heating period in a boiling water bath.

Reducing Power Assay. Reducing power activity was performed by using the methodology established by ref 22. In this assay, 100 μL of various levels of calli extract (50, 100, 200, 400, and 800 μg/mL), 1% potassium ferricyanide solution comprising 2.5 mL, was combined with 250 μL of a 0.2 mol/L sodium phosphate buffer solution. This resultant mixture was then incubated at a 50 °C temperature for a period of 30 min. To terminate the reaction, 2.5 mL of 10% trichloroacetic acid was added; after that, centrifugation was done at 3000 rpm for about 10 min. The supernatant was collected, and 0.5 mL of 0.1% ferric chloride solution and 2.5 mL of deionized water were added. Subsequently, the absorbance was measured by a spectrophotometer (specifically, Model U-2900) at 700 nm.

Investigation of Total Antioxidant Activity by the Phosphomolybdate Method. The assessment of the total antioxidant potential of the callus extract was conducted following the procedure outlined by ref 20. In this procedure, 0.5 mL of the sample solution was combined with 4.5 mL of reagent solution, which includes 4 mM ammonium molybdate, 0.6 M sulfuric acid, and 28 mM sodium phosphate. Following that, the sample mixture containing tubes was placed in a water bath at 95 °C for a period of 90 min, and the absorbance was checked with the help of a spectrophotometer at 695 nm wavelength. The total antioxidant activity of the sample was quantified based on the 695 nm absorbance.

Hypoglycemic Potential. *Glucose Uptake by Yeast Cells.* For glucose uptake assessment, 5 μg yeast was mixed in 1 mL of deionized water and vigorously vortexed for about 10−15 min. Afterward, the solution was centrifuged at 21,000 rpm for 5 min. Subsequently, a 10% (v/v) yeast suspension was prepared in deionized water. In the experimental setup, 100 μL of various concentrations of callus extract (50, 100, 200, 400, and 800 μg/mL) was taken and properly mixed with various concentrations (5, 10, and 25 mmol/L) of glucose solution (1 mL) and incubated at 37 °C for 10 min. A volume of 100 μL of yeast suspension was added for reaction initiation, followed by vortexing, and the entire solution was incubated for 1 h at 37 °C. The reaction mixture was then subjected to centrifugation at 3800 rpm (rpm) for 5 min, and the content of glucose was analyzed at 540 nm wavelength.[19] The percentage of glucose uptake by yeast cells was determined through the following formula

$$\text{hypoglycemic } \% = (\text{Abs control} - \text{Abs sample}/\text{Abs control}) \times 100$$

Glucose Adsorption Capacity. To determine the capacity of glucose adsorption, we followed the formula provided by ref 21. Various levels of callus extract (50, 100, 200, 400, and 800 μg/mL) were individually combined with 1 mL of glucose solution at concentrations of 5, 10, and 25 mmol/L. The resulting mixtures were gently mixed and placed in an incubator at 37 °C for 6 h. After incubation, the samples were subjected to centrifugation at 4000 rpm for 20 min. The supernatant was collected, and the glucose content was determined by using a glucose oxidase-peroxidase assay, and the absorbance was measured at 520 nm. In this experiment, acarbose served as the positive control.

Alpha-Amylase Inhibition Assay. Alpha-amylase inhibition assays were conducted in accordance with the procedure described by ref 22. Different concentrations (50, 100, 200, 400, and 800 μg/mL) of DMSO-mediated callus extract were used. The reaction sample was prepared by adding 3 mg of alpha-amylase in a reaction mixture consisting of 20 molar (mM) phosphate buffer at pH 6.7, along with 6.5 molar (mM) sodium chloride. Subsequently, 250 μL of this prepared sample solution was combined with the callus extract, and this resulting mixture was subjected to incubation at 37 °C for 30 min. After the initial incubation, 250 μL of a 0.5% starch solution in a 20 mM phosphate buffer at pH 6.9 was added, followed by further incubation at 37 °C. To terminate the reaction, 2 mL of 3,5-dinitrosalicylic acid (96 mM) color reagent was introduced. After that, a microplate was placed in a water bath at boiling temperature for 5 min and allowed to cool at room temperature. The sample absorbance was measured through a UV−visible spectrophotometer at 540 nm wavelength, and Glucobay was used as the reference medication.

https://doi.org/10.1021/acsomega.3c10222
ACS Omega 2024, 9, 20101−20118

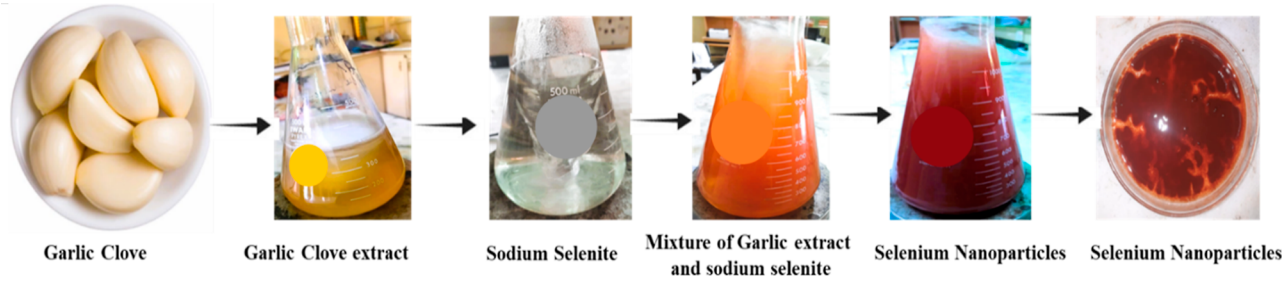


Figure 1. Visual representation of SeNPs biosynthesized *via* garlic clove extract. Sodium selenite salts were used as precursors in the synthesis. The bioactive compounds present in the garlic extract serve as reducing agents, donating electrons to the selenium precursor. This reduction step converts the soluble selenium ions into selenium atoms, which then aggregate to form nanoparticles.[2] This figure has been sourced from our previously published work, corresponding by Ali *et al.*,[2] 2023. Ali A, Mashwani Z-u-R, Raja NI, Mohammad S, Luna-Arias JP, Ahmad A and Kaushik P (2023). Phytomediated SeNPs and light regimes elicited *in vitro* callus cultures for biomass accumulation and secondary metabolite production in Caralluma tuberculata. Front. Plant Sci. 10.3389/fphar.2024.1325359 (https://www.frontiersin.org/legal/copyright-statement).

Alpha-Glucosidase Inhibition Assay. The alpha-glucosidase inhibition activity was conducted by following the protocol described by ref 23. Around 5 g enzyme powder was mixed in 1 mL of maleate buffer to form a dilute suspension. For the initiation of enzymatic reaction, 200 μL of the substrate was added specifically to *p*-nitrophenyl-α-D-glucopyranoside, which was present at a concentration of 2 mmol. Subsequently, this mixture was incubated at 37 °C for 30 min. Subsequently, to halt the reaction, the test tube containing the sample solution was placed in a water bath at boiling temperature for 5 min. Moreover, in the resulting reaction mixture, 0.1 M disodium hydrogen phosphate (100 μL) was added, and the absorbance was checked at 400 nm of the liberated *p*-nitrophenol by using a spectrophotometer. In this assay, the Glucobay drug was used as the standard.

Sucrase Inhibition Assay. In the assessment of sucrase inhibition potential, 10 μL of various concentrations of callus (50, 100, 200, 400, and 800 μg/mL) and crude enzyme solution were combined with 100 μL of maleate buffer. This solution mixture was incubated for 10 min at 37 °C. The enzymatic reaction commenced with the introduction of 100 μL of the substrate, which consisted of 60 mmol of sucrose. Subsequently, again, the mixture was kept for incubation at 37 °C for 0.5 h in a water bath. To halt the reaction, the sample mixture containing glass tubes was immersed in a boiling water bath for 10 min. The quantification of the glucose liberated during this process was carried out by using a glucometer. A control sample without callus extract was included, with Glucobay serving as the reference medication.[10]

Pancreatic Lipase Inhibition Assay. The procedure outlined by Kim *et al.*[24] served as the basis for the assessment of this activity. About 80 μL of each callus extract, spanning a range of various concentrations (3.125, 6.25, 12.5, 25, 50, 100, and 200 μg/mL), was combined with phosphate buffer (90 μL) and 20 μL of porcine pancreatic lipase (4 mg/mL). This resulting sample was then subjected to incubation for 37 min at 37 °C temperature. The reaction was initiated by introducing 10 μL of *p*-nitrophenyl butyrate substrate (10 mM *p*-NPB) dissolved in dimethylformamide. The sample was incubated at 37 °C for 0.5 h, and then the inhibitory potential of lipase was estimated by assessing the hydrolysis of *p*-NPB to *p*-nitrophenol. An ELISA microplate reader (BK-EL10 C) was used to measure at a 405 nm wavelength, and Orlistat was employed as the standard.

