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Phytochemical Profiling and Antidiabetic Potential of *Argemone mexicana* L. (Papaveraceae) Leaves: An *In-vitro* Study

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Abstract

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia, which, if left untreated, can lead to severe complications. The limitations of existing synthetic antidiabetic drugs have driven the interest in plant-based alternatives with fewer side effects. *Argemone mexicana*, a medicinal plant widely used in traditional medicine, has been explored for its potential antidiabetic properties. This study aimed to evaluate the phytochemical composition, nutritional profile, and *in vitro* antidiabetic potential of *Argemone mexicana.* The methanol extract of *A. mexicana* leaves was subjected to qualitative and quantitative phytochemical analyses to identify bioactive compounds. Proximate analyses were conducted to determine the nutritional composition of the plants. The antioxidant activity was evaluated using DPPH radical scavenging and total antioxidant capacity assays. Enzyme inhibition studies were performed to assess the inhibitory effects of the extract on α-amylase and α-glucosidase activity. Phytochemical analysis revealed the presence of significant amounts of alkaloids, flavonoids, tannins, and phenolic compounds. Proximate analysis indicated the presence of crude protein, fiber, and essential micronutrients. The extract exhibited strong antioxidant activity. The enzyme inhibition assays showed substantial inhibitory effects on α-amylase and α-glucosidase, with inhibition rates comparable to those of acarbose. These findings suggested that *A. mexicana* possesses bioactive properties that may contribute to diabetes management by modulating carbohydrate digestion and glucose absorption.

Keywords: Phytochemical analysis, *Argemone mexicana*, Antidiabetic activity, Enzyme inhibition, Antioxidant

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Introduction

Since ancient times, medicinal plants have played a crucial role in human health and have been widely used in traditional medicine for their healing and therapeutic properties. These plants serve as rich sources of phytochemicals, particularly secondary metabolites, which are responsible for their diverse therapeutic effects (Plaskova and Mlcek, 2023). The reliance on medicinal plants remains significant in many modern societies, especially in developing regions where people continue to harness the medicinal potential of wild plants (Hardy, 2021). Medicinal plants have been used to treat a wide range of diseases in humans and animals. Various plant families have been investigated for their anti-hyperglycemic properties, which are particularly beneficial for managing diabetes mellitus (Riyaphan *et al*., 2021).

Diabetes mellitus (DM) is a group of metabolic disorders characterized by high blood glucose levels over prolonged periods. Common symptoms include frequent urination, excessive thirst, and increased hunger (Kumar *et al*. 2020). Diabetes can be classified into several types: type 1, caused by autoimmune destruction of β-cells; type 2, resulting from a progressive loss of β-cell function; specific types due to other causes (e.g., monogenic diabetes syndromes or diseases of the pancreas); and gestational diabetes (ElSayed *et al.*, 2023). Globally, diabetes is a growing public health challenge affecting an estimated 537 million adults between the ages of 20 and 79, accounting for 10.5% of this age group. By 2030, the global diabetic population is expected to increase to 643 million, and by 2045, it is projected to reach 783 million (Kumar *et al*., 2024).

Among medicinal plants, Argemone *mexicana*, a poppy species belonging to the family Papaveraceae, has garnered attention for its medicinal potential. This annual herb thrives in drought-prone and nutrient-deficient soils and is widely cultivated in various parts of the world. Known for its high oil content, ranging from 30% to 40%, *Argemone mexicana* contains a combination of saturated fatty acids (stearic acid and palmitic acid) and unsaturated fatty acids (oleic acid and linoleic acid) (Ashine *et al.*, 2023). These phytochemical properties contribute to its therapeutic application, making it an important plant in the ongoing search for natural treatments for metabolic disorders, including diabetes.

This study aimed to evaluate the phytochemical composition, nutritional profile, and *in vitro* antidiabetic potential of *Argemone mexicana* by assessing its bioactive constituents and enzyme inhibitory activity against α-amylase and α-glucosidase, two key enzymes in carbohydrate metabolism.

