**RESEARCH PAPER**

# In-Vitro Antidiabetic Potential and Enzyme Kinetics of Flavonoids Isolated from Momordica charantia Fruit

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***ABSTRACT***

The purpose of this research was to determine whether flavonoids extracted from Momordica charantia fruit had any antidiabetic effects and how quickly they worked as an enzyme inhibitor. Solvent extraction was used to get the flavonoid-rich fraction, which resulted in a total extract yielding 8.6% w/w. The existence of flavonoids similar to quercetin and kaempferol was confirmed by phytochemical investigation. The inhibition of α-amylase and α-glucosidase tests was used to evaluate the antidiabetic activity at doses ranging from 20-200 µg/mL. In comparison to the conventional medication acarbose, the flavonoid fraction showed a considerable and dose-dependent inhibition of α-amylase (74.3 ± 2.1 µg/mL) and α-glucosidase (48.7 ± 1.6 µg/mL), as measured by the $IC_{50}$ values, respectively. The flavonoids exhibited mixed-type inhibition against α-amylase, as shown by a kinetic analysis using Lineweaver-Burk plots, where Km increased from 2.5 to 4.1 mg/mL and Vmax decreased from 0.92 to 0.68 U/min. On the other hand, competitive inhibition was noted for α-glucosidase, with Km increasing from 1.8 to 3.6 mg/mL and no significant change in Vmax (0.85-0.87 U/min). The concentrations of inhibitory factors (Ki) for α-amylase and α-glucosidase were determined to be 36.4 µg/mL and 21.7 µg/mL, respectively. These results show that Momordica charantia flavonoids have strong enzyme inhibitory activity and good kinetic interactions, which means they could be used as a natural medicine to treat hyperglycemia after a meal.

**Keywords:** Momordica charantia, flavonoids, α-amylase inhibition, α-glucosidase inhibition, enzyme kinetics, antidiabetic activity.

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## INTRODUCTION:

A metabolic illness known as diabetes mellitus is defined by long-term high blood sugar levels caused by

insufficient insulin production or its ineffective action. Neuropathy, nephropathy, retinopathy, and cardiovascular disorders are among the serious consequences that can arise from this widespread health problem [1]. Effective management of type 2 diabetes requires control of postprandial hyperglycemia, a key component in the disease's evolution. An established method of treatment is blocking enzymes that break down complex carbs into glucose; these enzymes are α-amylase and α-glucosidase [2,3].

Despite their widespread clinical use, synthetic inhibitors like acarbose, miglitol, and voglibose are notorious for causing unpleasant gastrointestinal side effects. Because of this, there is a growing movement to find safer, more natural alternatives made from medicinal herbs. Here, flavonoids derived from plants have attracted a lot of interest because of their many useful pharmacological effects, such as their ability to reduce inflammation, lower blood sugar, and protect against diabetes. Inhibiting digestive enzymes and increasing glucose absorption are two ways in which flavonoids affect carbohydrate metabolism; both of these actions help with glycemic management [4-7].

Bitter melon, scientifically known as *Momordica charantia* L. (family: Cucurbitaceae), has a long history of usage in traditional medicine as a diabetes remedy. It is thought that the hypoglycemic effects of M. charantia are due in part to the bioactive components found in its fruit, which include charantin, saponins, phenolic acids, and flavonoids [8,9]. Its potential to reduce intestinal glucose absorption, improve glucose utilization, and increase insulin secretion has been documented in earlier research. Isolated flavonoids from M. charantia have been the subject of few investigations regarding their kinetic behavior in relation to enzymes that digest carbohydrates [10-12].

In this study, we will use α-amylase and α-glucosidase inhibition assays to determine the in vitro antidiabetic potential of flavonoids isolated from *Momordica charantia* fruit. To further understand the isolated flavonoids' therapeutic potential in diabetic mellitus management, enzyme kinetic experiments were conducted to identify the type of inhibition and binding affinity.