Statistical Analysis. Each experiment was carried out using a set of three replicates. The obtained results were subjected to statistical analysis using SPSS 20, employing ANOVA (analysis of variance). To assess significant variations in means, Duncan's multiple range test was used to differentiate between them.

RESULTS AND DISCUSSION

Biosynthesis and Characterization of SeNPs. In our current research, we have confirmed that the use of garlic clove extract serves as an effective reducing, capping, and stabilizing agent for the synthesis of SeNPs. We employed various characterization techniques to validate the formulation of the phytosynthesized SeNPs. The initial confirmation of nanoparticle synthesis was evident through a distinct change in color to brick red upon the addition of garlic extract (Figure 1). According to this method, after the addition of garlic clove extract, SeNPs were fabricated, reducing selenite to selenium, as shown by the color change. At the beginning of the reaction, the solution was colorless and then became brick red after incubation for 5 days in the orbital shaker, and the intensity of red color increased after 2 additional days of incubation, and then no further color changes were experienced. The red color of the mixture was due to the excitation of the surface plasmon vibration of the SeNPs, indicating that elemental SeNPs were formed.[16] Additionally, a UV−visible absorption spectrum in the range of 200−500 nm further confirmed the synthesis of SeNPs, with characteristic peaks observed at 262 nm, indicative of surface plasma resonance in the biosynthesized selenium NPs. This finding aligns with previous scientific reports that have observed similar peaks in garlic clove extract-mediated SeNPs.[16] To further investigate the role of garlic extract in reducing, capping, and stabilizing SeNPs, FT-IR spectroscopy was conducted. Our results revealed peaks at 3434 cm^{-1}, confirming the presence of OH and NH groups, crucial in SeNP synthesis. Peaks at 2950, 1510, and 10220 cm^{-1} manifested the stretching of carbon and hydrogen bonds, stretching of the C═C alkene bonds, and stretching of the C−O group, suggesting the involvement of these functional groups in the reduction of sodium selenite by the clove extract. Additional absorption peaks at 1442 and 700 cm^{-1} may be attributed to the presence of aldehyde, carboxylic, oxygen, nitrogen, and amine groups. These findings align with earlier studies highlighting the importance of functional groups (C═C, O−H, N−H, and C═O) in the biosynthesis of SeNPs.[25,26] The size range of SeNPs was verified through SEM, which indicated a size range of 40−100 nm with a spherical shape. These observations are consistent with prior research on

https://doi.org/10.1021/acsomega.3c10222
ACS Omega 2024, 9, 20101−20118

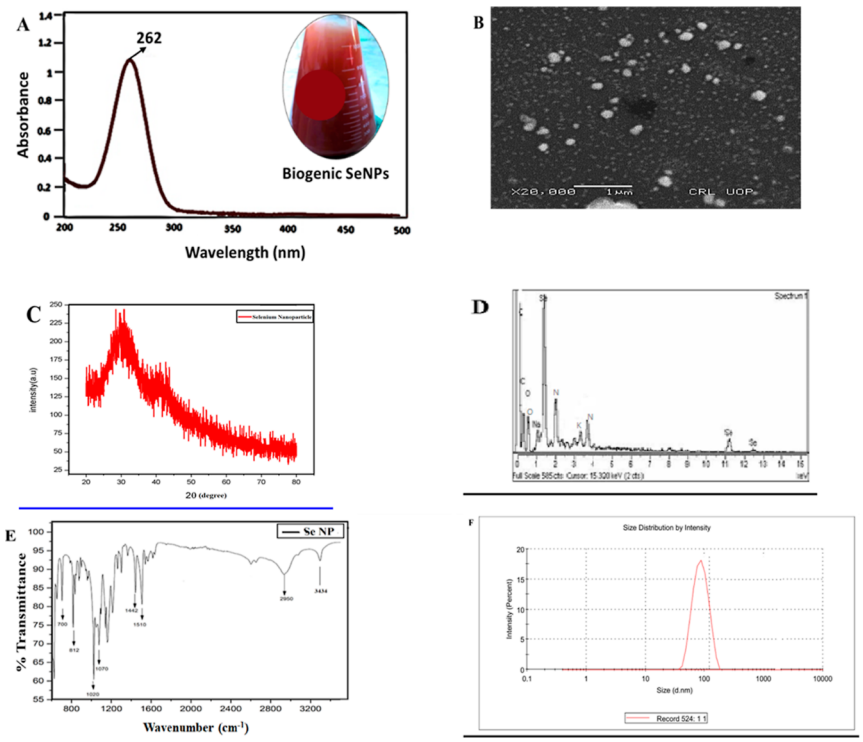


Figure 2. Analysis of SeNPs synthesized *via* plant-mediated process. (A) Illustration depicting UV−visible spectrophotometry evaluation of green synthesis of SeNP peak at 262 nm. (B) *SEM image illustrating SeNPs synthesized through a green process* with 40−80 nm size. (C) XRD image of green synthesized SeNPs. (D) Electron-dispersive X-ray (EDX) analysis image of SeNPs synthesized *via* a green approach, verifying the presence of selenium. (E) Peaks at the FT-IR image of phytosynthesized SeNPs. (F) SeNP size distribution by intensity.[2] This figure has been sourced from our previously published work, corresponding by Ali *et al.*,[2] 2023. Ali A, Mashwani Z-u-R, Raja NI, Mohammad S, Luna-Arias JP, Ahmad A and Kaushik P (2023). Phytomediated SeNPs and light regimes elicited *in vitro* callus cultures for biomass accumulation and secondary metabolite production in Caralluma tuberculata. Front. Plant Sci. 10.3389/fphar.2024.1325359 (https://www.frontiersin.org/legal/copyright-statement).

Brassica oleracea-mediated SeNPs, which exhibited similar spherical, rectangular, and irregular shapes, with sizes ranging from 10 to 25 nm.[27] Energy-dispersive X-ray analysis (EDX) further substantiated the presence of selenium in the SeNPs, as indicated by atomic signals of selenium in conjunction with those of carbon, oxygen, and sodium. XRD analysis unveiled the amorphous character of SeNPs. The XRD spectrum exhibited prominent peaks, which were ascribed to metallic SeNPs adopting a trigonal phase. Additional peaks in the pattern were elucidated as indicative of the presence of selenium oxide, a result of partial oxidation due to the presence of oxygen in the medium.[28,29] The assessment of the zeta potential played a crucial role in evaluating nanoparticle stability. Zeta potential, representing the potential at the shear plane, provides valuable insights into the stability of colloidal dispersion systems. In our case, the particle size of the SeNPs was determined to be 60.43 ± 0.75 nm. The zeta potential analysis yielded a significant finding, indicating a robust level of stability. The graph displayed the particle size distribution, with the abscissa representing the size in nanometers and the ordinate indicating the corresponding intensity in percentage terms, as shown in Figure 2.

Callus Proliferation. *C. tuberculata*, a plant recognized for its medicinal properties, edibility, and native status, carries substantial significance due to its abundance of bioactive metabolites, presenting a wide array of therapeutic possibilities. Nevertheless, sustainable cultivation of this plant confronts challenges like overexploitation, alterations in natural conditions, a sluggish growth rate, and insufficient biosynthesis of bioactive compounds in wild populations. Therefore, in our previous work, we meticulously examined the photocatalytic function of SeNPs in conjunction with optimized PGRs across various light conditions. We focused on their impact on the growth patterns and the production of bioactive antidiabetic compounds within the callus culture of *C. tuberculata*.[2] From this research, we observed that the synergistic application of phytosynthesized SeNPs alongside controlled light exposure resulted in significant disparities in both callus growth and the production of secondary metabolites (Figure 3). Among all the treatments we applied, the most favorable outcomes in terms of callus proliferation and secondary metabolite production were achieved with the judicious combination and appropriate concentrations of SeNPs[30] under specific culture conditions, involving a 2 weeks' incubation in a dark environment, followed by exposure to normal light. The calluses that developed under the same approach were utilized to check their antioxidant activities and antidiabetic potential through *in vitro* assay.