Materials and Methods

Sample Preparation and Extraction

Fresh plant materials were collected in June 2024, and identified in the Herbarium of University of Lagos by Dr. G. Nodza, and voucher number LUH 100207 was assigned. The plant material was washed and air-dried for one week. The dried material was coarsely powdered and 100 g was macerated in methanol at room temperature for 72 h with occasional stirring. The extract was filtered, concentrated using a rotary evaporator at a low temperature, and stored in labeled amber vials at 4°C for further analysis (Jaiswal *et al.*, 2023).

Qualitative Phytochemical Analysis

The presence of bioactive compounds in the extracts were assessed by standard methods (Nortjie *et al*. 2022).

Estimation of Phenolic Compound Content

The total phenolic content of the extracts was quantified using Folin-Ciocalteu reagent, following the method described by Singleton and Rossi (Singleton *et al*., 1999). A calibration curve was generated by mixing 1 ml aliquots of gallic acid solutions (10, 20, 50, 100, and 250 µg/ml) with 5.0 ml of Folin-Ciocalteu reagent (diluted 1:10) and 4.0 ml of sodium carbonate solution (75 g/l). Absorbance was measured at 765 nm after 30 min. For extract analysis (1 g/100 ml), 1 ml of each extract was treated with the same reagents as the calibration curve. After 1 h, the absorbance was recorded and the total phenolic content was calculated (Singleton *et al*., 1999).

Estimation of Total Flavonoid Content

The total flavonoid content was measured based on the formation of a flavonoid-aluminum complex with a maximum absorbance at 415 nm (Sembiring *et al*., 2018). For the assay, 100 µl of each extract in methanol (10 mg/ml) was mixed with 100 µl of 20% aluminum chloride in methanol and a drop of acetic acid, and diluted to 5 ml with methanol. After 40 min of incubation, absorbance was recorded at 415 nm. A blank was prepared using 100 µl of extract, acetic acid, and methanol. Rutin (0.5 mg/ml) was used as a standard under the same conditions. All tests were performed in triplicate, and the results are expressed as rutin equivalents (mg RE/g extract).

Estimation of Total Antioxidant Capacity (Phosphomolybdate Method)

A 0.1 ml aliquot of the extract (10 mg/ml) in water was combined with 1 ml of reagent solution, prepared by mixing 0.6 M sulfuric acid (3.14 ml in 100 ml), 28 mM sodium phosphate (397.49 mg in 100 ml), and 4 mM ammonium molybdate (494.36 mg in 100 ml). The mixture was incubated at 95°C for 90 min and then cooled to the ambient temperature. The absorbance was measured at 695 nm using a UV spectrophotometer (Thermospectronic BioMate 3, USA). Antioxidant capacity was expressed as the equivalent amount of ascorbic acid (Zengin *et al*., 2011).

Determination of Antioxidant Activity Using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method

A DPPH solution was prepared by dissolving crystalline DPPH in analytical-grade methanol and stored at 4°C. For the antioxidant assay, 2 mL of the DPPH solution was mixed with 2 mL of the extract, and tested at concentrations of 10, 25, 50, 100, and 250 µg/mL. The mixture was incubated in the dark for 30 minutes, followed by absorbance measurement at 517 nm using a UV-Vis spectrophotometer (Thermospectronic BioMate 3, USA). Methanol served as the blank (Sembiring *et al*., 2018).

Antioxidant activity was calculated using the following formula:

$$\%\ Inhibition\ = \left[\frac{\left(A_{control}\ -\ A_{sample}\right)}{A_{control}}\right] \times\ 100\%. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (2)$$

Proximate Analysis

Proximate analysis is used to determine the chemical composition of a compound, focusing on four key elements: moisture content, fixed carbon, volatile matter, and ash content. Ganogpichayagrai and Suksaard outlined the procedures used to determine these components (Ganogpichayagrai and Suksaard, 2020).

