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A Comparative Evaluation and Pharmacological Assessment of *Abelmoschus Manihot* Leaves Extract in Vitro Antidiabetic, Neuroprotective Activities, and their Effects on Glucose Uptake and DPP-4 Inhibition

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ABSTRACT

The present research was designed to pharmacologically evaluate the leaves of *Abelmoschus manihot* using various mechanistic models. The plant leaves were extracted using the Soxhlet extraction method, and the resulting extracts underwent preliminary phytochemical screening. The methanol extract (AMLE-M) was further assessed for its pharmacological activity in antidiabetic, antioxidant, and neuroprotective models. Phytochemical analysis confirmed the presence of phenols, flavonoids, alkaloids, and proteins in AMLE-M. The total phenolic content of the extract was 319.69 mg GAE/g. In mechanistic antidiabetic models, AMLE-M enhanced glucose utilization in L6 myoblasts in a dose-dependent manner, with a significant increase observed at 25 µg/mL. Inhibition of DPP-4, a key enzyme in diabetes management, was also observed, with a maximum inhibition of 42.79% at 125 µg/mL. In antioxidant models, AMLE-M significantly reduced intracellular reactive oxygen species (ROS) levels under H_2O_2-induced oxidative stress, with ROS levels decreasing to 166% at 48 µg/mL from 289% in the control. These findings suggest that AMLE-M possesses notable antioxidant, antidiabetic, and neuroprotective activities, highlighting its potential for therapeutic applications in oxidative stress and glucose-related disorders.

INTRODUCTION

Medicinal plants have been a valuable source of bioactive compounds for centuries, offering therapeutic benefits in the treatment and management of various diseases. Among such plants, *Abelmoschus manihot* (L.) Medik., commonly known as the Chinese hibiscus or ornamental okra, has gained attention for its potential medicinal properties [1, 2]. Historically used in traditional Chinese medicine for treating inflammation, infections, and cardiovascular diseases, the pharmacological value of *Abelmoschus manihot* is now being investigated scientifically for its bioactive compounds and therapeutic benefits, particularly in chronic conditions such as diabetes and neurodegenerative disorders [1-4].

Diabetes mellitus, a metabolic disorder characterized by chronic hyperglycemia, has become one of the most prevalent global health concerns. Its management often involves addressing both impaired glucose uptake and the regulation of key enzymes such as dipeptidyl peptidase-IV (DPP-4) [5-11]. This enzyme plays a significant role in glucose homeostasis by degrading incretin hormones, thereby reducing insulin secretion. Natural inhibitors of DPP-4, along with agents that promote glucose uptake, are therefore of considerable interest in developing alternative treatments for diabetes [6, 12-17].. In addition to diabetes, oxidative stress has been implicated in the development of various

chronic diseases, including neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Oxidative stress results from the overproduction of reactive oxygen species (ROS), which leads to cellular damage [16-18]. Compounds with antioxidant properties can mitigate oxidative stress by scavenging ROS and restoring cellular balance. Given the rising interest in neuroprotection and antioxidants, plants like *Abelmoschus manihot*, known to be rich in phenolic compounds, offer promising potential as therapeutic agents for both neuroprotective and antidiabetic applications [5, 7, 8]

The present study was designed to pharmacologically evaluate the extract of *Abelmoschus manihot* leaves using in vitro mechanistic models. The extracts were obtained through Soxhlet extraction, followed by a preliminary phytochemical evaluation to identify the presence of key bioactive compounds such as phenols, flavonoids, alkaloids, and proteins. The focus of the pharmacological assessment was on evaluating the effects in glucose uptake models, DPP-4 inhibition assays, and its antioxidant activity in mitigating ROS levels. Additionally, neuroprotective effects were assessed to explore the extract's ability to protect cells from oxidative damage, a key factor in neurodegenerative diseases. Through a comprehensive analysis of *Abelmoschus manihot* in antidiabetic and neuroprotective models, this study aimed to provide insights into its potential as a natural therapeutic agent. The findings may open new avenues for developing plant-based treatments for diabetes, oxidative stress, and related neurological conditions.

MATERIAL AND METHODS

Chemicals, Drugs, and Biochemical Kits

All chemicals and reagents used in this study were of analytical grade. Methanol, acetone, chloroform, petroleum ether, and water were used as solvents for extraction. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were sourced from Gibco (USA) for cell culture experiments involving L6 myoblasts. Hydrogen peroxide (H_2O_2) was obtained from Merck (Germany) for inducing oxidative stress. Diprotin A, a known DPP-4 inhibitor, was purchased from Sigma-Aldrich (USA). The glucose oxidase-peroxidase (GOD-POD) assay kit was used to measure glucose levels, while DPP-4 inhibition was evaluated using a commercially available DPP-4 assay kit (Bayer). The reactive oxygen species (ROS) were quantified using 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA), also from Sigma-Aldrich. All experiments were conducted following the manufacturer's protocols, and the reagents were stored under appropriate conditions to ensure their stability and efficacy.

