Int.J.Curr.Res.Aca.Rev.2025; 13(5): 120-133

EXCELLENT PUBLISHERS

International Journal of Current Research and Academic Review

ISSN: 2347-3215 (Online) Volume 13 Number 5 (May-2025)

Journal homepage: http://www.ijcrar.com

doi: https://doi.org/10.20546/ijcrar.2025.1305.015

A Drosophila-Based Platform for Screening Nanoparticles: Synthesis, Characterization, and Antidiabetic Activity of *M. cymbalaria* CaNPs

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Abstract

The convergence of green nanotechnology and traditional medicine offers new therapeutic strategies for metabolic disorders such as type 2 diabetes mellitus (T2DM). This study investigates the antidiabetic potential of calcium nanoparticles (CaNPs) synthesized using a green method with *Momordica cymbalaria*, an Ayurvedic plant renowned for its hypoglycemic properties. Nanoparticles were synthesized from both root and seed extracts and characterized via UV–Vis spectrophotometry. A *Drosophila melanogaster* high-sugar diet model was employed to simulate diabetic phenotypes. Diabetic flies exhibited impaired locomotor function and metabolic imbalance, which were significantly reversed upon treatment with CaNPs, particularly from root-derived formulations. Enzymatic assays demonstrated potent inhibition of α-amylase and α-glucosidase (non-competitive and mixed-type, respectively). CaNPs also showed high DPPH radical scavenging activity and restored catalase and glutathione peroxidase levels. Nitric oxide and glucose concentrations were markedly reduced, with glucose levels dropping from ~196.1 to ~16.2 mg/dL. These results highlight the therapeutic potential of plant-based CaNPs and validate *Drosophila* as a preclinical screening model for Ayurvedic nanomedicine.

Article Info

Received: 15 March 2024
Accepted: 24 April 2024
Available Online: 20 May 2025

Keywords

Drosophila melanogaster, nanobiotechnology, green synthesis, *Momordica cymbalaria*, diabetes mellitus, calcium nanoparticles, phytotherapy, enzyme inhibition, oxidative stress, glucose control.

Introduction

In today's fast-paced world, human health is increasingly affected by changing lifestyles characterized by sedentary behavior, poor dietary habits, stress, and a lack of physical activity. These factors contribute to a rise in lifestyle-related diseases, with non-communicable conditions like obesity, hypertension, and particularly Diabetes becoming more prevalent. Urbanization and technology-driven work environments have reduced physical exertion, while processed and high-sugar foods have become a dietary norm. As a result, metabolic disorders are emerging at earlier ages, affecting not just adults but also children and adolescents (Leroux *et al.*, 2014).

Among these lifestyle diseases, Diabetes stands out as a significant global concern. Type 2 diabetes, in particular, is directly linked to lifestyle factors and is on the rise due to insulin resistance caused by excess body fat and inactivity.

This chronic condition leads to serious complications such as cardiovascular disease, kidney failure, and nerve damage. The management of Diabetes has improved significantly with advancements in medicine, including oral hypoglycemic agents, insulin therapy, continuous glucose monitoring devices, and personalized treatment plans. Despite these developments, Diabetes remains a long-term burden for patients, often requiring lifelong management and behavioral adjustments (Hu, 2003).

Ongoing research in Diabetes focuses on understanding its molecular basis, developing improved therapeutic options, and identifying preventive strategies. Animal models, particularly rodents such as mice and rats, as well as fruit flies (*Drosophila melanogaster*), are widely utilized to investigate the pathophysiology of Diabetes and evaluate potential treatments. These models allow researchers to investigate genetic, biochemical, and cellular mechanisms underlying diabetic complications. Studies using animal models also help assess the effects of natural products, nanoparticles, and gene-editing technologies, offering hope for more effective and less invasive therapies in the future (Covington and Chen 2024). The fruit fly, *Drosophila melanogaster*, is used as a model organism to study disciplines ranging from fundamental genetics to the development of tissues and organs. The *Drosophila* genome is 60% homologous to that of humans and is less redundant. Approximately 75% of the genes responsible for human diseases have homologs in flies (Ugur *et al.,* 2016). These features, together with a brief generation time, low maintenance costs, and the availability of powerful genetic tools, make the fruit fly an ideal model for studying complex pathways relevant to biomedical research, including Diabetes. Indeed, publications that use flies to model Diabetes have exponentially increased in the last 10 years.

Notwithstanding the ubiquity and utility of vertebrate models of Diabetes (especially rodent models), and when compared with other experimental models of Diabetes, *Drosophila* has clear advantages. Despite sometimes ill-defined parameters in diet regimes and environment, the model strengths, namely, a robust, extensive, and highly developed genetic system, with a plethora of isolated and characterized insulin pathway and general metabolism genes, ease of manipulation and use, low cost, high fertility, and numbers, short generation time, high evolutionary conservation, and homogeneous genetic backgrounds, among other positive characteristics, make the fly a premier system for insulin pathway and metabolic studies (Alvarez *et al.,* 2018).