Biological Activities (Antioxidant and Antihypoglycemic Potential) of SeNP-Mediated *In Vitro* Callus Culture Extract of *C. tuberculata*. *C. tuberculata* plant was specifically used in the treatment of diabetes mellitus ailments. Plant phenolic compounds are one of the most diverse and widespread groups of natural antioxidants and minimize the adverse effect of free radicals in organisms, cosmetics, and food products. The current study was focused on the biological properties of the *in vitro* callus material to confirm the ethnopharmacological applications. Therefore, we have used the SeNP- and light regime-mediated callus culture extract for antioxidant and antihypoglycemic activities compared with

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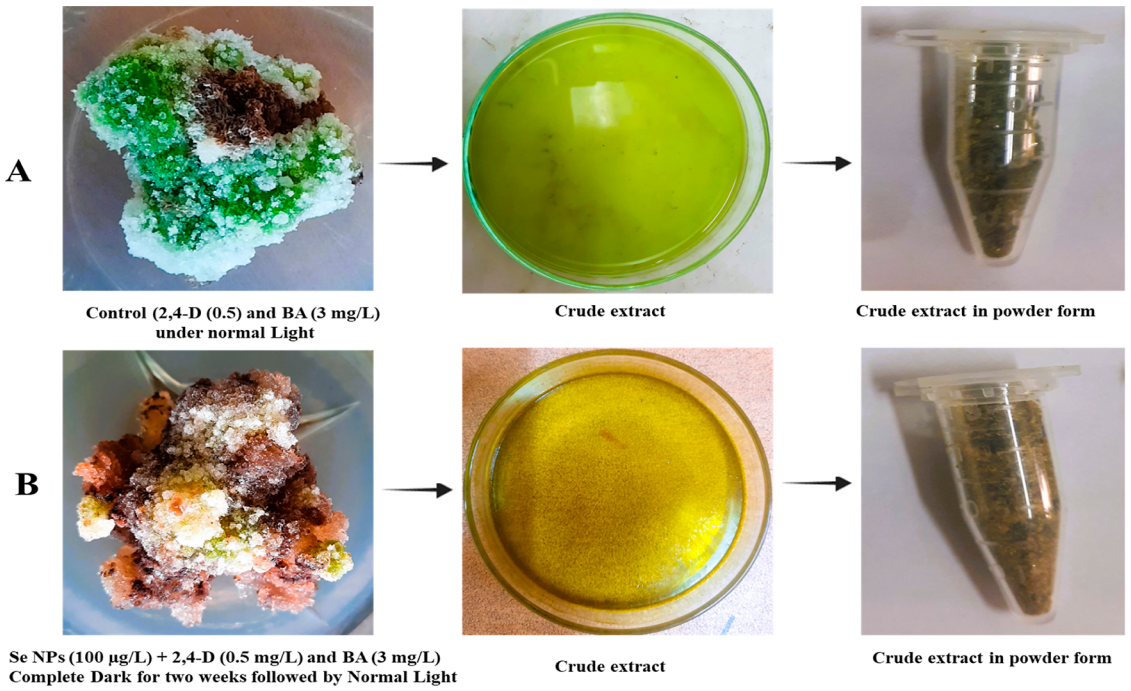

Figure 3. *Caralluma tuberculata* Callus cultures were utilized for antioxidant and hypoglycemic activities. Callus grown on MS media supplemented with 2,4-D and BA (0.5 + 3 mg/L) under normal light (A). Callus grown on MS media supplemented with Se NPs (100 μg/L) + 2,4-D and BA (0.5 + 3 mg/L) subjected to complete darkness for 2 weeks, after which normal light conditions were restored (B). Ali A, Mashwani ZU, Raja NI, Mohammad S, Luna-Arias JP.[2] Phytomediated SeNPs and light regimes elicited *in vitro* callus cultures for biomass accumulation and secondary metabolite production in Caralluma tuberculata. Front in Plant Sci. 2023 Sep 22; 14:1253193.[2] This figure has been sourced from our previously published work, corresponding to Ali *et al.*,[2] 2023. Ali A, Mashwani Z-u-R, Raja NI, Mohammad S, Luna-Arias JP, Ahmad A and Kaushik P (2023). Phytomediated SeNPs and light regimes elicited *in vitro* callus cultures for biomass accumulation and secondary metabolite production in Caralluma tuberculata. Front. Plant Sci. 10.3389/fphar.2024.1325359 (https://www.frontiersin.org/legal/copyright-statement).

untreated calli extract. Overall, SeNPs and light-treated callus extract showed strong antioxidant and antidiabetic potential due to the abundant amount of polyphenols and antidiabetic compounds (Figure 3).

Antioxidant Activities. *Reducing Power.* The presence of antioxidants is strongly correlated with reducing properties, contributing hydrogen atoms, disrupting free-radical chains, and exerting their antioxidative effects.[30] Consequently, it is plausible that *C. tuberculata* boasts an elevated antioxidant content, which interferes with radical chain reactions by engaging free radicals to establish stability. The robust antioxidant profile of *C. tuberculata* is elucidated by its performance in the reduction power assay. Thus, the present study delved into the effects of SeNP- and light-mediated conditions on *C. tuberculata* calli. The treated calli extract exhibited a reduced power activity of 26.29%. By comparison, the reducing power assay yielded values of 17.39% for untreated calli extract and 40.47% for the ascorbic acid scavenging potential (Figure 4a). The outcomes from the treatment involving SeNP- and light-facilitated callus extract were slightly below those of ascorbic acid, yet they displayed efficacy in quenching radicals as the extract concentrations increased. It is worth noting that the reducing power analysis presented concentration dependence and sensitivity to culture conditions.[31] In our study, calli exposed to the nanoparticles and light treatment exhibited a higher reducing power compared to untreated callus extracts. This contrast arises from the enhanced antioxidant systems developed in response to stress induced by ROS in the culture,.[32] The *in vitro*-cultivated *C. tuberculata* extract simulates the functions of diverse antioxidant enzymes such as catalase, superoxide dismutase, and peroxidase. These enzymes initiate various biological outcomes, including counteracting intracellular ROS toxicity. Through the transfer of hydrogen atoms, these antioxidants thwart lipid peroxidation and disrupt the propagation of free-radical chains.[33] This efficient reducing capability swiftly extinguishes any radical species present in the environment surrounding the *C. tuberculata* methanolic extract. Similar observations emerged from another study involving callus extracts from *C. tuberculata*, where reducing power activity was exhibited.[34] Likewise, the reducing power of callus extracts from five distinct *in vitro*-cultivated Ocimum species (*Ocimum sanctum*, *Ocimum kilimandscharicum*, *Ocimum gratissimum*, *Ocimum basilicum*, and *Ocimum americanum*) was also explored. The study unveiled notable activity primarily attributed to the heightened accumulation of polyphenols within the callus extracts.[35]

ABTS Antioxidant Assay. The radical scavenging capability of the *in vitro*-cultivated callus from *C. tuberculata* underscores its effectiveness in ABTS + sanitation efforts. The ABTS + appraisal gauges the capacity of antioxidants to neutralize oxidizing agents generated by ABTS+. The formation of ABTS + radicals occurs through a potent interaction between ABTS + salt and potassium persulfate. As the potential of antioxidants to scavenge increases, there is a reduction in absorbance. The initial blue/green color of the ABTS + solution transitions to pale yellow and eventually becomes colorless. In the context of this study, the percentage scavenging efficacy of ascorbic acid registered at 45.24%. In comparison, the percentage for SeNP- and light-treated callus extract reached 42.51%, while untreated

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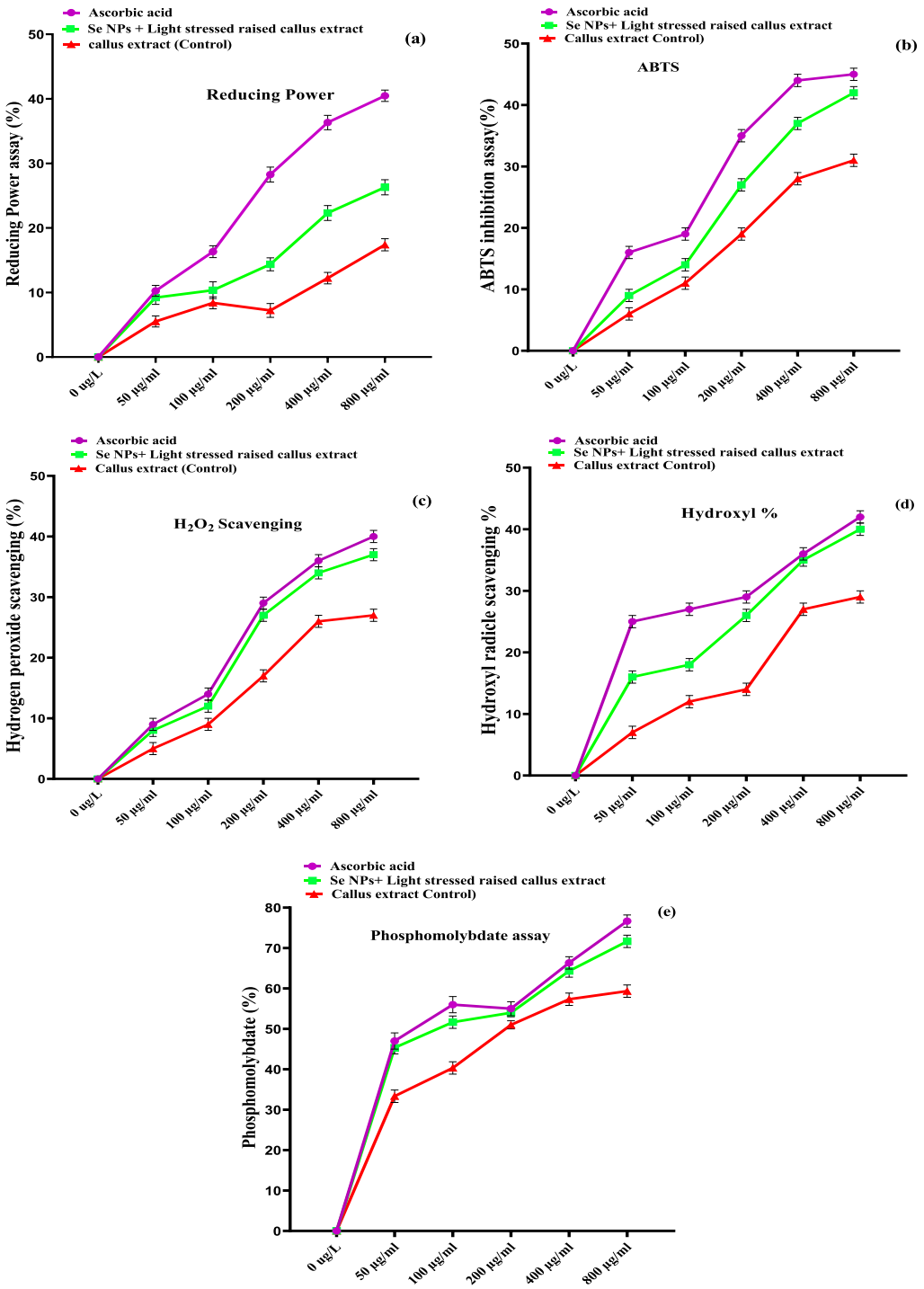