Collection of the plant material

The leaves of the medicinal plant *Abelmoschus manihot* (L.) Medik. were collected from the Majhitar region in Sikkim for the study. The plant was identified and authenticated by Dr. Gaurav Pant, a botanist from the Department of Botany. To ensure future reference and validation, a herbarium specimen of the plant, labeled with the accession number AMMK120922/AS/231, has been meticulously prepared and deposited in the pharmacognosy laboratory. This preserved specimen will serve as an important reference point for ongoing and future research related to this plant.

The Preparation of Extracts

The leaves of the plant were dried in the shade over several days to preserve their phytochemical properties. Afterward, the dried leaves were ground into a coarse powder using a mechanical grinder. A total of 500 grams of the powdered plant material was subjected to successive extraction using a Soxhlet apparatus. The extraction process was performed with five different solvents in sequence: petroleum ether, chloroform, acetone, methanol, and water. Each extraction cycle was carried out for several hours to ensure maximum extraction of the plant's bioactive compounds. The extracts obtained from each solvent were then concentrated using a rotary vacuum evaporator (KM Traders, India) at a controlled temperature of less than 45°C to avoid the degradation of heat-sensitive components. The five extracts, labelled as petroleum ether (AMLE-P), chloroform (AMLE-C), acetone (AMLE-A), methanol (AMLE-M), and water (AMLE-W), were stored at 4°C to maintain their stability until further use. Subsequently, all five extracts were evaluated for their total phenolic content. The percentage yield of each extract was determined using a standard formula to quantify the efficiency of the extraction process. This step was crucial in determining the overall extraction efficiency and guiding further phytochemical investigations [19, 20].

% Yield= Extract Amount (gm)/ Initial dry powder drug amount (gm) ×100

Preliminary phytochemical Screening

All the extracts underwent preliminary phytochemical screening to identify the presence of various bioactive compounds. This screening aimed to detect a wide range of phytochemicals, including alkaloids, glycosides, flavonoids, phytosterols, phenols, saponins, proteins, carbohydrates, and others. Standardized phytochemical tests were employed for this purpose, ensuring reliable identification of the different chemical constituents present in each extract. These tests provide a foundational understanding of the plant's chemical profile, offering insight into the potential therapeutic applications of the extracts based on the identified compounds [21].

Total Phenolic Compounds assay

The total phenolic content of the extracts was determined using the Folin-Ciocalteu (FC) method [22]. To begin, the Folin-Ciocalteu reagent was diluted in distilled water at a ratio of 1:10, and a 7.5% sodium carbonate solution was prepared in distilled water. Gallic acid was used as the standard phenolic compound, and a calibration curve was established using different concentrations of gallic acid ranging from 100 to 1000 microg/mL. For each extract, approximately 1 mg was dissolved in 1 ml of methanol to form a stock solution, which was further diluted to an appropriate concentration for the assay. The assay procedure involved mixing 0.5 ml of the extract or standard solution with 2.5 ml of the diluted Folin-Ciocalteu reagent. The mixture was allowed to incubate at room temperature for 5 minutes. After incubation, 2 ml of the prepared 7.5% sodium carbonate solution was added to the mixture, which was then vortexed to ensure thorough mixing. The reaction was further incubated for 30 minutes in the dark at room temperature. Following the incubation period, the absorbance of each sample and standard solution was measured at 765 nm using a UV-visible spectrophotometer. The total phenolic content was calculated by comparing the absorbance values of the samples with the standard calibration curve of gallic acid. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract (mg GAE/g). This method provided a reliable quantification of the phenolic compounds in the extracts, which are known for their antioxidant potential and possible therapeutic properties.

Antidiabetic activity

Utilisation of Glucose by L6 Myoblasts: An Experimental Approach

To determine how glucose was used by L6 myoblast cells, the methods described elsewhere were applied [23]. At a density of 4,000 cells per well, L6 myoblast cells were seeded into 104-well growth plates to carry out the glucose utilization assay. The cells were initially allowed to adhere to the plate and proliferate until they reached approximately 95% confluence. This level of confluence was essential to ensure uniformity in the assay results. During this setup, two rows of the plate were intentionally left cell-free to serve as blanks for the glucose utilization measurements, which would help in eliminating any non-specific readings from the medium. Once the cells reached 95% confluence, the growth medium was replaced with Dulbecco's Modified Eagle Medium (DMEM) containing 2% fetal bovine serum (FBS) to maintain the viability of the cells during the glucose utilization experiment. The cells were then cultured for an additional five days under these conditions. The analysis for glucose utilization was performed 48 hours prior to refreshing the growth medium to ensure that glucose depletion or changes in metabolism due to aging medium were not a confounding factor. To evaluate glucose utilization, varying concentrations of the plant extract (15 µg/ml, 25 µg/ml, and 65 µg/ml) were prepared and added to specific wells. These concentrations were chosen to assess the dose-dependent effect of the extracts on glucose metabolism. In parallel, insulin was used as a positive control at a concentration of 6 µg/ml, known to stimulate glucose uptake in cells. The cells were then incubated for an additional 48 hours with the plant extracts or insulin, providing sufficient time for the