Traditional Indian medicine, particularly Ayurveda, has been practiced for millennia and remains a vital part of healthcare in India. Ayurvedic medicine offers a holistic approach to managing chronic diseases, including diabetes mellitus (*Madhumeha*), using a variety of herbal formulations, mineral preparations, and lifestyle interventions. Numerous medicinal plants, including *Gymnema sylvestre, Momordica charantia, Trigonella foenum-graecum, and Azadirachta indica*, have demonstrated hypoglycemic effects in preclinical and clinical studies. Despite the long-standing empirical use and widespread clinical application, the lack of rigorous scientific evaluation and limited understanding of molecular mechanisms hinders the global acceptance and integration of these traditional therapies. In this context, the *Drosophila melanogaster* model provides a promising platform for systematically evaluating the antidiabetic potential of Ayurvedic medicines. It enables high-throughput screening of bioactive compounds and the dissection of genetic and metabolic pathways conserved with mammals. Leveraging Drosophila in Ayurvedic research may accelerate the discovery of effective compounds and elucidate mechanisms underlying traditional treatments, thus bridging traditional knowledge with modern biomedical Science (Patwardhan *et al.,* 2005; Subramoniam, 2016). In this paper, we introduce the signal pathways involved in glucose homeostasis regulation and energy metabolism, which are shared between fruit flies and humans. We describe the methods used to establish Drosophila Diabetes models and utilize them in pharmacodynamic evaluations.

Recent advances in nanotechnology have opened new avenues in the field of medicine, particularly in enhancing the efficacy, stability, and bioavailability of plant-derived compounds. Green synthesis of nanoparticles using plant extracts has gained attention due to its eco-friendliness, cost-effectiveness, and biocompatibility. Incorporating *M. cymbalaria*-based nanoparticles into novel delivery systems, such as medicated jellies, can offer a palatable and efficient method for diabetes management.

Momordica cymbalaria Hook. F. (Family: Cucurbitaceae) is a lesser-known yet auspicious plant with a history of use in traditional Indian medicine for managing Diabetes and related metabolic disorders. Commonly referred to as Karchikai (Kannada), Athalakkai (Tamil), or Kasarakayee (Telugu), this plant is native to the tropical regions of India and Southeast Asia. Phytochemical investigations have revealed that *M. cymbalaria* is rich in charantin, saponins, flavonoids, and other compounds known for their hypoglycemic and antioxidant effects (Tomoyuki Oki *et al.,* 1999; Matsuda *et al.,* 2002).

Different parts of *M. cymbalaria* have traditionally been used to treat several ailments. The fruits are utilized as a stimulant, tonic, laxative, and stomachic. They can be used for treating gout, rheumatism, spleen, and liver

diseases. The plant is used in local folk medicine to treat diabetes mellitus. The juice of fruits and tea leaves of *M. cymbalaria* is used to treat Diabetes, malaria, colic, sores, wounds, and infections. The juice is also used against worms and parasites. They are also helpful as an emmenagogue for measles, hepatitis, and fever.

The roots of *M. cymbalaria* possess abortifacient and aphrodisiac activities. The roots are also utilized to treat constipation, indigestion, Diabetes, diarrhoea, and rheumatism. The juice of the fruits and leaves of *M. cymbalaria* possesses antihelmintic properties (Mahantesh *et al.,* 2021).

The nutrient contents of *M. cymbalaria are* summarized and correlated with the nutritional value of *Momordica charantia* in Table 1. *Momordica charantia,* commonly known as bitter gourd or bitter melon, is a highly regarded plant in the Ayurvedic system of medicine for treating hyperglycemic conditions.

It contains carbohydrates, protein, calcium, potassium, sodium, iron, copper, manganese, zinc, phosphorus, vitamin C, and α-carotenes. It also maintains normal cardiac rhythm, blood coagulation, muscle contraction, and nerve responses.

Momordica cymbalaria contains a higher amount of Calcium than *M. charantia*. The iron content in both vegetables is almost the same. Potassium, sodium, copper, manganese, and zinc are also high in *M. cymbalaria,* whereas α-carotenes content in *M. cymbalaria* is very low. The fruits of *M. cymbalaria* are reported to contain citric acid, malic acid, and vitamin C (Parvathi *et al.,* 2002).

Phytochemical screening has revealed the presence of several biologically active compounds in *Momordica* plant parts, characterized by a wide diversity of bioactive compounds, including phenolic acids, flavonoids, carotenoids, cucurbitane triterpenoids, and phytosterols (Koneri *et al.,* 2014). Many therapeutically active substances exist in *M. cymbalaria*, i.e., tannins, alkaloids, amino acids, vitamin C, carbohydrates, and α-carotenes.

The fruits of *M. cymbalaria* contain citric acid, maleic acid, and vitamin C (Gopu *et al.,* 2022). The fixed oil present in the fruits of *M. cymbalaria* also contains palmitic acid, oleic acid, stearic acid, α-eleostearic acid, and γ-linolenic acid (Parvathi *et al.,* 2002; Mohammed *et al.,* 2024). These compounds are distributed in the fruits, roots, and leaves, with the highest concentration of antidiabetic agents found in the fruits and roots.

Extraction of Nanoparticles from the crude extract of *M. cymbalaria*

Green nanotechnology involves utilizing environmentally friendly methods to synthesize nanoparticles without the use of harmful chemicals or extreme physical conditions. It promotes sustainability by utilizing biological entities, such as plant extracts, bacteria, fungi, or enzymes, as reducing and stabilizing agents. This approach is gaining popularity due to its cost-effectiveness, scalability, and biocompatibility.

Materials and Methods

Collection and Preparation of Plant Material

Fresh *Momordica cymbalaria* plants were collected as whole plants from Raketla village, Uravakonda (M), Anantapur District. Andhra Pradesh, India. The collected plant parts (fruits, roots, and leaves) were thoroughly washed with distilled water to remove dirt and debris, shade-dried for 10–15 days, and then ground into a fine powder using a mechanical grinder. The powdered samples were stored in airtight containers for extraction analysis and further use.