Figure 4. Antioxidant activities (a) demonstrate the reducing power of *Caralluma tuberculata* callus extract. (b) Illustration of the scavenging potential of *Caralluma tuberculata* callus extract against ABTS + radicals. (c) Exhibition of the quenching power of *Caralluma tuberculata* callus extract. (d) Display of the potential of *Caralluma tuberculata* callus extract to cleave hydroxyl radicals. (e) Depiction of the results of the phosphomolybdate assay for *Caralluma tuberculata* callus extract. For all antioxidant activities, the level of significance was set at $p < 0.05$. Significance was determined by a *p*-value less than 0.05, and this determination was confirmed through post hoc testing.

in vitro-cultivated callus extract exhibited a scavenging potential of 31.37%. This noteworthy scavenging potential holds promising implications for commercial applications (Figure 4b). Corroborating prior findings, Noreen *et al.*[36] demonstrated that extracts derived from *C. tuberculata* shoots exhibited the highest percentage reduction in ABTS + radical species. This phenomenon implies that callus extracts selectively counteract ROS within normal cells, safeguarding them from oxidative stress.[37] The outcomes of this investigation unveil that *C. tuberculata* callus has a significant medicinal value and beneficial phytochemicals boasting heightened antioxidant potential. This capacity to avert radical chain reactions stands out. Similarly, the extract from *Caralluma flava* demonstrated noteworthy radical-scavenging

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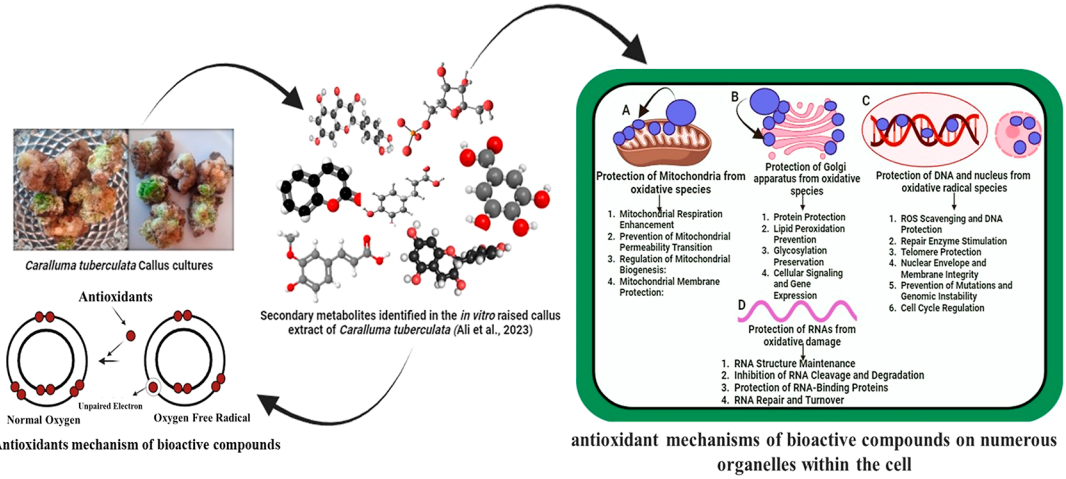

Figure 5. Schematic diagram illustrates the antioxidant mechanisms of the bioactive constituents derived from *C. tuberculata*, focusing on their impact across various cellular organelles. These bioactive metabolites effectively traverse the plasma membrane, subsequently interacting with and binding to diverse organelles. This orchestrated interaction plays a pivotal role in neutralizing free-radical species. When a particular organelle generates ROS, the resultant byproducts are conveyed to other organelles. As these byproducts travel and reach different organelles, the bioactive compounds seamlessly associate with ROS molecules, preemptively mitigating any potential disruptions. This intricate interplay serves as a buffer against the emergence of oxidative disturbances. Consequently, these secondary metabolites emerge as crucial agents in diminishing oxidative stress, reinforcing cellular equilibrium.

attributes in ABTS assays, positioning *C. flava* as a promising reservoir of antioxidants, both enzymatic and nonenzymatic.[38] A study conducted by ref 90 examined the antioxidant and inhibitory capabilities of the methanolic extract from *Secamone afzelii* leaves. The findings revealed the extract's remarkable total antioxidant activity, along with significant inhibition of DPPH, and ABTs. These inhibitory effects are attributed to the abundant presence of polyphenols, suggesting potential applications in the food and pharmaceutical sectors.

Hydrogen Peroxide Scavenging Assay. Hydrogen peroxide instigates the generation of hydroxyl radicals, which in turn induce lipid oxidation within uncovered cells, leading to DNA impairment and cell demise. Swift contact with hydrogen peroxide poses a significant risk to one's well-being, as it triggers skin irritation where they intersect. Mitochondria, pivotal organelles governed by a specific enzyme influencing cellular development and mortality, play a crucial role. Fortunately, the enzyme responsible for breaking down hydrogen peroxide into harmless components is naturally present within cells.[39] In essence, the synthesis of hydrogen peroxide within bodily cells serves as a safeguard against more perilous agents such as superoxide radicals. However, when the body faces conditions of illness, these enzyme functions wane. Consequently, hydrogen peroxide levels surge beyond safe thresholds, diminishing scavenging capacity and culminating in lipid peroxidation. Recent research has illuminated the efficacy of SeNP- and light-mediated *in vitro* callus extracts from *C. tuberculata* in effectively curtailing hydrogen peroxide levels within cells. The potency of callus extract in scavenging hydrogen peroxide was contingent on the concentration (ranging from 50 to 800 μg/mL), as elucidated in Figure 4c. Remarkably, the scavenging potential of nanoparticle-treated and light-regulated callus extracts registered at 37.26%, signifying a slightly lower activity compared to the antioxidant prowess of ascorbic acid at 40.18%, and untreated callus extract at 27.53%. In comparison to earlier findings, the methanolic extract of *Randia echinocarpa*, *Spondias pinnata*, *Gymnema sylvestre*, and *R. arvensis* exhibited superior H2O2 scavenging abilities when evaluated for biological activities[40] The methanolic extract derived from the callus culture of *C. tuberculata* impressively quells hydrogen peroxide, potentially due to its phenolic groups, capable of donating electrons to neutralize hydrogen peroxide into harmless water (H2O) (Figure 5).