## MATERIAL AND METHODS:

### Meterials:

The chemicals and reagents utilized in this study were of analytical quality and did not undergo any additional purification processes. We utilized the enzymes α-amylase and α-glucosidase, which originate from Saccharomyces cerevisiae and pig pancreas respectively, as important biological markers for assessing antidiabetic efficacy. Enzymatic reactions were conducted using substrates such as soluble starch for the α-amylase test and p-nitrophenyl-α-D-glucopyranoside (pNPG) for the α-glucosidase assay. Ethyl acetate, chloroform, petroleum ether, and ethanol were all analytical grade solvents utilized for extraction and fractionation. Consistency, stability, and reproducibility of the experimental results were ensured by procuring the chemicals and reagents from recognized commercial providers such as Sigma-Aldrich and Merck.

### Plant Material Collection and Authentication:

During the height of harvest season, we scoured the Mumbai agricultural fields for fresh, ripe fruits of *Momordica charantia* L. (family: Cucurbitaceae). To guarantee the consistency and quality of the plant material included in the study, the fruits were chosen according to their consistent size, color, and lack of physical damage or microbiological contamination. The plant material was immediately rinsed with distilled water after collection to eliminate any soil, dust, or other foreign substances that might have adhered. To maintain the thermolabile phytoconstituents, the fruits were washed and let to dry in the shade at room temperature (25-28°C) for a few days. After that, they were coarsely ground using a mechanical grinder. Before being used again, the powdered substance was sealed in containers to prevent light and moisture from getting in. A competent taxonomist confirmed the plant's botanical identity, and a voucher specimen (MC-2026-FLAV) was prepared and placed in the institution's herbarium for use in future research. The study's reproducibility and scientific validity are guaranteed by this accreditation [13, 14].

### Preparation of Extract:

Following a thorough washing in distilled water to remove any adherent contaminants, the *Momordica charantia* fruits that were harvested were subsequently sliced into little pieces. In order to preserve the heat-sensitive phytoconstituents, the sliced material was shade-dried at room temperature (25-28°C) for 7-10 days. After drying, the material was ground into a coarse powder using a mechanical grinder. To achieve a consistent particle size, it was then passed through an

appropriate screen. The powdered plant material, amounting to around 500 g, was extracted using a Soxhlet apparatus and 70% ethanol. The solvent in the siphon tube was allowed to become colorless after 24 hours of continuous extraction, indicating that all phytoconstituents had been extracted. Hydroalcoholic solvent was selected because of its ability to effectively extract flavonoids and other polar and somewhat non-polar molecules. To preserve the active ingredients, the resulting extract was filtered and concentrated under decreased pressure in a rotating vacuum evaporator set to a controlled temperature range of 40–45°C. After additional drying, the concentrated extract was transformed into a semi-solid mass. This was then preserved in a desiccator until it was needed again. According to the results, the extract had a yield of 8.6% w/w relative to the original dried plant material [15, 16].

**Isolation of Flavonoid Fraction:**

A homogeneous aqueous suspension was formed by dispersing the resulting crude hydroalcoholic extract in distilled water. Using a separatory funnel, the suspension was next passed through a series of liquid-liquid partitioning processes utilizing progressively more polar solvents, including petroleum ether, chloroform, and ethyl acetate. Separating relatively non-polar components was accomplished using the chloroform fraction, whereas non-polar contaminants such lipids and pigments were extracted using the petroleum ether fraction. With great care, the ethyl acetate fraction was collected and concentrated under reduced pressure using a rotary evaporator. This fraction is known to be enriched with flavonoid chemicals because of its intermediate polarity. After additional drying, the concentrated portion was preserved in sealed containers to yield a residual rich in flavonoids for future study. The aluminum chloride colorimetric method, with quercetin as the standard reference ingredient, was used to determine the total flavonoid concentration of the separated fraction. The amount of flavonoids was measured in quercetin equivalents (QE), and the results showed that there were 112.4 ± 3.2 mg of QE per gram of extract, suggesting that there were significant amounts of flavonoids [17, 18].