Extraction Techniques

Two methods were employed for the extraction of bioactive constituents:

Soxhlet Extraction

The plant powder was first Soxhlet-extracted with ethanol (50g of powder in 500ml of ethanol) for a 6-hour period, repeated three times. The ethanolic extract was rotary evaporated and then subjected to saponification until a viscous liquid was formed. The extract was then dried and hydrolyzed with 0.1 N KOH, and the resulting mixture was extracted using diethyl ether twice to obtain the upper organic layer. After centrifugation at 3000 rpm for 3 minutes, the supernatant was collected as the final solution.

Cold Maceration

For the extraction process, 10 g of the plant powder is steeped in conical flasks containing 100 mL of methanol. The mixture was agitated thoroughly with magnetic

beads and left undisturbed for five hours to ensure sufficient extraction. Following the extraction, the solution was filtered through a Whatman No. 1 filter paper to obtain a clear filtrate. This solution was used for future use.

Synthesis of Nanoparticles

Calcium nanoparticles were synthesized using the plant extracts of the Root and Fruit samples:

Calcium Nanoparticles (CaNPs)

50 mL of the $CaCl_2$ (5×10^{-2} mol/L) solution is then added to 25 mL of the extracted solution. Adjust the pH of the solution to 8.5 by adding a 1 mol/L ammonia (NH_3) solution (if needed). Stir the mixtures for 2 hours using magnetic beads. After the precipitate is formed, CaNP formation will be confirmed by its colour change. This mixture was centrifuged, washed, and dried.

Characterization Techniques

The reduced CaNPs solutions were centrifuged at 8000 rpm for 30 minutes. The supernatant and pellets of pure CaNPs were collected separately for further characterization and applications using UV-VIS Spectrophotometer instrumentation.

UV-VIS spectrophotometer Analysis of CaNPs

Methanolic solution of 0.1%. 0.05 mg of the powdered root and fruit nanoparticles are measured and mixed with 5 mL of methanol to form a 0.1% solution, which is used as the sample. Methanol is used as the blank solution to create the baseline. The samples were tested between 200 and 900nm.

Confirmatory In-Vivo Studies on Animals

Drosophila Melanogaster as Experimental Model

The fruit fly, Drosophila melanogaster, is a widely used model organism in genetics and biomedical research due to its short lifespan, genetic similarity to humans, and low care costs. Approximately 75% of human disease-related genes have counterparts in Drosophila, making it a valuable model organism for studying metabolic disorders, such as diabetes. We used two strains of D. melanogaster: the CS Wild type and the Vestigial Mutant type, obtained from Dr. Gurudatta Baraka's lab at the National Institute of Mental Health and Neurosciences in Bangalore, Karnataka, India.

Experimental Groups and Diet

Flies were cultured in *Drosophila* culture medium (cornmeal, agar, yeast and sucrose mixed with ethanolic solution of p-hydroxy methyl benzoate and propionic acid) and were reared at room temperature (20 to 25 ◦C) under the 12–12 h (for CS and transgenic strains) or the natural light–dark cycle for the wild caught strain. Flies were starved for 22 to 24 h (with access to water, but no food) before using them in food choice and food consumption assays.

Flies were divided into four groups:

1. **Normal Control (NC)** – Fed a standard diet.
2. **Diabetic Control (DC)** – Fed a high-sugar diet (30% sucrose).
3. **Extract-Treated Group** – Diabetic flies treated with *M. cymbalaria* extract.
4. **CaNP-Treated Group** – Diabetic flies treated with calcium nanoparticles.

Parameters Assessed

Climbing Assay (Negative Geotaxis Test)

This behavioural test evaluates motor function. Healthy flies climb upwards in a vial; diabetic flies show impaired climbing ability. Improvements in treated groups indicate enhanced neuromuscular performance. At the end of the various treatment periods, the climbing ability of flies exposed to the different experimental diets was assessed as described by Gargano *et al.,* (2005). To evaluate the effects of varying sucrose diets on Drosophila, flies were exposed to the respective diets for 15 days. Following this, their climbing ability was assessed using a climbing assay. Briefly, ten flies per vial from each treatment were transferred into an empty vial. The vial was tapped on a surface three times to bring all flies to the bottom, and a picture was taken immediately after twelve seconds. This procedure was repeated 10 times, with flies allowed to recover for 120 seconds between taps. The relative climbing index was calculated by dividing the climbing height into five equal sections and scoring them from zero (at the bottom) to four (highest section). The height climbed was calculated by summing up the number of flies per treatment in the different sections.

Invitro Enzyme Inhibition Assays

α Amylase assay

In vitro inhibitory assays were conducted for the enzymes α-amylase and α-glucosidase. The alpha amylase assay was carried out using the DNSA reagent. The reaction mixtures were prepared as follows: The substrate concentration was varied from 2 mg/mL to 10 mg/mL, and the activity was measured. Two milliliters of Phosphate buffer (pH 6.0) was added to all test tubes, along with 0.5 mL of 2% Amylase solution. 0.5 mL of inhibitor (prepared as a 100 mg/mL solution of crude extract) was added to each test tube in a separate set, and the activities were measured by adding 1 mL of DNSA reagent and placing the tubes in a boiling water bath. O.D was measured at 540 nm. Kinetic profiles of the enzymes were studied and compared with and without the inhibitor. Lineweaver-Burk plots were developed to determine the type of inhibition, along with the Km and Vmax.