Hydroxyl Radical (OH•)-Scavenging Activity. Hydroxyl radicals are highly reactive chemical species with a fleeting existence.[41] Radicals lacking oxygen possess unpaired electrons, rendering them inherently unstable. Among ROS in biological systems, hydroxyl radicals cause the most damage. The decomposition of H2O2 primarily generates hydroxyl radicals. A significant concern is the absence of any enzymatic defense against hydroxyl radicals in living systems, unlike superoxide dismutase which can quench superoxide radicals.[42] Living organisms continuously produce primary ROS entities, namely O2− radicals and OH radicals, by converting oxygen into water through a reduction process.[39] Moreover, hydroxyl radicals possess a particularly detrimental trait in their aptitude to disrupt protein disulfides and denature fibrinogen. Consequently, proteins undergo abnormal unfolding and refolding, leading to structural deformities.[43] *In vitro*, cultivated callus extracts, treated with SeNPs and specific light conditions, exhibited a 40.23% degradation potential against hydroxyl radicals. Similarly, at the highest concentration of 800 μg/mL, ascorbic acid displayed a 42.47% degradation potential. Callus extracts grown without nanoparticle treatment exhibited a degradation potential. These radical species interact with antioxidants, which can effectively neutralize them before initiating harm.[44] *C. tuberculata* produces various secondary metabolites, many of which possess antioxidant properties. Thus, the current study aimed to verify the callus extract's ability to scavenge free radicals (Figure 4d). Similarly, extracts from *Schima wallichii* demonstrated concentration-dependent inhibition of hydroxyl free-radical generation. Various plant extracts have also exhibited the capability to scavenge hydroxyl free radicals in a concentration-dependent manner.[45,48]

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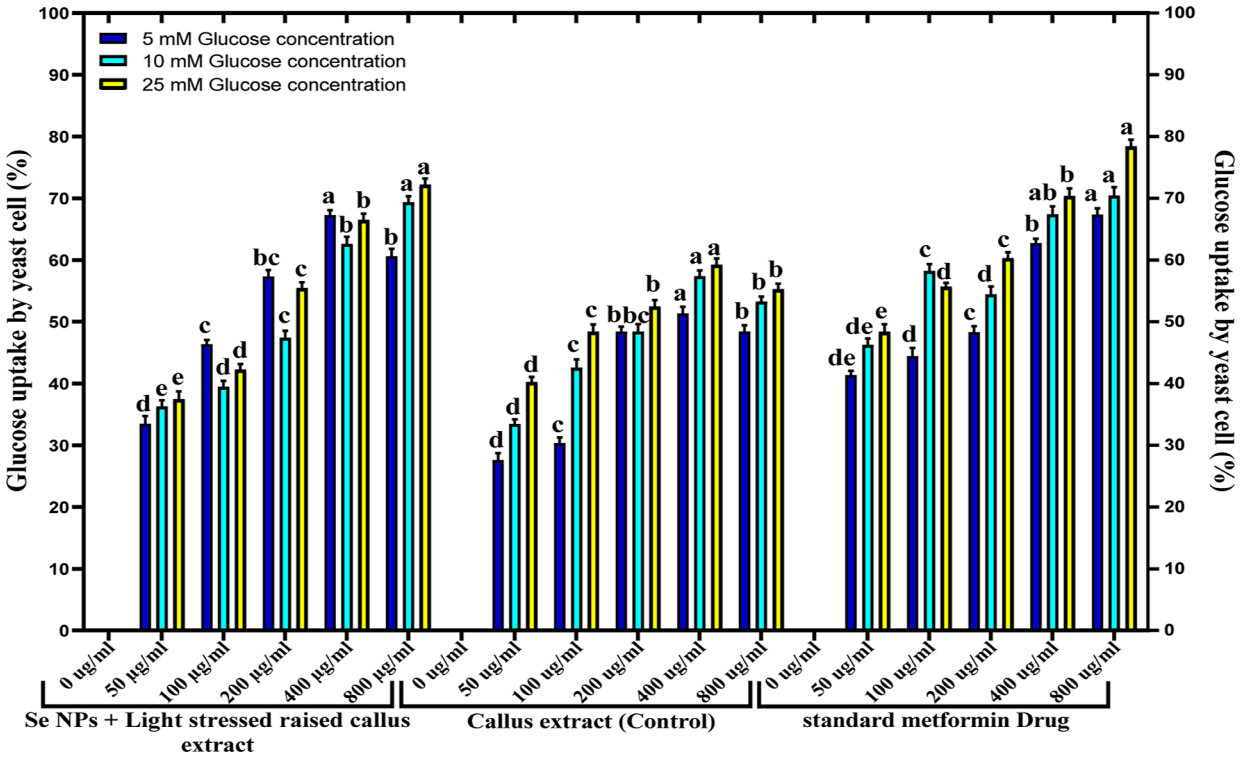

Figure 6. Graph illustrates the percentage values represented graphically for *C. tuberculata* callus extract and the standard metformin (5, 10, and 25 mmol/L). The significance level for assessing glucose uptake using the yeast cell assay was set at $p < 0.05$. A p-value below 0.05 was considered statistically significant, and this determination was supplemented with a post hoc test.

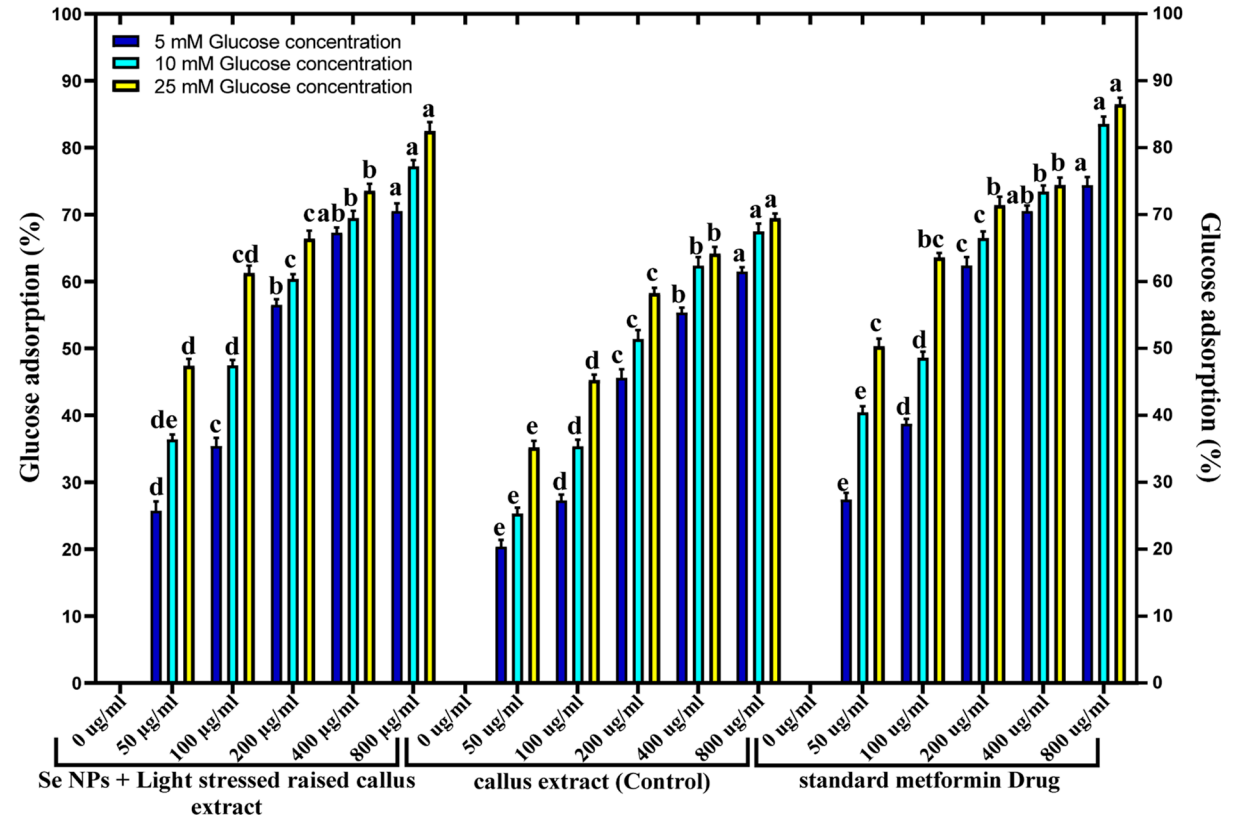

Figure 7. Graph illustrating the percentage values represented graphically for *C. tuberculata* callus extract and the standard metformin (5, 10, and 25 mmol/L). The significance level for assessing glucose adsorption assay was set at $p < 0.05$. A p-value below 0.05 was considered statistically significant, and this determination was supplemented with a post hoc test.

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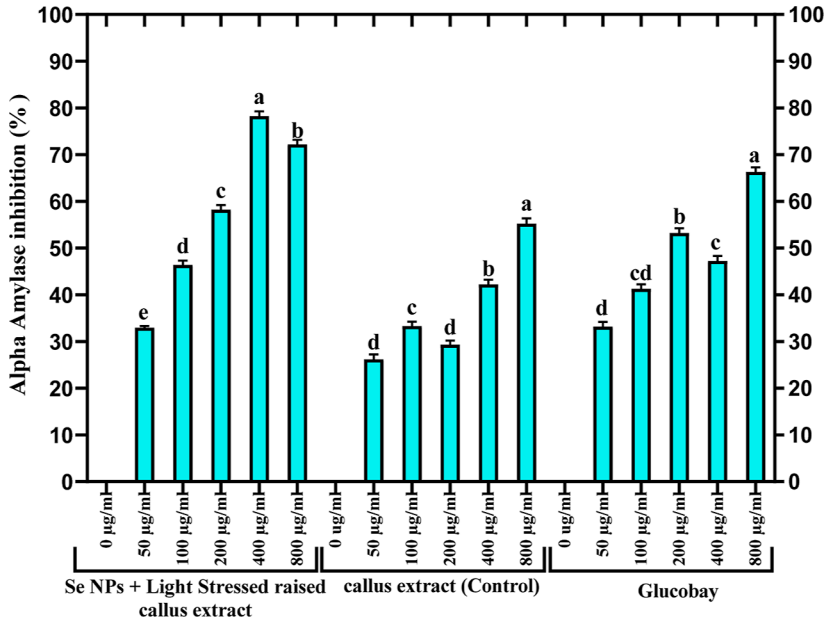

Figure 8. Graph illustrating the percentage values represented graphically for *C. tuberculata* callus extract and the standard Glucobay (5, 10, and 25 mmol/L). The significance level for assessing the alpha-amylase inhibition assay was set at $p < 0.05$. A p-value below 0.05 was considered statistically significant, and this determination was supplemented with a post hoc test.