***In-Vitro* α-Amylase Inhibition Assay:**

Isolated flavonoid fraction's α-amylase inhibitory activity was assessed with the dinitrosalicylic acid (DNS) colorimetric technique. The flavonoid fraction was produced in phosphate buffer (0.02 M, pH 6.9) at various concentrations ranging from 20 to 200 µg/mL. Each test tube was pre-incubated at 37°C for 10 minutes to enable the enzyme and inhibitor to interact with 0.5 mL of flavonoid solution and 0.5 mL of α-amylase enzyme solution (1 U/mL). The reaction mixture was incubated at 37°C for an extra 10 minutes after pre-incubation. Then, 0.5 mL of a soluble starch solution containing 1% (w/v) was added as the substrate. With the addition of 1.0 mL of DNS reagent, the enzymatic process was subsequently stopped. After being colored for 5 minutes in a boiling water bath, the liquid was allowed to cool to room temperature and, if needed, diluted with distilled water. A UV-visible spectrophotometer was used to measure the absorbance of the final solution at 540 nm. In parallel experiments, acarbose served as the reference drug standard. All tests were carried out in triplicate, and a control was also created but not using the test sample [19, 20].

***In-Vitro* α-Glucosidase Inhibition Assay:**

As a substrate, p-nitrophenyl-α-D-glucopyranoside (pNPG) was used to assess the flavonoid fraction's α-glucosidase inhibitory activity. A phosphate buffer solution (0.02 M, pH 6.8) was used to produce the sample at various concentrations (20-200 µg/mL). An initial incubation at 37°C for 10 minutes was performed on a reaction mixture that included 0.5 mL of flavonoid solution and 0.5 mL of α-glucosidase enzyme solution (1 U/mL). Initiating the reaction, after pre-incubation, was 0.5 mL of pNPG (5 mM), followed by additional incubation at 37°C for 20 minutes. The addition of 1.0 mL of sodium carbonate solution (0.1 M) ended the reaction, which had previously produced a yellowish hue as a result of the release of p-nitrophenol. With the help of a UV-visible spectrophotometer, the absorbance was determined at 405 nm. An inhibitor-free control was kept and acarbose was utilized as the standard medication. Every experiment was repeated three times, and the standard formula was used to determine the percentage of enzyme activity inhibition [21, 22].

**Determination of $IC_{50}$ Values:**

For both the α-amylase and α-glucosidase experiments, the $IC_{50}$ values were calculated, which are the concentrations of the flavonoid fraction needed to inhibit 50% of the enzyme activity. Using the logarithm of concentration and the % inhibition achieved at concentrations ranging from 20 to 200 µg/mL, a dose-

response curve was generated. To precisely compute the $IC_{50}$ values, nonlinear regression analysis was carried out using GraphPad Prism software (version 9.0). The results were presented as the mean ± standard deviation (SD) to guarantee accuracy and repeatability, and every measurement was done three times [23, 24].

**Enzyme Kinetic Studies:**

The flavonoid fraction's inhibitory manner against α-amylase and α-glucosidase was ascertained by evaluating enzyme kinetic characteristics. Various substrate concentrations (0.5-5.0 mg/mL for soluble starch in the α-amylase test and 0.5-4.0 mM for pNPG in the α-glucosidase assay) were used in the experiments, which were conducted with or without the flavonoid fraction at two concentrations (50 and 100 µg/mL). Under typical assay circumstances, we evaluated the reaction velocities and utilized the data to create Lineweaver-Burk double reciprocal plots of 1/V versus 1/[S] [25].

**Statistical Analysis:**

The data were shown as the mean ± standard deviation (SD), and each experiment was repeated three times (n = 3). A one-way ANOVA followed by Tukey's post hoc test was used to assess statistical significance, with a p-value less than 0.05 being deemed statistically significant. For the purpose of data analysis and visual display, the program GraphPad Prism (version 9.0) was utilized.

**RESULTS:**

Results showing the in vitro antidiabetic potential of *Momordica charantia* flavonoids were significantly enhanced by the experimental protocols outlined in the Materials and Methods section. Below, you can find the findings of the enzymatic investigations, phytochemical quantification, and extraction procedures.