α-Glucosidase assay

A similar assay was carried out using PNPG as a substrate for the α-glucosidase inhibitory assay (Yeast Maltase). 200 µl of Phosphate buffer was added along with varying amounts of the substrate (from 0.1 to 0.5 mg). 50 µL of inhibitor (prepared as a 100 mg/mL solution of crude extract) was added to all the reaction mixtures in a separate set. 200 µL of 0.1 M Sodium Carbonate was added to develop the color, and the O.D. was taken at 415 nm. Activity was calculated by measuring the amount of p-nitrophenol liberated. Kinetic profiles of the enzymes were studied and compared with and without the inhibitor. Lineweaver-Burk plots were developed to determine the type of inhibition, along with the Km and Vmax.

Biochemical Assays

DPPH Radical Scavenging Assay

Aliquots of 10µL of the sample homogenate were transferred into a test tube. Each well was supplemented with 125 µL of DPPH solution. A volume of 50 µL of absolute methanol was added to each well to adjust the reaction volume. The test tubes were incubated in the dark at room temperature for 20 minutes. The absorbance was measured at 517 nm using a UV spectrophotometer. The percentage of DPPH scavenging activity was calculated using the formula:

$$\text{Percentage of DPPH scavenging} = \left(\text{ABS}_{\text{control}} - \text{ABS}_{\text{sample}}/\text{ABS}_{\text{control}}\right) \times 100$$

Glutathione Peroxidase

To prepare the reagents for the glutathione peroxidase assay, a 50 mM sodium phosphate buffer was prepared by dissolving 2.76 g of NaH2PO4 and 2.04 g of Na2HPO4 in 80 mL of Milli-Q water. The pH was then adjusted to 7.0 with HCl or NaOH, and the solution was diluted to 100 mL. Additional reagents included 10 mM sodium azide (6.5 mg in 10 mL MilliQ water), 4 mM reduced glutathione (0.012 g in 10 mL MilliQ water), and 0.25 mM H_2O_2 (5 µL stock H_2O_2 in 9995 µL of sodium phosphate buffer). Trichloroacetic acid (TCA) was prepared at 100 mM by dissolving 1.639 g in 100 mL of MilliQ water, while 0.3 M sodium hydrogen phosphate required 4.258 g in 100 mL of MilliQ water. For the assay, 0.05% DTNB was prepared by dissolving 0.05 g in 100 mL of 1% sodium phosphate buffer with 0.1 M EDTA, and 0.08 mM EDTA was made by dissolving 23.3 mg in 100 mL of milliQ water. The procedure involved homogenizing the tissue in a buffer containing phosphate buffer, sodium azide, EDTA, H_2O_2, reduced glutathione, the protein sample, and Milli-Q water. The mixture was split into two tubes, incubated at 37°C for 90 and 180 minutes, and treated with TCA to precipitate proteins. After centrifugation at 10,000 rpm for 10 minutes at 4°C, the supernatant was collected and stored at 4 °C. For analysis, 200 µL of supernatant was mixed with 120 µL of sodium hydrogen phosphate and 100 µL of DTNB, with a blank prepared using sodium hydrogen phosphate and DTNB. Absorbance readings were taken at 412 nm using a microplate reader.

Results and Discussion

The investigation into the antidiabetic potential of *Momordica cymbalaria* was conducted through the green synthesis of Calcium (CaNPs) nanoparticles, their characterization, and study of their effect in Drosophila, yielding comprehensive results.

Extraction of Plant Material

This study employed Soxhlet and cold maceration methods to extract bioactive compounds from the plant's leaves, roots, and fruits. Extracts were prepared using methanol, yielding nine extracts that all showed positive results for flavonoids, phenolics, steroids, and terpenoids. Initial extraction using the Soxhlet method

was performed with methanol to yield extracts from the leaves, roots, and fruits of *Momordica cymbalaria*. However, exposure to high temperatures led to the denaturation of key phytochemicals. To preserve bioactivity, a cold maceration method using methanol was adopted for further extraction. Vaghasiya and Chanda (2007) have also extracted fourteen Indian medicinal plants using the same organic solvents and reported higher extraction in methanol compared to acetone.

Nanoparticle Synthesis

Synthesis of Calcium Nanoparticles (CaNPs)

Calcium nanoparticles were synthesized by adding calcium chloride (CaCl2) solution to the plant extract under alkaline conditions (pH adjusted to 8.5 using ammonia). The mixture was stirred for several hours, followed by incubation at 27°C. A milky white precipitate indicated the formation of calcium carbonate nanoparticles were shown in Fig.1. These were collected by centrifugation, washed, and dried to obtain CaNPs in powder form.

- Calcium Nanoparticles (CaNPs): Observed as a cloudy precipitate after reaction with $CaCl_2$ and Na_2CO_3. UV-Vis peaks at 326–328 nm signaled successful synthesis.
- Visual and spectral changes confirmed nanoparticle formation and stability. The green synthesis approach proved efficient and eco-friendly.

Characterization of Nanoparticles

UV-VIS spectrophotometer Analysis of CaNPs

The formation and stability of Calcium nanoparticles (CaNPs) synthesized using *Momordica cymbalaria* extract were initially confirmed through UV–Visible spectrophotometry, a primary characterization technique for nanoparticle detection. The spectral analysis revealed distinct surface plasmon resonance (SPR) peaks corresponding to each nanoparticle type. CaNPs displayed a broader, less intense absorption band, typically in the 650 nm range, as shown in Fig.2, which is consistent with earlier reports on calcium-based nanostructures (Kumar *et al.,* 2017). The comparatively lower intensity and broader nature of the CaNP spectrum suggest different electronic transitions, possibly related to surface defects and variable particle size distribution. The distinct absorbance patterns of CaNPs confirm the influence of the phytochemical profile of *M. cymbalaria* in reducing and stabilizing the metal ions into nanoparticles.