Determination of Moisture Content

To determine the moisture content, 2 g of the dry powdered sample was placed in a pre-weighed 50 ml porcelain crucible and heated in an oven at 105°C for 3 to 5 h. After heating, the crucible was removed and allowed to cool in a desiccator at room temperature before weighing. The crucible was then returned to the oven, and the process was repeated two to three times for 30 min each time, until a constant weight was achieved (Ganogpichayagrai and Suksaard, 2020). The moisture content was calculated

Determination of Total Ash Content

A precise amount (2 g) of the sample was placed in a pre-ignited and tared silica dish. The material was evenly spread and then ignited in a muffle furnace (incinerator), and the temperature was gradually raised between 500 and 600°C until it turned a greyish-white color, indicating that all carbon was removed. After cooling in a desiccator, the dishes were weighed (Ganogpichayagrai and Suksaard,
2020). The percentage of the total ash in the dried material was calculated.

Crude
Protein

About one gram of sample material was accurately weighed and placed in a decomposition flask. To this,
1 g of Na_2SO_4, 0.1 gram of $CuSO_4$, 1-2 selenium boiling granules, and concentrated H_2SO_3 (20 mL) were added. The mixture was boiled until it became almost colourless or light green, typically for 3–6 h for inorganic substances, with an additional 30 min of boiling. Care was taken to ensure that no part of the Kjeldahl flask was heated to above the level of the decomposition mixture. After boiling, the flask was allowed to cool slightly to prevent solidification, and
200 ml of water was added to bring the total volume up to 250 ml. Boiling granules were added, if necessary, to prevent foaming. About 50mL of the solution was transferred into a 500 ml Erlenmeyer
flask using a pipette, and 50 ml of 40% NaOH solution was slowly introduced along the side of the flask until a distinct layer formed beneath the digestion mixture. The flask was then connected to the distillation apparatus, which contained 50 ml of–
2-4% boric acid solution with two drops of Conway's indicator. The flask was swirled to ensure thorough mixing of contents. Heating was applied until all ammonia was distilled into the boric acid solution, and approximately 150 ml of the distillate was collected. Care was taken not to disturb the bulb and precautions were taken to avoid boiling or distilling
the acid too quickly to prevent ammonia loss. The condenser tip was rinsed, and the distillate was titrated with a 0.05-0.5 M standard HCl solution (Ganogpichayagrai and Suksaard, 2020).

Determination of Crude Lipids

Dried and ground samples were extracted using an organic solvent (diethyl ether, hexane, or petroleum ether) to dissolve fats, oils, pigments, and other fat-soluble substances. After the extraction, the solvent was evaporated from the fat solution. The remaining residue was weighed and recorded as either ether extract or crude fat content (Ganogpichayagrai and Suksaard, 2020). The crude fat percentage (essential extract) was calculated.

Alpha Glucosidase and Alpha-Amylase Assays

The enzyme inhibitory potential of the extract was determined using alpha-glucosidase and alpha-amylase inhibitory assays as described by Man *et al*. (2022), with slight modifications.