Phosphomolybdate Assay. The assessment of the total antioxidant capacity of the callus extract from *C. tuberculata* was conducted using the phosphomolybdate assay, as outlined by Prieto *et al.*[20] This method hinges on the capability of an extract containing antioxidant compounds to contribute an electron to Mo (VI), leading to its reduction to Mo (V) and the consequent formation of a green phosphomolybdenum V complex. This complex exhibits a maximum absorbance at 695 nm. Figure 4e illustrates the antioxidant capacity of the methanolic extract derived from the *in vitro-grown* callus of *C. tuberculata*. Notably, calli extract treated with SeNPs and specific light conditions displayed a remarkable antioxidant capacity of 71.66%. This was followed by ascorbic acid at 76.66%, while the untreated calli extract exhibited an activity of 59.33%. It is worth noting that ascorbic acid was employed as a positive control and proved to be comparatively more effective than that of the *in vitro* calli extracts (Figure 5e). In a similar vein, Khan *et al.*[49] delved into the phosphomolybdate assay applied to Launea procumbens. Their study unveiled a notable correlation between the results of the phosphomolybdenum assay and the polyphenolic content present in plant extracts. The results derived from the phosphomolybdate assay conducted on the callus extract of *C. tuberculata*, as reported in this study, potentially suggest the extract's substantial richness in the antioxidant content.[46,47]

Hypoglycemic Potential of SeNP-Mediated *In Vitro* Callus Culture of *C. tuberculata*. *Glucose Uptake by Yeast Cells.* Insulin plays a crucial role in glucose regulation by facilitating its absorption from the bloodstream and storing it in glycogen stored in muscular and hepatic cells. Additionally, glucose undergoes utilizing glycolysis for ATP production, and any surplus glucose is converted into fat for long-term energy reservoir. However, in cases of hyperglycemia, such as when pancreatic cells oxidize and there is increased glucose intake through the diet, elevated blood sugar levels can occur due to reduced insulin levels, which impairs glucose absorption into liver cells.[50] While various medications are available for hyperglycemia, the growing number of diabetic patients motivates researchers to explore novel diabetes management approaches. Plant extracts are harnessed to mimic the insulin mechanism involving capturing excess glucose from the external environment and facilitating its absorption into yeast cells. Metformin, a widely recognized medication for hyperglycemia, served as the standard drug in our study. We tested three different glucose concentrations (5, 10, and 25 mmol/L), and at a concentration of 800 μg/mL, selenium nanoparticle-mediated callus extract exhibited a remarkable 72.18% glucose absorption for the 25 mmol/L glucose solution. For 5 and 10 mmol/L glucose concentrations, the same callus extract demonstrated glucose absorbance percentages of 60.58 and 69.33%, respectively (Figure 6). Metformin exhibited glucose absorption percentages of 78.37, 70.43, and 67.33% for the corresponding glucose concentrations (Figure 7). The affinity of the callus extract for glucose increased with higher glucose concentrations in the solution. In summary, SeNP-mediated and light-treated callus extract from *C. tuberculata* displayed a strong attraction to glucose molecules, equivalent to metformin. Correspondingly, Rehman *et al.*[51] reported that extracts from Cassia nimophila enhanced glucose uptake through the plasma membrane of yeast cells, with a linear increase observed with increasing test sample concentrations. In another study, Rehman *et al.*[51] found that the methanolic extract of *Butea monosperma* significantly promoted glucose uptake by yeast cells compared to the control. The antidiabetic properties of the methanolic extract from callus culture can likely be attributed to the presence of bioactive compounds in *C. tuberculata*, as confirmed by our previous study.[2]

Glucose Adsorption. The assessment of a natural drug's potential as an antidiabetic agent often involves *in vitro* assays to determine its ability to adsorb glucose. These assays not only offer insights into its *in vivo* antidiabetic potential but can also shed light on the drug's mechanism of action. Thus, our study aimed to evaluate the glucose adsorption capacity of the *in vitro C. tuberculata* callus extract in comparison to a

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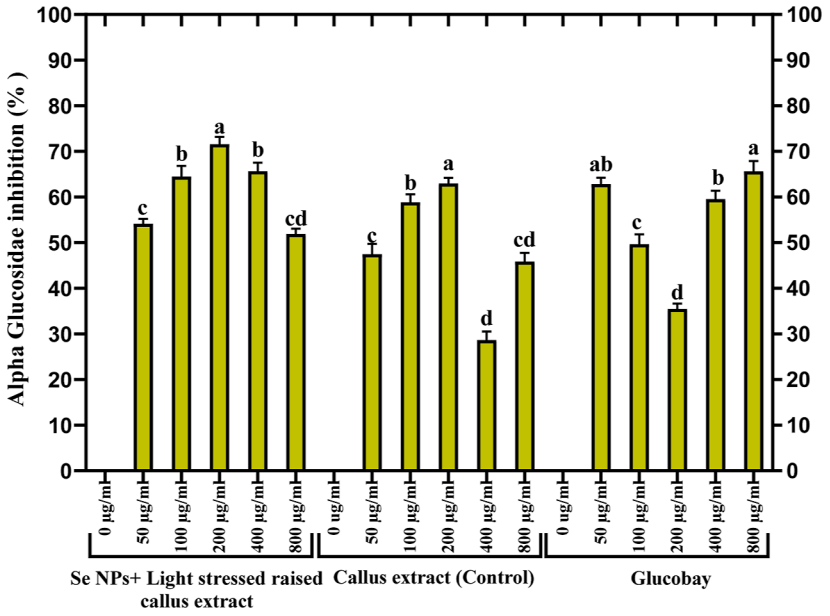

Figure 9. Graph illustrating the percentage values represented graphically for *C. tuberculata* callus extract and the standard Glucobay (5, 10, and 25 mmol/L). The significance level for assessing alpha-glucosidase inhibition assay was set at $p < 0.05$. A p-value below 0.05 was considered statistically significant, and this determination was supplemented with a post hoc test.

synthetic drug. The impact of the callus extract on *in vitro* glucose adsorption is illustrated in Figure 8. Our research revealed that the extract displayed a notable ability to adsorb glucose at various concentrations. Additionally, we observed a direct correlation between the amount of glucose and the extent of adsorption in the test sample. At a concentration of 800 μg/mL, the callus extract mediated by SeNPs displayed notable glucose absorption percentages of 70.51, 77.19, and 82.47% for glucose concentrations of 5, 10, and 25 mmol/L, respectively (Figure 7). Metformin, in comparison, demonstrated glucose absorption rates of 74.37, 83.55, and 86.46%, corresponding to the respective glucose concentrations (Figure 8). This suggests that the test extract possesses the capability to bind glucose even at lower concentrations. The ability of the callus extract to adsorb glucose is probably linked to the presence of secondary metabolites contained within the extract. Within the intestinal lumen, this glucose adsorption by the extract might play a role in mitigating the postprandial increase in blood glucose levels.[52] Comparable findings have been documented for fractions obtained from *Averrhoa carambola*, which exhibited significant glucose adsorption properties, aiding in the control of blood glucose concentration.[53]

Alpha-Amylase Inhibition Assay. Alpha-amylase, an enzyme found in the saliva of all animals, including humans, is an essential element of the gastrointestinal system that is released by the parotid glands. It functions optimally within a pH range of 6.7−7.0.[43] This intestinal enzyme plays a vital role in breaking down the 1,4 glycosidic linkage of polysaccharides and producing their monosaccharides, facilitating their incorporation into the bloodstream[43] (Figure 12). Salivary amylase initiates the initial phase of chemical food digestion. In our study, we investigated the ability of *C. tuberculata* callus extract to suppress the enzymatic function of alpha-amylase, thereby reducing the hydrolysis of polysaccharides and subsequently limiting the release of glucose into the bloodstream.[54] The antidiabetic metabolites present in the callus extract bind to the enzyme's catalytic site, preventing substrate