In-Vivo Antidiabetic Activity Using Drosophila Melanogaster

Physical Assay

Climbing Assay

The flies were gently tapped to the bottom of the cylinder to ensure they started from a consistent position. Under ambient light conditions, their climbing activity was recorded for 2 minutes. The number of flies crossing the 17.5 cm mark was documented. This assay was repeated across all groups to quantify locomotor differences.

Table 1 illustrates that the high sucrose diet led to a greater proportion of flies displaying impaired locomotor abilities, especially in the Intermediate (Starvation) and Diabetic categories, suggesting diet-induced physiological changes. The results obtained from the climbing assay indicate that both the Wild type and VS groups exhibit similar trends in climbing performance across different conditions. In both groups, the Normal condition yields the highest climbing performance, followed by the Intermediate condition, with the Diabetic condition exhibiting the lowest performance. The statistical significance level of 0.05 indicates that the observed differences in climbing performance are statistically significant and shown in Fig.3. This demonstrates the impact of normal, intermediate, and diabetic conditions on the physical abilities of the two groups.

Inhibition assay

α Amylase assay

The alpha-amylase assay results highlight the enzyme kinetics of root and seed nanoparticles (NP) in the presence and absence of an inhibitor. For both Root NP and Seed NP, the Km (Michaelis constant) and Vmax (maximum reaction velocity) values were measured, indicating non-competitive inhibition. Specifically, the Km values with inhibitors were significantly more negative compared to those without inhibitors, while Vmax values showed a slight decrease when inhibitors were present. This suggests that the inhibitor affects the enzyme activity in both root and seed nanoparticles, providing insights into their biochemical interactions and

potential applications. The graphical representations are shown in Fig. 4 and further illustrate these changes, confirming the non-competitive nature of the inhibition. To find the linear regression from the Table 2 plot to calculate unknown maltose concentrations in α-amylase assays, Table 3 to determine the substrate concentration of α-amylase activity, and the results obtained are shown in Table 5.

α-Glucosidase assay

The α-glucosidase inhibitory activity was assessed using PNPG as a substrate, with and without the addition of a crude plant extract as the inhibitor. The enzyme exhibited increased activity with increasing substrate concentration from 0.1 to 0.5 mg, as indicated by the rise in absorbance values at 415 nm due to the liberation of p-nitrophenol. In the presence of the inhibitor, a noticeable decrease in enzyme activity was observed across all substrate concentrations, indicating effective inhibition. The kinetic parameters were determined using Lineweaver-Burk plots. In the absence of the inhibitor (Table 6), the Km and Vmax values were calculated to be [e.g., Km = 0.25 mM, Vmax = 0.80 µmol/min], whereas in the presence of the inhibitor (Table 7), the Km increased to 0.40 mM and Vmax decreased to 0.60 µmol/min, suggesting a mixed-type inhibition. These results confirm that the crude extract contains bioactive compounds capable of modulating alpha-glucosidase activity, supporting its potential role in regulating carbohydrate metabolism.

The alpha-glucosidase activity was evaluated by varying the concentration of the p-nitrophenyl (PNP) substrate and measuring the amount of p-nitrophenol released, indicated by absorbance at 415 nm after color development with sodium carbonate. As expected, no absorbance was detected in the control samples (tubes 1A to 5A) containing only phosphate buffer without the enzyme, confirming that substrate hydrolysis did not occur in the absence of alpha-glucosidase.

In the test samples (1B to 5B), a progressive increase in absorbance was observed with increasing substrate concentration, indicating a dose-dependent increase in enzyme activity. The absorbance values ranged from 0.092 at 0.08 mg PNP to 0.536 at 0.40 mg PNP. This trend demonstrates that alpha-glucosidase catalyzes the hydrolysis of the substrate more effectively at higher concentrations, consistent with classical enzyme kinetics. The results support the enzyme's substrate-dependent catalytic behavior and validate the assay conditions for studying enzyme activity. This data can be used to construct a Michaelis-Menten curve and, subsequently, a Lineweaver-Burk plot to determine kinetic parameters such as Km and Vmax. These parameters are essential for understanding the enzyme's affinity toward the substrate and its maximum catalytic efficiency under the given experimental conditions.

Biochemical Assays

DPPH Radical Scavenging Assay

The DPPH assay results highlight the significant oxidative stress induced by Diabetes in both mutant (VS) and wild-type (WT) strains. Under normal dietary conditions, both strains exhibit moderate baseline oxidative stress, as reflected by O.D. average values of 0.12 for the mutant strain and 0.147 for the wild-type. However, in the diabetic condition, oxidative stress markedly increases, with O.D. avg rising to 0.22 and 0.25 for the mutant and wild-type strains, respectively, and no scavenging activity is observed (0%). This underscores the elevated oxidative damage associated with the diabetic state, as illustrated in Table 8.

Treatment with calcium nanoparticles (CaNPs) derived from seeds and roots demonstrated exceptional antioxidant activity. In the mutant strain, CaNPs reduced O.D. avg to 0.024 (seed) and 0.023 (root), achieving scavenging activities of 89.09% and 89.55%, respectively. Similarly, in the wild-type strain, CaNPs lowered O.D. avg to 0.036 (seed) and 0.023 (root), with scavenging activities of 85.6% and 90.8%. These findings suggest that CaNPs are highly effective in neutralizing free radicals, likely due to their nanoscale size and enhanced bioavailability.