Alpha amylase assay

The extract was prepared at a concentration of 20 mg/mL, and various dilutions were prepared from this solution, resulting in final concentrations of 10, 20, 50, 100, and 250 µg/mL using phosphate buffer (pH 6.9) as a diluent to achieve a final volume of 1 mL. Each mixture was then incubated at 37°C for 30 min. After incubation, 1 mL of the potato starch solution was added to each sample, followed by an additional incubation period of 20 min. Subsequently, 1 mL of 3,5-Dinitrosalicylic acid (DNSA) was added and the samples were placed in a hot water bath at 85-90°C for 5 min to facilitate colour development. After cooling, the absorbance of each sample was measured at 540 nm using a UV spectrophotometer in triplicates to evaluate the inhibitory effects on α-amylase activity. The methanol extract of *Argemone mexicana* was prepared at a concentration of 20 mg/mL by mixing120 µL of the extract with 480 µL of distilled water. Serial dilutions were made from this solution, yielding final concentrations of 10, 20, 50, 100, and 250 µg/mL, using phosphate buffer (pH 6.9) as the diluent to achieve a final volume of 1 mL. Each mixture was then incubated at 37°C for 30 min. After incubation, 1 mL of the potato starch solution was added to each sample and incubated for an additional 20-minute incubation. Subsequently, 1 mL of 3,5-Dinitrosalicylic acid (DNSA) was added and the samples were incubated in a hot water bath at 85-90°C for 5 min to facilitate colour development. After cooling, the absorbance of each sample was measured at 540 nm in triplicates using a UV spectrophotometer to assess the inhibitory effects on alpha-amylase activity. The level of inhibition (%) was then calculated.

Alpha glucosidase assay

Reaction mixtures were prepared by mixing varying concentrations of the extract (10 µg/mL, 20 µg/mL, 50 µg/mL, 100 µg/mL, 250 µg/mL) with 3,5-dinitrosalicylic acid and 1.0 U/mL of α-glucosidase in sodium phosphate buffer (pH 8.0). Acarbose was used as the positive control. After a 5-minute incubation at 37°C, the reaction was terminated by boiling the mixtures for an additional 5 min. The experiments were conducted in triplicate, and the absorbance was measured at 540 nm using a spectrophotometer to assess enzyme activity. The level of inhibition (%) was then calculated.

Results

The phytoconstituents of *A. mexicana* are alkaloids, anthraquinones, terpenoids, tannins, cardiac glycosides, phenolic compounds, flavonoids, reducing sugars, and steroids. The amount of Phenolics (21.16 mg/100g of sample), Flavonoid (67.45 mg/100g of sample) and the Total Antioxidant Capacity (57.70 mg/g Ascorbic Acid) were quantified (Table 1)

Antioxidant activity

The DPPH scavenging activities of *argemone mexicana* as a percentage inhibition against concentration and that of the standard, ascorbic acid, were recorded. The IC_{50}. were 3.962847 µg/ml for *A. mexicana* (figure 1) and 1.157671 µg/ml for ascorbic acid (figure 2)

Table 1: Quantitative analysis

Phytoconstituents	Mean ± Sem
Phenolic compound	21.16 ± 0.1887 mg/100g of sample
Flavonoids	67.45 ± 0.5563 mg/100g of sample
Total Antioxidant Capacity	57.70 ± 0.248 mg/g Ascorbic Acid

Results of the Proximate analysis

Proximate analysis showing the nutritional composition, including moisture (7.50), ash (19.00), crude protein (6.55), fat (3.78), and fiber (22.19) contents in *A. mexicana were* determined and expressed as percentages (Table 2).

Table 2: Proximate analysis

SAMPLE / PARAMETERS	MEAN ± SEM (g/100g of dry sample)
Moisture Content (%)	7.50 ± 0.5
Ash Value (%)	19.00 ± 1.00
Crude Fibre (%)	22.19 ± 0.49
Crude Fat/Lipid (%)	3.78 ± 1.02
Crude Protein (%)	6.545 ± 0.435