compounds to exert their potential effects on glucose utilization. After the 48-hour incubation period, the growth medium was carefully removed from the wells and replaced with an incubation buffer consisting of 10 milliliters of glucose, 0.2% bovine serum albumin (BSA), and diluted RPMI medium in phosphate-buffered saline (PBS). The buffer was designed to provide a controlled environment for glucose uptake. The cells were then incubated at 37°C for an additional three hours, allowing for interaction between the cells and the available glucose in the buffer. Following the incubation, 6 µl of the incubation medium was extracted from each well to assess glucose consumption. To quantify the glucose concentration remaining in the medium, 220 µl of glucose oxidase reagent (Bayer) was added to each well. The glucose oxidase reagent interacts with glucose in the medium, enabling the quantification of the glucose concentration. The plates were then incubated for 20 minutes at 37°C to allow the reaction to complete. The absorbance of each well was measured at 522 nm using a Multiscan microtitre plate reader (Lab Systems). The absorbance readings from the wells containing cells were compared to the blank wells (without cells) to determine the glucose utilization by the cells. The difference between the wells with cells and the blanks was used to calculate the glucose consumption. The percentage of glucose absorbed by the cells was determined by comparing the results with the untreated control group, serving as a baseline for normal glucose metabolism. Finally, to ensure that the observed glucose utilization was a result of cellular activity and not due to cell death, the MTT assay was performed on representative wells. This viability test confirmed the metabolic activity of the cells, validating the glucose utilization data obtained from the experiment [24].

DPP-4 Inhibition Assay

The DPP-4 inhibition assay was carried out using a modified version of the method proposed by Al-Masri et al. in 2009 [25]. This assay aimed to evaluate the ability of test compounds to inhibit the DPP-4 enzyme, which is a target for managing conditions such as type 2 diabetes. In this procedure, 16 µl of a human recombinant DPP-4 enzyme solution (with an activity of 55 µU/µl in Tris buffer) was first added to the designated wells of a 104-well plate. The enzyme solution was followed by the addition of 36 µl of AMLE-M (test compound) in varying concentrations (ranging from 65 µg/ml to 125 µg/ml) to evaluate the dose-dependent inhibition effect. For comparison, a positive control, diprotin A, at a concentration of 60 µg/ml, was also added to specific wells. This served as a reference to assess the effectiveness of the test compound in inhibiting the enzyme. After setting up the plate, the enzymatic reaction was initiated by adding 55 µl of Gly-Pro-pNA, a chromogenic substrate specific to DPP-4, which was dissolved at a concentration of 22 mM in Tris buffer. However, before adding the substrate, the reaction mixture was incubated for 7 minutes at 37°C to allow the test compounds and enzyme to interact. Following this, the substrate was introduced, and the reaction was allowed to continue for an additional 35 minutes at the same temperature, ensuring optimal enzyme activity and substrate conversion. Once the incubation was complete, the enzymatic reaction was halted by adding 26 µl of a 24% acetic acid solution to each well. This step ensured that the reaction stopped precisely, preventing further enzyme activity. Immediately after stopping the reaction, the absorbance of each well was measured at a wavelength of 411 nm using a microplate reader. The absorbance readings provided a quantitative measure of how much of the chromogenic substrate had been cleaved by the enzyme, giving insight into the enzymatic activity remaining after treatment with the test compounds or controls. In addition to the experimental wells, controls were included to account for any background absorbance that could skew the results. A sample blank was prepared by substituting 36 µl of the AMLE-M with the buffer solution, ensuring that the test compound itself did not contribute to any non-specific absorbance. Similarly, an enzyme blank was created by replacing 16 µl of the enzyme solution with the buffer, ensuring that any non-enzymatic processes did not influence the absorbance readings. The percentage inhibition of the DPP-4 enzyme by each test compound or control was calculated using the following formula:

% inhibition = (Control Absorbance –Test Absorbance) / Control Absorbance × 100

This formula compared the absorbance of the control wells, which represented full enzymatic activity, to the absorbance of the wells treated with the test compound. The result provided a percentage of enzyme inhibition, indicating how effectively the test compound inhibited DPP-4 activity relative to the control. This assay provided valuable data on the potential of the AMLE-M extract and the positive control, diprotin A, in inhibiting DPP-4, which is crucial for exploring therapeutic options for managing glucose levels in diseases such as type 2 diabetes.

Neuroprotective activity

Cell culture and treatment

To investigate the neuroprotective properties of WS, SK-N-SH cells, a human neuroblastoma cell line sourced from the American Type Culture Collection (ATCC), were cultured in Dulbecco's Modified Eagle Medium (DMEM). The medium was supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin to support cell growth. SK-N-SH cells were seeded into 96-well plates (Corning) at a density of 1.5×10^4 cells per well and incubated at 37°C in a humidified environment with 5% CO_2 to allow the cells to adhere and proliferate. After 24 hours, the media was completely removed from the wells, and the cells were transferred to serum-free DMEM, which still contained antibiotics (penicillin and streptomycin). The removal of serum was done to create a stressful environment for the cells, facilitating the assessment of WS's neuroprotective effect under adverse conditions. Subsequently, acrolein, a neurotoxic agent, was introduced at a concentration of 20 µM to induce cellular stress and damage. The cells were exposed to acrolein for 24 hours, providing ample time for the neurotoxic effects to take place, thus creating conditions suitable for evaluating the potential protective effects of WS against neurotoxicity [26, 27].