Table.1 Summarizes the climbing ability of Drosophila under different diet conditions

Climbing Ability Category	WT Group	VS Group
Normal	4	6
Intermediate	5	4
Diabetic	1	2

Table.2 Construction of Maltose calibration curve

Volume of standard Maltose solution (3 mg/ml) in ml	Volume of Distilled Water in ml	Amount of Maltose in mg	Volume of 1% DNSA	Incubation in a boiling water bath for 10 minutes	Volume of Distilled Water	Absorbance at 540 nm
0	2	0	**1**		17	0
0.2	1.8	0.6				0.132
0.4	1.6	1.2				0.242
0.6	1.4	1.8				0.359
0.8	1.2	2.4				0.456
1	1	3				0.574

Table.3 Determination of substrate concentration of Alpha amylase activity

Test Tube	Starch solution (ml)	Distilled water (ml)	Amount of Starch in mg	Phosphate Buffer (ml)	Pre - incubate at 37℃ for 10 minutes	Phosphate Buffer/ Enzyme (ml)	Incubate at 37℃ for 10 minutes	Volume of 1% DNSA reagent	Incubation in a boiling water bath for 10 minutes	Distilled water (ml)	Absorbance at 540 nm
1A	0.4	1.6	2	2mL		0.5 PB		**1mL**		14.5 mL	0
1B						0.5 AE					0.181
2A	0.8	1.2	4			0.5 PB					0
2B						0.5 AE					0.372
3A	1.2	0.8	6			0.5 PB					0
3B						0.5 AE					0.553
4A	1.6	0.4	8			0.5 PB					0
4B						0.5 AE					0.751
5A	2	0	10			0.5 PB					0
5B						0.5 AE					0.921

Table.4 Determination of Inhibitor concentration of Alpha amylase activity

Test Tube	Starch solution (ml)	Distilled water (ml)	Amount of Starch in mg	Phosphate Buffer (ml)	Inhibitor in ml (100 ug/ml of crude extract)	Pre-incubate at 37℃ for 10 minutes	Phosphate Buffer/ Enzyme (ml)	Incubate at 37℃ for 10 minutes	Volume of 1% DNSA reagent	Incubation in a boiling water bath for 10 minutes	Distilled water (ml)	Absorbance at 540 nm
1A	0.4	1.6	2	1.5mL	0.5mL		0.5 PB		**1mL**		14.5 mL	0
1B							0.5 AE					0.03
2A	0.8	1.2	4				0.5 PB					0
2B							0.5 AE					0.16
3A	1.2	0.8	6				0.5 PB					0
3B							0.5 AE					0.27
4A	1.6	0.4	8				0.5 PB					0
4B							0.5 AE					0.35
5A	2	0	10				0.5 PB					0
5B							0.5 AE					0.43

Table.5 Results obtained from the LB Plot

Sample Type	Condition	Km	Vmax
Seed NP	Without Inhibitor	12.2 mM	55 µmol/min
	With Inhibitor	-6.1 mM	50 µmol/min

Table.6 PNP Calibration graph

Sl. No	Vol. of std PNP (2 mg/ml) in µl	Vol of Distilled Water in µl	Amount of PNP in mg	Volume of 1M Sodium Carbonate in µl	Absorbance at 415 nm
1	0	100	0	100	0
2	20	80	0.04	100	0.069
3	40	60	0.08	100	0.138
4	60	40	0.12	100	0.205
5	80	20	0.16	100	0.282
6	100	0	0.2	100	0.356

Table.7 Determination of substrate concentration of Alpha Glucosidase activity

Test Tube	PNP solution (µl)	Distilled water (µl)	Amount of PNP in mg	Phosphate Buffer (µl)	Pre - incubate at 37℃ for 10 minutes	Phosphate Buffer/ Enzyme (ml)	Incubate at 37℃ for 10 minutes	Volume of 1M Sodium Carbonate reagent (µl)	Absorbance at 415 nm
1A	20	80	0.08	50		0.5 PB		100	0
1B						0.5 AE			0.092
2A	40	60	0.16			0.5 PB			0
2B						0.5 AE			0.185
3A	60	40	0.24			0.5 PB			0
3B						0.5 AE			0.356
4A	80	20	0.32			0.5 PB			0
4B						0.5 AE			0.441
5A	100	0	0.4			0.5 PB			0
5B						0.5 AE			0.536

Table.8 DPPH Radical Scavenging Assay – Sample Preparation Table

Sl.No	Sample (in µL)	Conc. (in µg/mL)	DW (in µL)	DPPH (in µL)	Methanol (in µL)
Solvent	0	0	0	0	250
Sample	10	2.5	90	125	50
DPPH_Control	125	0.1 mM		125	125

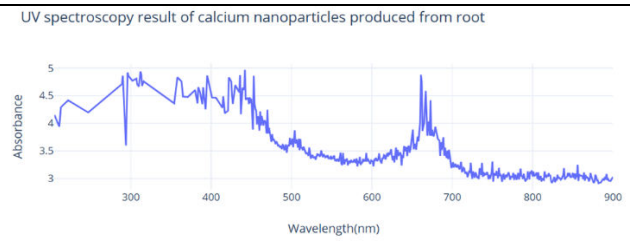
Figure.1 A& B: Visual observation of the Calcium nanoparticle formation. At the beginning of the reaction (A) and after 3 Days (B).