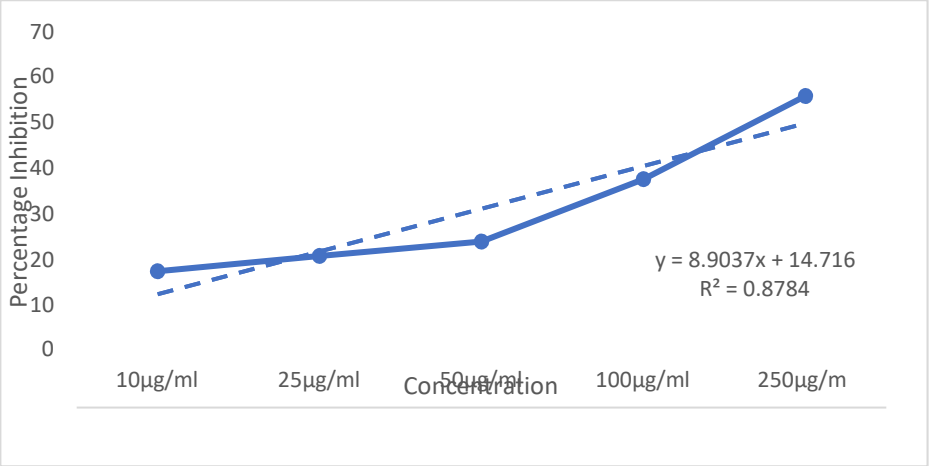

Figure 1: DPPH Scavenging Activity of *A. Mexicana*

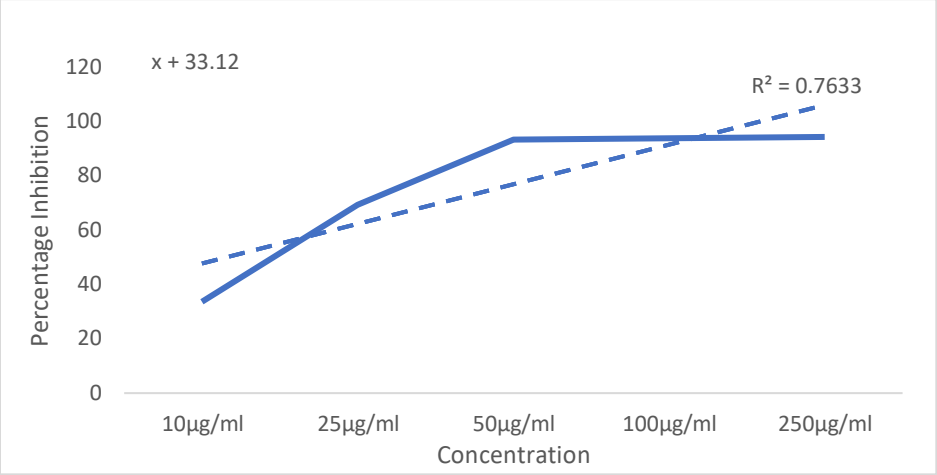

Figure 2: DPPH Scavenging Activity of Ascorbic Acid

Effect on alpha amylase activity

The percentage inhibition of alpha amylase by the extract and acarbose (used as a standard) at different concentrations (10–250 µg/ml) suggested a dose-dependent increase with increasing concentrations, although ascorbic acid inhibited alpha amylase at a higher rate than the extract (figure 3).

Effect on alpha glucosidase activity

The percentage inhibition of α-glucosidase by the extract showed a close and comparable increase as the concentration increased, similar to that of the standard (figure 4).