Intracellular reactive oxygen species level

The intracellular levels of reactive oxygen species (ROS) were measured to evaluate the antioxidant activity of the compound AMLE-M in SK-N-SH cells. This was achieved by using the fluorescent dye 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA), a well-established method for detecting ROS in cells. DCF-DA is a non-fluorescent, cell-permeable compound that enters the cells and is enzymatically deacetylated by intracellular esterases to form DCFH, a non-fluorescent intermediate. In the presence of reactive oxygen species, DCFH is oxidized to 2',7'-dichlorofluorescein (DCF), a highly fluorescent compound. The intensity of the fluorescence emitted by DCF is directly proportional to the amount of ROS present within the cells. For the experiment, SK-N-SH cells were treated with AMLE-M, and after incubation, DCF-DA was added to the cells to assess the compound's ability to scavenge intracellular ROS. The fluorescence intensity was measured using a fluorescence microplate reader, with higher fluorescence indicating higher levels of ROS. Conversely, a reduction in fluorescence after treatment with AMLE-M would suggest its potential antioxidant activity, as it would demonstrate the compound’s ability to reduce intracellular ROS levels. This method provided a quantitative assessment of the compound’s ROS scavenging ability, which is crucial in determining its potential as an antioxidant [27, 28].

Statistical analysis

The statistical analysis of the data was performed using GraphPad Prism version 8. The results were expressed as mean ± standard deviation (SD) to reflect the variability within each dataset. To compare the means between different groups, a One-Way Analysis of Variance (ANOVA) was conducted, followed by Dunnet's *post hoc* test to identify significant differences between the groups. A p-value of less than 0.05 ($P < 0.05$) was considered statistically significant, indicating that the differences between the groups were not due to random chance.

RESULTS AND DISCUSSION

Preliminary phytochemical Screening

The preliminary phytochemical screening results for the various extracts of *Abelmoschus manihot* (L.) Medik. revealed a diverse presence of bioactive compounds across different solvent extracts, including petroleum ether (AMLE-P), methanol (AMLE-M), water (AMLE-W), chloroform (AMLE-C), and acetone (AMLE-A)

(Table 1 and Figure 1). Carbohydrates were consistently present in all extracts, indicating that this compound group was extractable in each of the solvents. Alkaloids were found only in the methanol (AMLE-M) and chloroform (AMLE-C) extracts, suggesting their solubility in these specific solvents. Phenolic compounds were detected in all extracts except for chloroform, with petroleum ether, methanol, water, and acetone showing positive results. Similarly, flavonoids were found in all extracts except for chloroform, highlighting that these compounds are widely present in the plant but are not easily extracted with chloroform. Methanol was the only solvent capable of extracting glycosides, indicating that it is particularly effective for this class of compounds. Phytosterols were present in petroleum ether, methanol, water, and chloroform extracts but were absent in acetone, showing that acetone is not effective for extracting phytosterols. Interestingly, saponins were absent in all extracts, suggesting either a low presence in the plant or that these solvents are unsuitable for their extraction. Terpenoids were consistently found in all extracts, indicating that they are a dominant compound in the plant and readily extractable by any of the solvents tested. Tannins were only detected in the petroleum ether extract, indicating that this solvent is particularly effective for extracting tannins, while the other solvents were not. Lastly, proteins were found in petroleum ether, methanol, water, and acetone extracts but not in chloroform, suggesting that chloroform may not be suitable for extracting proteins. Overall, methanol emerged as the most versatile solvent, effectively extracting a wide range of phytochemicals, while chloroform showed more limited extraction capabilities, particularly for flavonoids, phenols, and proteins.

Tablet 1. The preliminary pharmaceutical screening results for all the extracts

Phytochemical test	AMLE-P	AMLE-M	AMLE-W	AMLE-C	AMLE-A
Carbohydrates	+	+	+	+	+
Alkaloids	-	+	-	+	-
Phenols	+	+	+	-	+
Flavonoids	+	+	+	-	+
Glycosides	-	+	-	-	-
Phytosterols	+	+	+	+	-
Saponins	-	-	-	-	-
Terpenoids	+	+	+	+	+
Tannins	+	-	-	-	-
Proteins	+	+	+	-	+