Day 1

Day 3

Figure.2 UV–Visible absorption spectra of Calcium nanoparticles (CaNPs) synthesized using *Momordica cymbalaria* extract. A. The CaNPs produced from the root exhibit a broad peak in the 650nm region, and B. The CaNPs produced from the seed exhibit a broad peak in the 650 nm region, reflecting polydispersity and variable particle sizes.

A



B

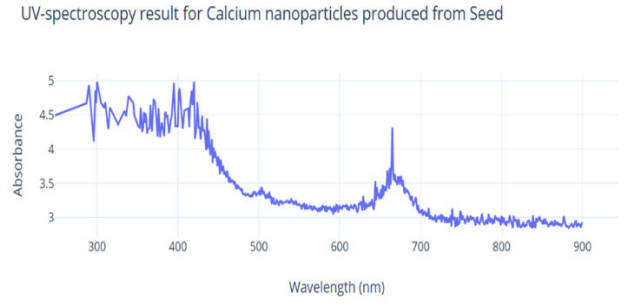


Figure.3 The Bar graph indicating the Climbing Assay Results

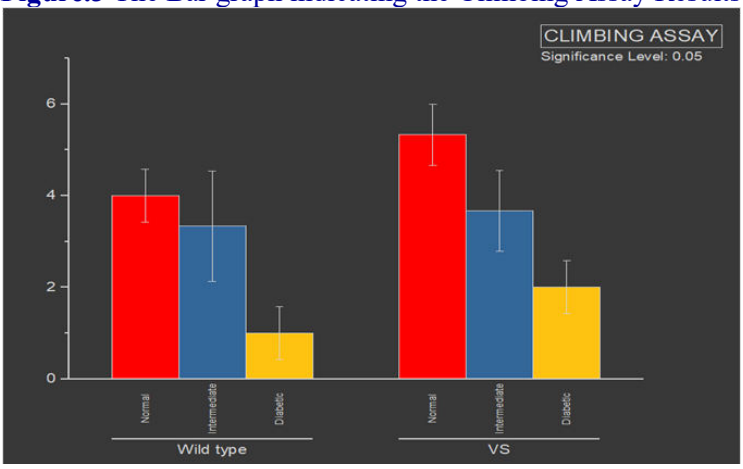


Figure.4A Maltose calibration curve

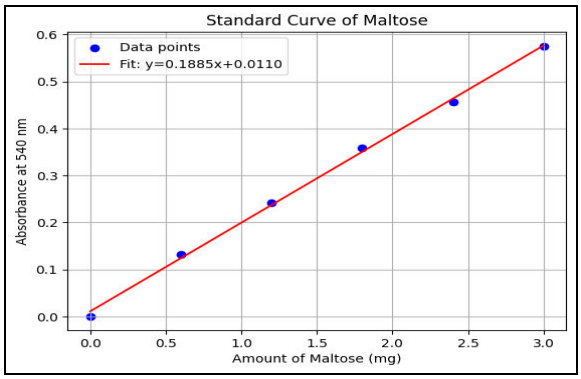
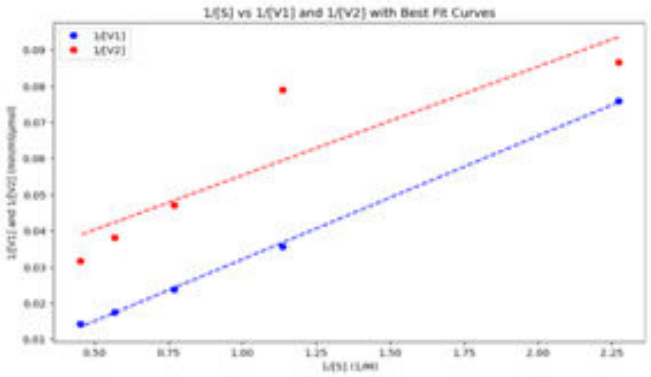


Figure.4B Plot to compare the Km and Vmax values with and without inhibitors.



The blue line represents the case without an inhibitor, while the red line represents the case with an inhibitor.