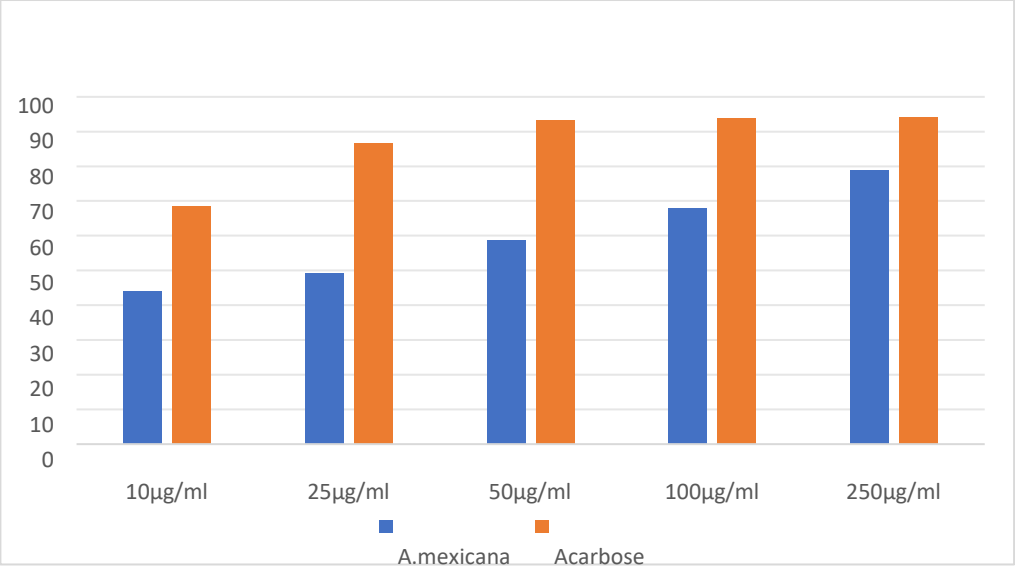

Figure 3: Alpha Amylase Inhibitory Activity of *Argemone mexicana and* Acarbose at various Concentrations

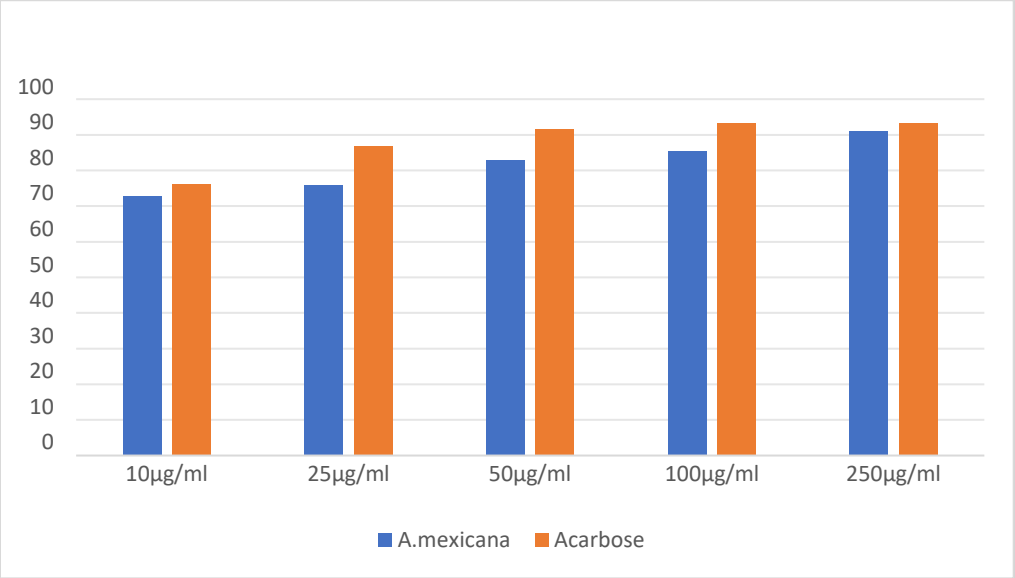

Figure 4: Alpha Glucosidase Inhibitory Activity of *Argemone mexicana and Acabose* at various Concentrations

Discussion

This study aimed to evaluate the antidiabetic study potential of *Argemone mexicana* leaves through comprehensive phytochemical screening and enzyme inhibition assays. The results confirmed the presence of a diverse array of bioactive compounds,

consistent with previous studies and reinforcing the medicinal relevance of the plant, particularly in diabetes management. These phytochemical findings are consistent with those of Haruna (2019), who identified similar phytochemicals in *A. mexicana*. However, the absence of saponins in this study contrasts with some literature, such as Khan and Bhadauria (2019), which reported their presence. This discrepancy may be attributed to differences in extraction methods, plant maturity, or environmental factors affecting phytochemical composition.