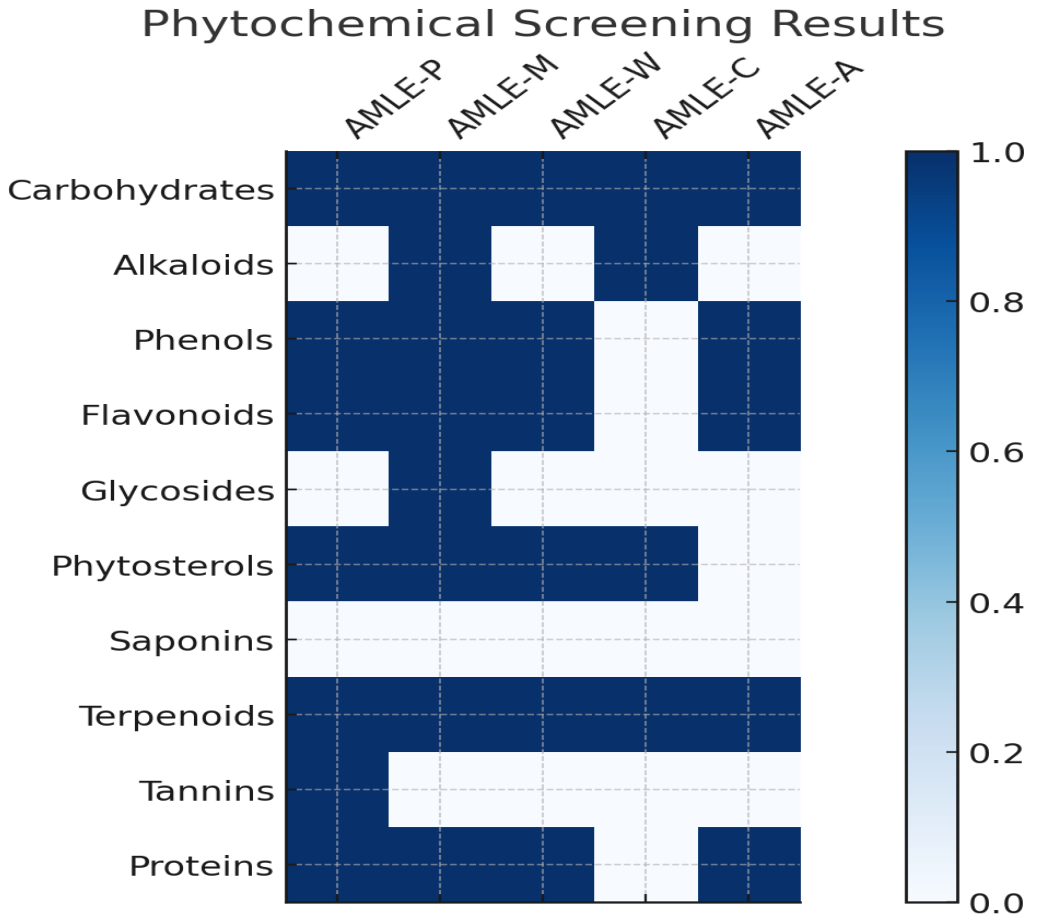

Figure 1. A heatmap representing the phytochemical screening results for the different extracts of *Abelmoschus manihot* (AMLE-P, AMLE-M, AMLE-W, AMLE-C, and AMLE-A).

Total Phenolic Compounds assay

The data from Table 2 presents the total phenolic content in different extracts of *Abelmoschus manihot* (L.) Medik. and the corresponding linear regression equations. The total phenolic content is expressed in gallic acid equivalents (GAE) per gram of extract, and the regression equations indicate the relationship between absorbance and concentration for each extract, with high R^2 values suggesting a good fit for the data. Among the

extracts, methanol (AMLE-M) exhibited the highest total phenolic content at 319.69 mg GAE/g, indicating that methanol was the most effective solvent for extracting phenolic compounds. The linear regression equation for AMLE-M is y=0.0052x+0.0887y = 0.0052x + 0.0887y=0.0052x+0.0887 with an R^2 value of 0.9687, demonstrating a strong correlation between the data points. The acetone extract (AMLE-A) also showed a high phenolic content of 296.22 mg GAE/g, with the regression equation y=0.0091x+0.0652y = 0.0091x + 0.0652y=0.0091x+0.0652 and an R^2 of 0.9568. This suggests that acetone is also highly effective at extracting phenolic compounds, though slightly less than methanol. The chloroform extract (AMLE-C) had a total phenolic content of 227.87 mg GAE/g, with a regression equation of y=0.0085x+0.0989y = 0.0085x + 0.0989y=0.0085x+0.0989 and an R^2 of 0.9562, indicating moderate phenolic content and a reliable linear relationship. Petroleum ether (AMLE-P) and water (AMLE-W) extracts showed lower total phenolic contents of 224.67 mg GAE/g and 213.86 mg GAE/g, respectively. AMLE-P had a regression equation of y =0.0036x+0.07845y = 0.0036x + 0.07845y = 0.0036x + 0.07845 with an R^2 of 0.9883, and AMLE-W had an equation of y = 0.0072x+0.0889y = 0.0072x + 0.0889y=0.0072x+0.0889 with an R^2 of 0.9745 (Table 2 and Figure 2). Despite lower phenolic content, these extracts still demonstrated a strong correlation in their regression models. In summary, methanol was the most efficient solvent for extracting phenolic compounds, followed by acetone. Chloroform, petroleum ether, and water showed lower but still substantial phenolic content, with all regression models exhibiting high R^2 values, suggesting a good fit for the data across all extracts.