Figure.5 Bar graph indicating the results of DPPH Radical Scavenging Assay

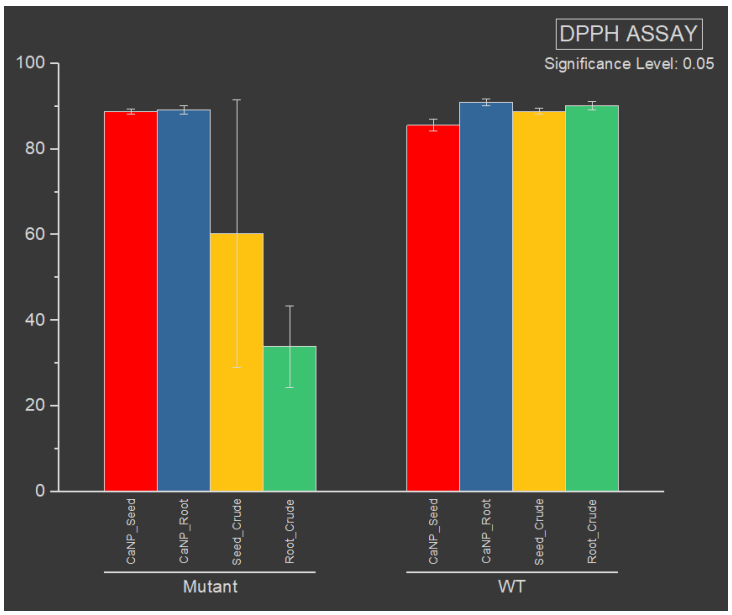
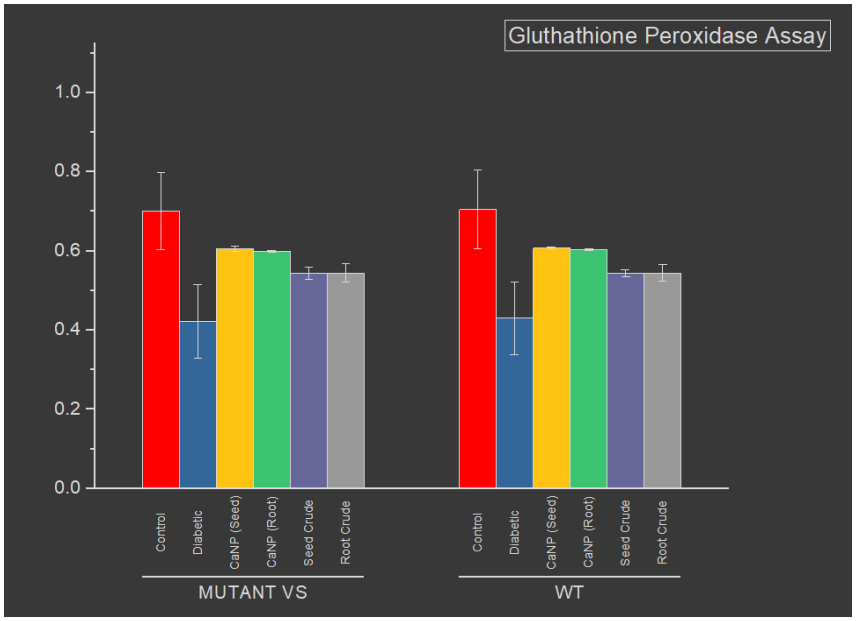


Figure.6 Bar graph indicating the results of the Glutathione Peroxidase Assay



Crude extracts from seeds and roots also displayed significant antioxidant potential, although less pronounced than CaNPs. In the mutant strain, the seed crude extract showed an O.D. avg of 0.086 with 60.91% scavenging activity, while the root crude extract had an O.D. avg of 0.143 and 35% scavenging activity.

For the wild-type strain, both seed and root crude extracts performed comparably to CaNPs, with scavenging activities of 88.8% and 90%, respectively. Notably, the seed extract was more effective in the mutant strain, while the root extract outperformed in the wild-type strain.

Both treatments effectively reduce oxidative stress by neutralizing free radicals through hydrogen atom or electron donation, as reflected in the high scavenging percentages and results are shown in Fig 5. The wild-type strain showed slightly better responsiveness to antioxidant treatments, possibly due to differences in metabolic or genetic factors. Overall, this study highlights the therapeutic potential of CaNPs and plant-derived extracts in managing oxidative stress associated with Diabetes, with CaNPs emerging as the most potent antioxidants.

Glutathione Peroxidase

The glutathione peroxidase assay results indicate a clear trend in oxidative stress levels across different treatment groups. As expected, the diabetic flies exhibited a significant reduction in O.D. values (0.356–0.488) compared to the control group (0.632–0.77), confirming oxidative stress-induced depletion of glutathione. However, treatment with calcium nanoparticles (CaNPs) derived from both seed and root extracts significantly restored O.D. levels (0.598–0.611), bringing them close to normal values, suggesting their strong antioxidative potential.

In contrast, crude seed and root extracts also improved O.D. values (0.528–0.561) but were slightly less effective than their nanoparticle counterparts, likely due to differences in bioavailability and efficacy. Overall, these findings demonstrate that nanoparticle-based treatments exhibit a more potent restorative effect on glutathione levels in diabetic flies, highlighting their potential as effective antidiabetic agents.

The results of the glutathione peroxidase assay are clearly demonstrated in Fig. 6 and the impact of oxidative stress in diabetic flies, as seen in their significantly lower O.D. values. The treatment with calcium nanoparticles (CaNPs) effectively reversed this depletion, restoring glutathione levels to near-normal levels, which highlights their strong antioxidative potential.

Compared to crude seed and root extracts, CaNPs exhibited superior efficacy, likely due to improved bioavailability and interaction at the cellular level. This suggests that nanoparticle-based formulations may offer

a more efficient therapeutic approach for managing oxidative stress and related complications in diabetic conditions, emphasizing their promise as potential antidiabetic agents.

Conclusion

In conclusion, the study highlights that calcium nanoparticles synthesized from medicinal plant roots possess potent antidiabetic, antioxidant, and enzyme-inhibitory properties. Their enhanced bioactivity over crude extracts suggests that nanoparticle formulation enhances the delivery and efficacy of bioactive compounds. The *Drosophila* model proves to be a valuable and efficient tool for the preclinical evaluation of traditional formulations, paving the way for further molecular studies and potential therapeutic applications in diabetes management.

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How to cite this article:

Devaki Devi, K., C. Pavan Kumar and Sreedevi, E. 2025. A Drosophila-Based Platform for Screening Nanoparticles: Synthesis, Characterization, and Antidiabetic Activity of *M. cymbalaria* CaNPs. *Int.J.Curr.Res.Aca.Rev.* 13(05), 120-133. doi: https://doi.org/10.20546/ijcrar.2025.1305.015