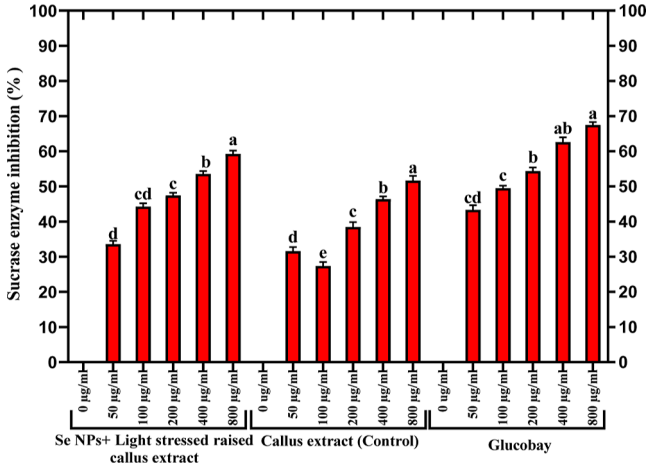

Figure 10. Graph illustrating the percentage values represented graphically for *C. tuberculata* callus extract and the standard Glucobay (5, 10, and 25 mmol/L). The significance level for assessing the sucrase inhibition assay was set at $p < 0.05$. A p-value below 0.05 was considered statistically significant, and this determination was supplemented with a post hoc test.

binding and the initiation of catalytic activity. This leads to a reduction in the breakdown of the sugar compounds. In our *in vitro* experiments, we examined the impact of SeNP-mediated *in vitro* cultures of *C. tuberculata* on the alpha-amylase activity across a range of concentrations, from 50 to 800 μg/mL. Notably, the nanoparticle-mediated *in vitro* callus extract demonstrated potent inhibition of alpha-amylase, reaching an impressive 78.24 inhibition in a dose-dependent manner. Moreover, subjecting the solution to incubation in a vigorously boiling water bath led to a transformation in color, shifting from reduced sugar to a brown-red hue. In this exploratory study, we compared the performance of our approach with Glucobay, a well-known medication for hyperglycemia. Glucobay achieved a 66.31% reduction in sugar, which was notably less effective than the NP-grown callus extract (Figure

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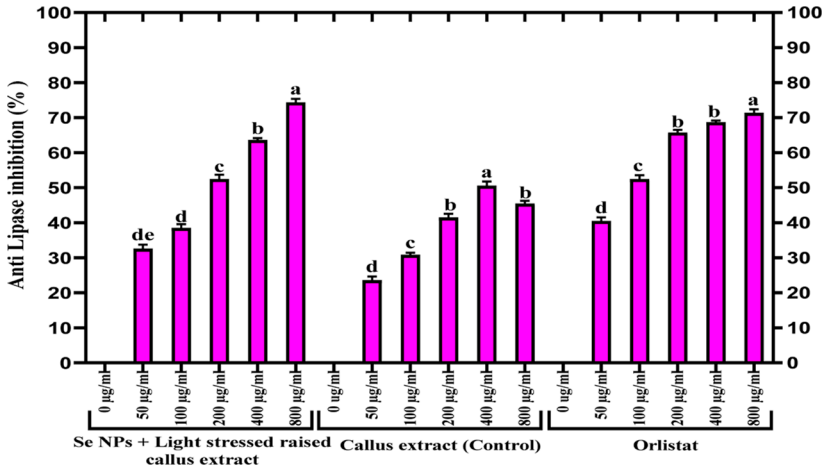

Figure 11. Graph illustrating the percentage values represented graphically for *C. tuberculata* callus extract and the standard Orlistat at three different concentrations (5, 10, and 25 mmol/L). The significance level for assessing antilipase inhibition activity was set at $p < 0.05$. A p-value below 0.05 was considered statistically significant, and this determination was supplemented with a post hoc test.

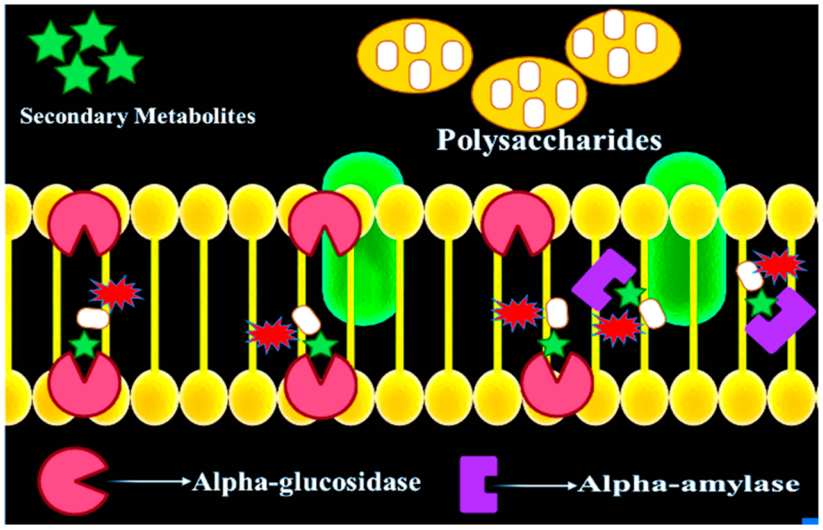

Figure 12. Illustration of the inhibitory capacity of secondary metabolites in *C. tuberculata* against alpha-glucosidases and alpha-amylase is described schematically. These secondary metabolites operate by diminishing the creation of the complex formed between the enzyme and substrate through the occlusion of the enzyme's active site. This decline in metabolic activity results in a diminished liberation of glucose from the bloodstream.

8). Untreated calli exhibited 55.24% inhibition of alpha-amylase activity. Notably, several species within the Caralluma genus, such as *C. tuberculata*, *Caralluma umbellata*, *Caralluma lasiantha*, *C. sinaica*, and *Caralluma edulis* have previously demonstrated antidiabetic properties in various studies.[21,55,56] Through phytochemical analysis, polyphenols have been identified in these plants, which may contribute to their ability to lower blood sugar levels. For example, *C. fimbriata* leaf extract has exhibited the capacity to inhibit enzymes responsible for carbohydrate digestion, including alpha-amylase. The inhibition of these enzymes, which are crucial for glucose metabolism, provides an effective strategy for combating postmeal hyperglycemia.[57]

Alpha-Glucosidase Inhibition Assay. Alpha-glucosidases show a pivotal role in the breakdown of macromolecules such as starch and carbohydrates, converting complex polysaccharides into readily accessible monosaccharides, primarily glucose.[58] These enzymes specifically target the terminal alpha-1,4-linkages found in a number of carbohydrate residues. Alpha-glucosidase is predominantly synthesized in the small intestine, where it resides on the microvilli lining of the intestinal walls. Its primary function is to catalyze the cleavage of alpha (1 → 4) bonds within polysaccharides, thereby releasing monosaccharides into the surrounding environment[59] (Figure 12). In our current study, we employed phytosynthesized SeNPs and induced light stress during *in vitro* callus culture to serve as inhibitors of alpha-glucosidase activity. This inhibition effectively reduces the hydrolysis of polysaccharides, indirectly regulating blood sugar levels. Our research results revealed that these nanoparticle- and light-stressed calli exhibited a remarkable 51.28% inhibition of alpha-glucosidase activity at an 800 μg/mL concentration. Furthermore, we compared these findings to the performance of Glucobay, a widely recognized drug for hypoglycemia, which demonstrated 65.58% inhibition at 800 μg/mL concentration. Notably, at a lower concentration of 200 μg/mL, the enzyme inhibition achieved with our callus extract was 71.55%, whereas Glucobay achieved 45.83% inhibition, underscoring the effectiveness of

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ACS Omega 2024, 9, 20101−20118

our approach (Figure 9). The inhibitory percentages obtained with our callus extract closely approached the results obtained with the standard medication. The activity of α-glucosidase inhibited by the methanolic extract of *C. tuberculata* callus in our study likely stems from a combination of metabolites. The secondary metabolites present in *C. tuberculata* are known to interact with the various amino acids of α-glucosidase, either modifying or reducing its enzymatic efficacy.[60] This decrease or slowdown of enzyme activity causes a reduction in carbohydrate digestion, subsequently leading to lower glucose availability for intestinal absorption.[61] Prior studies have also shown that 37 out of 45 plant extracts of various plants possess the ability to inhibit α-glucosidase activity compared to acarbose.[62] There are three enzymes, alpha-amylase, sucrase, and alpha-glucosidase, responsible for the carbohydrates' breakdown. Inhibition of these enzymes may cause carbohydrate digestion to be delayed and glucose absorption to be reduced; as a result, the blood glucose increase after a meal is reduced. Alpha-amylase and alpha-glucosidase are inhibited by drugs like acarbose, voglibose, and miglitol, but they create some unfavorable side effects in the body like bloating, intestinal discomfort, diarrhea, and so forth.[87] In consideration of this problem, an ethnopharmacological study lists over 1200 plants with antidiabetic activity that are used to treat DM, demonstrating the significance of conventional folk medicines.[88] Secondary metabolites in *C. tuberculate* may structurally resemble the natural substrates of these enzymes. By having a structure similar to that of the substrates, these metabolites can compete with them for binding to the active site of the enzyme. When the metabolite occupies the active site, it prevents the enzyme from interacting with its usual substrate, thus inhibiting the enzymatic reaction. Some bioactive compounds act as noncompetitive inhibitors by binding to a site on the enzyme other than the active site. This binding induces a conformational change in the enzyme that alters its catalytic efficiency. Noncompetitive inhibition does not involve direct competition with the substrate, but it still hinders the enzyme's ability to function effectively. It can also influence gene expression, leading to a reduction in the level of production of enzymes involved in carbohydrate digestion. This indirect effect decreases the overall activity of alpha-amylase, sucrase, and glucosidase.[89]

Additionally, it is well-documented that plants with notable antioxidant activities often exhibit reasonable α-glucosidase inhibitory effects, which can vary depending on the composition of active phytoconstituents and the specific culture conditions in which plant cells are grown[63] (Figure 12).