Table 2. The amount of total phenolic contents and the linear regression analysis equations for all the extracts

Extracts	Regression Equation	Total Phenolic content in GAE per gram of extract
AMLE-P	y = 0.0036x + 0.07845 R^2 = 0.9883	224.67
AMLE-M	y = 0.0052x + 0.0887 R^2 = 0.9687	319.69
AMLE-W	y = 0.0072x + 0.0889 R^2 = 0.9745	213.86
AMLE-C	y = 0.0085x + 0.0989 R^2 = 0.9562	227.87
AMLE-A	y = 0.0091x + 0.0652 R^2 = 0.9568	296.22

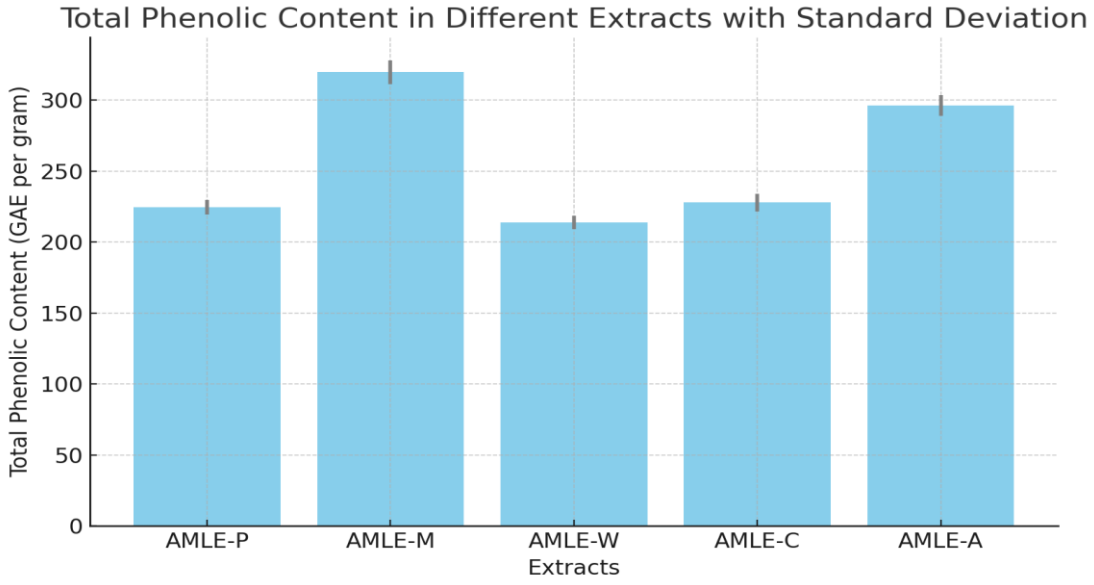

Figure 2. The amount of total phenolic contents in terms GAE per gram for all the extracts

Antidiabetic activity

Glucose Utilization in L6 Myoblast

The glucose utilization data in L6 myoblast cells indicate a clear response to both insulin and varying concentrations of the methanol extract of *Abelmoschus manihot* (AMLE-M) (Figure 3). In the control group, the baseline glucose utilization was 99.99 ± 2.29, which served as the reference point for evaluating the effects of the treatments. Insulin, at a concentration of 6 µg/mL, significantly enhanced glucose uptake, with a recorded value of 144.95 ± 3.67, demonstrating its potent effect on increasing glucose utilization in muscle cells. When treated with AMLE-M at 15 µg/mL, glucose utilization increased to 115.04 ± 2.77, showing a moderate improvement compared to the control group. As the concentration of AMLE-M was increased to 25 µg/mL, glucose uptake further improved to 122.99 ± 2.82, suggesting a dose-dependent effect. However, at the highest concentration of AMLE-M (65 µg/mL), glucose utilization was 116.99 ± 2.89, which did not show a significant improvement over the 25 µg/mL concentration, indicating that higher doses of AMLE-M may not continue to enhance glucose uptake beyond a certain threshold. In summary, insulin exhibited the most substantial increase in glucose utilization, while AMLE-M also demonstrated a positive effect, particularly at 25 µg/mL. The data suggest that AMLE-M could potentially improve glucose uptake, although its efficacy appears to plateau at higher concentrations.