The identified phytochemicals underscored the medicinal potential of *A. mexicana*. Alkaloids, which are abundant in the extract, have been extensively studied for their hypoglycemic effects, primarily through enzyme inhibition and glucose metabolism modulation (Rout *et al*., 2011). Flavonoids, tannins, and phenolic compounds, which are known for their strong antioxidant properties, may contribute to the plant's antidiabetic activity by reducing oxidative stress, which plays a critical role in insulin resistance and pancreatic β-cell dysfunction. These compounds may improve insulin sensitivity and glucose uptake by mitigating oxidative damage and thereby enhancing glycemic control.

A key finding of this study was the high total antioxidant capacity of *A. mexicana*, of 57.703 mg/g of ascorbic acid (AA). This is a notable concentration, considering that vitamin C is widely recognized for its role in reducing blood glucose levels, enhancing insulin synthesis, and protecting against oxidative damage associated with type 2 diabetes mellitus (T2DM) (Mason *et al*., 2021)]. Given that citrus fruits, one of the richest dietary sources of vitamin C typically contain 50–70 mg/100 g (or 0.5–0.7 mg/g) (Rishi *et al*., 2023), *A. mexicana* presents itself as a competitive natural source of ascorbic acid. This suggests potential applications not only in diabetes management, but also in nutritional supplementation, particularly in regions where the plant is traditionally consumed.

To further assess the antidiabetic properties of *A. mexicana*, its ability to inhibit key carbohydrate-digesting enzymes, α-amylase and α-glucosidase, was evaluated and compared with that of acarbose, a standard pharmaceutical inhibitor. Acarbose competitively inhibits α-amylase and α-glucosidase, delaying carbohydrate breakdown into glucose and thereby reducing postprandial hyperglycemia (Matsuda and DeFronzo, 1999).

The results demonstrated that *A. mexicana* exhibited significant inhibitory effects against α-amylase, with increasing concentrations leading to progressively lower absorbance values, indicating stronger enzyme inhibition. The highest concentration tested (250 µg/mL). The percentage inhibition of the extract ranged from 43.9% to 78.8%, suggesting a dose-dependent effect, which implies that *A. mexicana* could potentially slow the digestion of complex carbohydrates, reduce the rapid release of glucose into the bloodstream, and mitigate postprandial hyperglycemia.

Similarly, an α-glucosidase inhibition assay confirmed the strong antidiabetic potential of the plant. There was a dose-dependent increase in inhibitory activity as the concentration increased. The inhibition percentage ranged from 72.8% to 90.9%, suggesting that *A. mexicana* effectively delayed the final step of carbohydrate digestion and glucose absorption in the intestine. This mechanism is crucial in diabetes management as it helps prevent rapid spikes in blood glucose levels after meals.

Compared to acarbose, *A. mexicana* demonstrated a promising profile for inhibiting both α-amylase and α-glucosidase, making it a potential natural alternative for glycemic control. Although acarbose exhibited slightly higher inhibition percentages, its plant extract activity at higher concentrations indicated that it could be an effective complementary therapy. Moreover, synthetic enzyme inhibitors, such as acarbose, are often associated with gastrointestinal side effects, such as bloating and diarrhea due to excessive carbohydrate fermentation in the colon. Plant-based inhibitors, on the other hand, may offer a better safety profile with additional health benefits owing to their antioxidant and anti-inflammatory properties.

The findings of this study reinforce the potential of *A. mexicana* as a natural anti-diabetic agent. Incorporating these extracts into dietary regimens or developing standardized formulations could provide innovative strategies for glycemic control. However, while *in vitro* results are promising, further research, including in vivo animal studies and clinical trials, is essential to confirm these effects, determine the optimal dosages, and assess long-term safety.

Conclusion

This study demonstrates the significant antidiabetic potential of *Argemone mexicana* leaves, and enzyme inhibition assays further confirmed *Argemone mexicana*'s capacity to effectively inhibit both α-amylase and α-glucosidase, indicating its potential as a natural alternative for controlling postprandial blood sugar levels.

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