Sucrase Inhibition Assay. Every day, the human body relies on a multitude of enzymes that serve a wide range of functions. These enzymes play distinct roles in regulating various aspects of cellular metabolism. Among them, sucrase is a crucial enzyme responsible for breaking down sucrose, which is a major component of our daily dietary intake.[58] Sucrase is an enzyme responsible for cleaving alpha 1−4 glycosidic bonds, liberating glucose and fructose molecules within the organism. These monosaccharides are efficiently captivated through the microvilli's surfaces during digestion, specifically at the tips or apex of the small intestine epithelium. The process of disaccharide breakdown into monosaccharides and their subsequent absorption into the bloodstream lead to an elevation in blood glucose levels. This can give rise to metabolic challenges in the form of hyperglycemia, as observed in prior research by Yakoob *et al.*[19] in 2016 and Preety *et al.* in 2020.[91] The inhibition of sucrase by *in vitro*-raised *C. tuberculata* extract from callus leads to a decrease in sucrose breakdown, indirectly resulting in a reduction in glycemic levels. At elevated concentrations of 800 μg/mL Se NPs and light stress-treated calli extract, the inhibitory potential was 59.24% and that of standard Glucobay was 67.48%, respectively (Figure 10). While untreated calli extract showed 51.66% inhibition. Se NP- and light-stress-mediated calli extract inhibitory effect were observed to tightly bind to the enzyme's active site, rendering it incapable of performing its catalytic function. While numerous studies have explored the sucrase activity of plant extracts, it is unfortunate that no data are reported regarding the *in vitro* sucrase activity of callus extracts from *C. tuberculata*. These results align with the previous study, which also observed sucrase-inhibitory activity in the tested plants *Azadirachta indica*, *Ocimum tenuiflorum*, and *Rhodiola crenulata* and were examined to diminish the catalytic efficacy of the sucrase enzyme, thereby reducing its ability to catalyze the hydrolysis of sucrose.[31,64] Food-inspired peptides from spinach Rubisco have been tested for their antioxidant and enzyme inhibition activity *in vitro* and *in vivo* to evaluate their ability to maintain or lose the original activity.[92] The peptides show the best antioxidant and inhibitory activities suggesting a possible use in the food and pharmaceutical industry as a potential drug in new nutraceutical properties.

Antilipase Inhibition Activity. Obesity is a severe metabolic disease that is characterized by irregularities in calorie consumption and expenditure. Efficient lipid digestion is crucial, and pancreatic lipase plays a key role in this process that is produced and released by the pancreas. The exploration of medicinally important plants and their phytoconstituent's effects on lipase enzymes and triglyceride absorption has gained significant attention. Natural compounds found in plants have shown promise in combating obesity, and current strategies are shifting toward managing obesity with these natural products, which contain numerous phytochemicals that can synergistically act against antiobesity.[65] The antilipase potential is one of the extensively researched mechanisms for evaluating the potential effectiveness of natural products as agents to combat obesity.[12] In our recent research study, we prepared methanolic extracts from the callus culture of *C. tuberculata* and investigated their antilipase activity by using various concentrations from 0 to 800 μg/mL. The inhibitory activities against lipase enzymes are depicted in Figure 11. Both the Orlistat drug and the callus extracts exhibited dose-dependent inhibitory potential. As the concentration of Orlistat and callus extracts increased, the lipase inhibition activity also increased. Notably, among all the examined extracts at various concentrations, the phytosynthesized SeNP- and light stress-mediated *in vitro* callus extract demonstrated significant *in vitro* inhibition of porcine pancreatic lipase activity (74.26%) at 800 μg/mL concentration. Furthermore, the methanolic extract from untreated calli showed 50.48% inhibition of porcine pancreatic lipase. The outcomes of the current study were compared to the standard antilipase compound, Orlistat, which exhibited 71.30% antilipase inhibition activity. Orlistat, a pharmaceutical compound derived from the *Streptomyces toxytricini* bacterium, is a conventional medication in the market recognized for its ability to block the activity of pancreatic lipase enzymes. This enzyme plays a crucial role in breaking down dietary triglycerides (DTG) in the small intestine. Nevertheless, the

https://doi.org/10.1021/acsomega.3c10222
ACS Omega 2024, 9, 20101−20118

prolonged use of Orlistat has been linked to undesirable consequences, such as cardiovascular and gastrointestinal issues.[66] Plants are believed to exert antiobesity effects through various mechanisms, including the digestion and absorption of lipids, enhanced energy expenditure, reduced lipogenesis, and increased triglyceride breakdown. Previous studies have explored the efficacy of various medicinal and edible plant extracts, affirming their potential as antiobesity natural agents when compared to Orlistat.[67] Additionally, the *Ocimum dayi* organic extract also demonstrated antilipase potential.[68] Vinifera leaf extracts have shown robust antilipase activity compared to Orlistat.[69] Similarly, our results support the notion that *in vitro* cultured callus extracts of *C. tuberculata* possess significant antilipase potential. In conclusion, the identification of plant-based natural phytomolecules capable of combating weight gain offers a promising and timely opportunity for the development of cost-effective and safe clinical approaches to address obesity and diabetes.

CONCLUSIONS

The findings from this research study established the first comprehensive assessment regarding the scavenging potential of free radicals in the context of oxidative stress and hypoglycemic activity of *in vitro*-raised callus extracts. The applications of phytosynthesized SeNP- and light-mediated callus cultures of *C. tuberculata* have revealed their maximum potential in terms of antioxidant and hypoglycemic effects. Notably, the response of the callus extract exhibits a dose-dependent relationship, with concentrations of 200 and 800 μg/mL validating the effectiveness of the callus extract in various assays such as ABTs (42.51%), reducing power assay (26.29%), hydrogen peroxide scavenging assay (37.26%), hydroxyl radical scavenging assay (40.23%), and phosphomolybdate assay (71.66%), and the results closely align with the standards. Furthermore, in antienzyme assays, SeNP- and light-mediated callus extracts exhibit superior inhibition of alpha-amylase (78.24%), alpha-glucosidase (71.55%), antisucrase (59.24%), antilipase (74.26%), glucose uptake by yeast cells (72.18%), and glucose adsorption assay (86.49%) compared to Glucobay and metformin standards. The hypoglycemic potential of the callus extract functions as a competitive inhibitor, hindering the catalytic activity of enzymes responsible for breaking down disaccharides and polysaccharides. This action leads to a decrease in the release of glucose in the body. The study suggests that phytosynthesized and light stress-mediated *in vitro* callus culture may act synergistically to enhance antioxidant and hypoglycemic potential. The results from *in vitro* assays shed light on the antidiabetic and antioxidant properties of *C. tuberculata* callus extracts, laying the groundwork for drug development. The connection between *in vitro* assay concentrations and dosage and exposure patterns relevant to animals or humans in real-life situations remains unclear. Additionally, determining the extent to which the phytochemicals employed in the *in vitro* model reach the actual site of action poses a challenge. Therefore, animal trials are required to validate and develop an effective and potent bioactive compound for the management of type-2 DM treatment.

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Author Contributions

Amir Ali, Zia-ur-Rehman Mashwani designed the research. Amir Ali collected the data. Amir Ali, Zia-ur-Rehman Mashwani, Sher Mohammad processed the data. Amir Ali, Juan Pedro Luna-Arias drew and beautified data images. Amir Ali wrote the original manuscript. Juan Pedro Luna-Arias, Naveed Iqbal Raja, M. Sheeraz Ahmad provided suggestions and improved the manuscript. Zia-ur-Rehman Mashwani provided resources and supervision. All authors have read and agreed to publish the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors are deeply grateful to the staff of PMAS Arid Agriculture University Rawalpindi and the Agriculture Research Institute Tarnab Farm Peshawar for their continuous stimulating support to these studies.

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