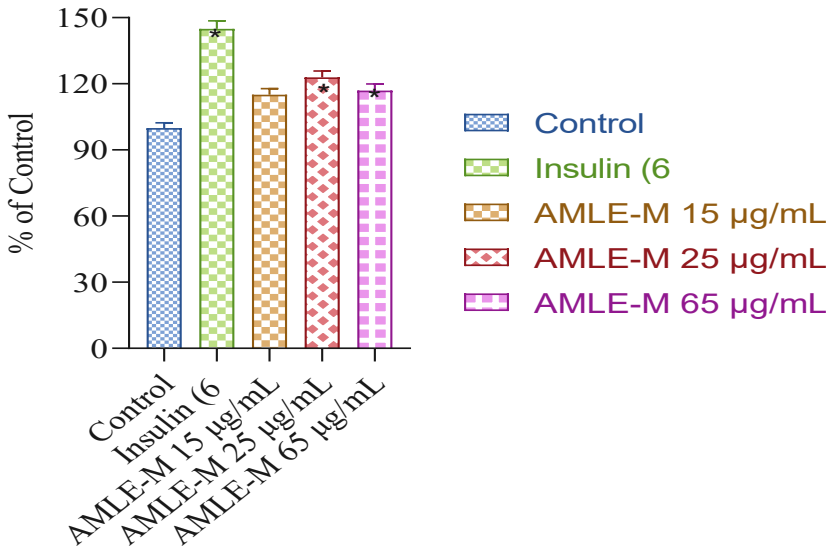

Figure 3. Impact of AMLE-M on L6 myoblast glucose uptake. The plant extract was applied to the cells either at a constant concentration orat different concentrations for 48 hours.

Inhibition Assay of DPP-4

The DPP-4 inhibition assay results show a clear comparison between the control, Diprotin A, and different concentrations of the methanol extract of *Abelmoschus manihot* (AMLE-M) (Figure 4). The control group exhibited 0% inhibition (0 ± 0), indicating no DPP-4 inhibition in the absence of any active compound. Diprotin A, a known DPP-4 inhibitor used as the positive control at 65 µg/mL, demonstrated a strong inhibition of 89.69 ± 1.75%, confirming the effectiveness of the assay and validating its use as a benchmark for DPP-4 inhibitory activity. At 65 µg/mL, AMLE-M showed moderate inhibition of DPP-4, with a percentage inhibition of 19.92 ± 1.02%, indicating some capacity to inhibit the enzyme, though significantly lower than Diprotin A. When the concentration of AMLE-M was increased to 125 µg/mL, the inhibition rose to 42.79 ± 1.11%, demonstrating a dose-dependent effect. The increase in DPP-4 inhibition at higher concentrations suggests that AMLE-M has potential as an enzyme inhibitor, though it remains less potent than Diprotin A. In summary, while Diprotin A provided the highest level of DPP-4 inhibition, AMLE-M also displayed notable inhibitory effects, particularly at higher concentrations, suggesting its potential for further exploration as a DPP-4 inhibitor.

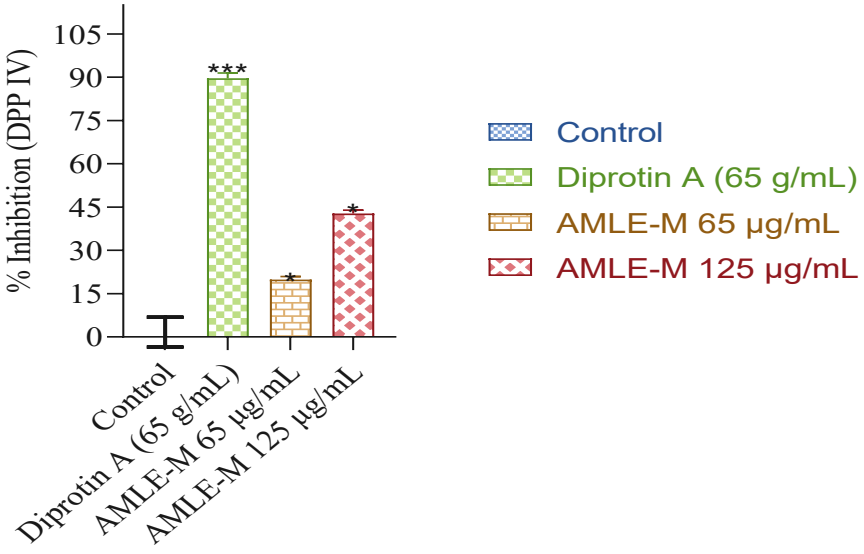

Figure 4. The impact of AMLE-M on the percentage of DPP-4 activity inhibition.

Neuroprotective activity

Intracellular reactive oxygen species levels are lowered by AMLE-M

The data on the impact of *Abelmoschus manihot* methanol extract (AMLE-M) on intracellular reactive oxygen species (ROS) levels indicates a dose-dependent reduction in ROS, particularly under oxidative stress induced by hydrogen peroxide (H_2O_2). The control group, which represents normal cellular conditions, had a baseline ROS level set at 100. When H_2O_2 (500 µM) was applied, it caused a significant increase in ROS levels to 289 ± 7.58, confirming the induction of oxidative stress. At a low concentration of 3 µg/ml, AMLE-M showed minimal effect on ROS levels (288 ± 6.98), suggesting that the antioxidant activity of the extract was not yet prominent. However, at 6 µg/ml, a slight reduction in ROS was observed (248 ± 6.31), indicating that AMLE-M started to exhibit some antioxidant effects. As the concentration increased to 12

µg/ml, ROS levels decreased further to 230 ± 5.08, showing a more noticeable reduction in oxidative stress. At 24 µg/ml, the ROS levels dropped significantly to 170 ± 4.17, demonstrating strong antioxidant activity at this concentration. The highest concentration tested, 48 µg/ml, resulted in the greatest reduction in ROS levels (166 ± 4.27), confirming that AMLE-M has potent antioxidant properties that increase with concentration. Overall, AMLE-M effectively reduced intracellular ROS in a concentration-dependent manner, with the most substantial reduction observed at higher concentrations, particularly at 24 µg/ml and 48 µg/ml. This indicates that AMLE-M could play a significant role in mitigating oxidative stress in cells (Figure 5).

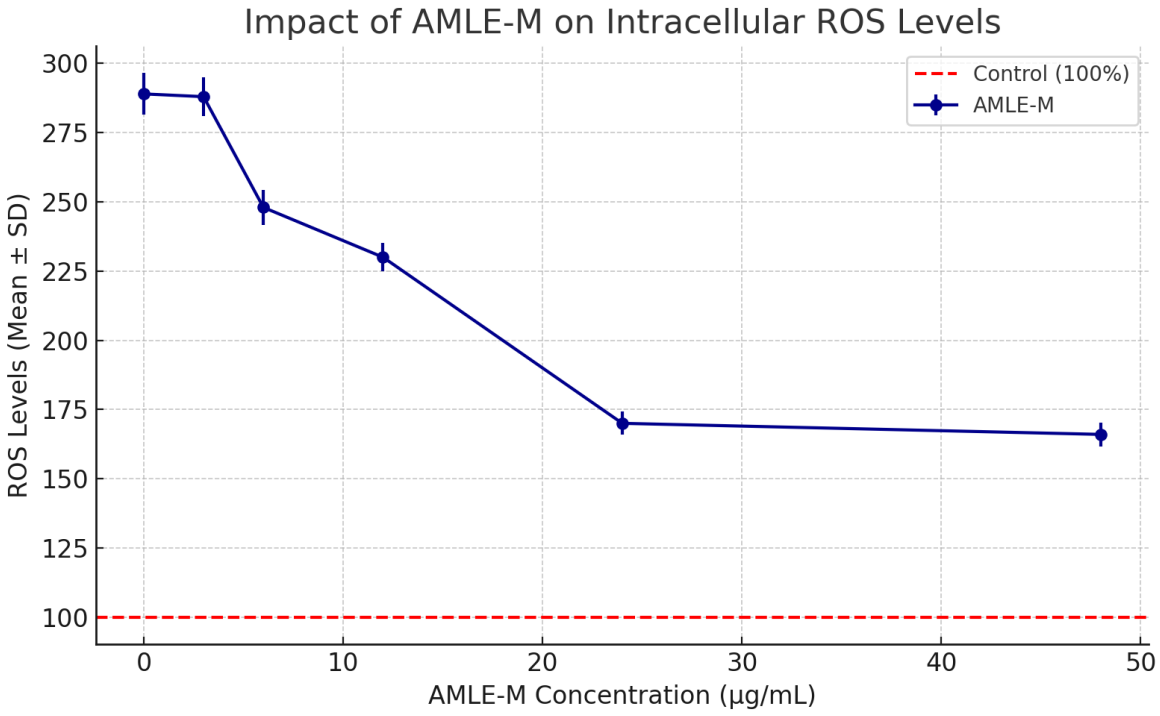

Figure 5. The amount of intracellular reactive oxygen species is reduced by AMLE-M.

CONCLUSIONS

The methanol extract of *Abelmoschus manihot* (AMLE-M) demonstrated promising bioactive properties, including antioxidant, antidiabetic, and DPP-4 inhibitory effects. Phytochemical analysis confirmed the presence of essential compounds such as phenols, flavonoids, and alkaloids, which contribute to its biological activities. AMLE-M exhibited the highest total phenolic content (319.69 mg GAE/g), indicative of its strong antioxidant capacity. In intracellular ROS assays, AMLE-M significantly reduced oxidative stress in a dose-dependent manner, with ROS levels declining to 166% at the highest tested concentration of 48 µg/mL, compared to 289% in the H_2O_2-treated control. In glucose utilization studies on L6 myoblasts, AMLE-M enhanced glucose uptake, with the most notable improvement seen at 25 µg/mL. This suggests that the extract has potential benefits for managing glucose metabolism. Additionally, the DPP-4 inhibition assay revealed that AMLE-M moderately inhibits the enzyme, with a maximum inhibition of 42.79% at 125 µg/mL, indicating its potential role in regulating blood glucose levels by inhibiting DPP-4, a critical enzyme involved in glucose homeostasis. These findings collectively highlight *Abelmoschus manihot* as a valuable natural source for the development of therapeutic agents targeting oxidative stress, glucose regulation, and enzyme inhibition in conditions such as diabetes.

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