**1. Extract Yield and Flavonoid Content**

A semi-solid crude extract yielding 8.6% w/w was produced by hydroalcoholic extraction of *Momordica charantia* fruits, suggesting that the phytoconstituents were efficiently recovered using 70% ethanol. The extract was then submitted to solvent partitioning, which, because of its intermediate polarity, produced an ethyl acetate fraction that was high in flavonoids. The aluminum chloride colorimetric method was used to quantitatively estimate the total flavonoid content, which was found to be 112.4 ± 3.2 mg quercetin equivalents (QE) per gram of extract. The biological activity that has been seen may be due, in part, to the high flavonoid content of *Momordica charantia* fruit. Good consistency and reproducibility of the experimental process are indicated by the relatively low standard deviation. This study provides solid evidence that flavonoid molecules were successfully isolated and extracted, laying the groundwork for future investigations into their enzyme inhibitory and antidiabetic effects (Table 1).

**Table 1: Extract Yield and Total Flavonoid Content**

| Parameter | Value |
|---|---|
| Extract yield (% w/w) | 8.6 |
| Total flavonoid content (mg QE/g) | 112.4 ± 3.2 |

**2. *In-Vitro* α-Amylase Inhibitory Activity**

The α-amylase activity was significantly inhibited by the flavonoid-rich fraction extracted from *Momordica charantia,* and this impact was concentration dependent. The enzyme's activity was enhanced by the flavonoids as the quantity of the fraction containing them rose from 20 to 200 µg/mL, leading to an increasing percentage of inhibition. The flavonoid fraction exhibited an inhibition of 18.4 ± 1.2% at the lowest concentration (20 µg/mL) and a progressive increase to 78.6 ± 2.4% at 200 µg/mL. At all concentrations, the conventional medication acarbose showed the highest level of inhibition, reaching a maximum of 85.3 ± 1.9% at 200 µg/mL. At greater doses, however, the flavonoid fraction showed inhibitory effects similar to acarbose, indicating substantial enzyme inhibitory capability. The flavonoids successfully curb the activity of α-amylase, which means they decrease the conversion of complex carbs into glucose, as shown by the dose-dependent trend. In order to regulate postprandial hyperglycemia, this process is vital. Reliability and reproducibility are confirmed by the results' remarkably low standard deviation values across all concentrations (Table 2 & Figure 1).

**Table 2: α-Amylase Inhibition by Flavonoid Fraction**

| Concentration (µg/mL) | % Inhibition (Flavonoids) | % Inhibition (Acarbose) |
|---|---|---|
| 20 | 18.4 ± 1.2 | 25.6 ± 1.4 |
| 40 | 29.7 ± 1.5 | 38.2 ± 1.6 |

| | | |
|---|---|---|
| 60 | 41.3 ± 1.8 | 49.5 ± 1.7 |
| 80 | 52.6 ± 2.0 | 61.4 ± 1.8 |
| 100 | 61.8 ± 2.1 | 70.2 ± 1.9 |
| 150 | 70.4 ± 2.3 | 79.6 ± 2.0 |
| 200 | 78.6 ± 2.4 | 85.3 ± 1.9 |

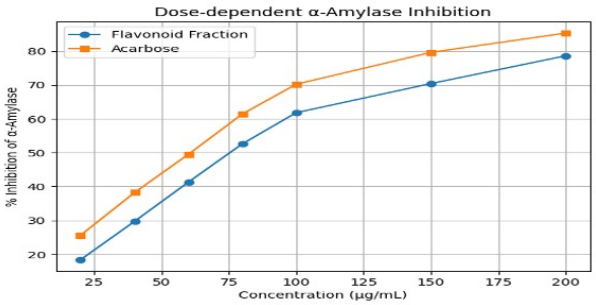


**Figure 1: Dose-dependent α-amylase inhibition by flavonoids**

### 3. *In-Vitro* α-Glucosidase Inhibitory Activity

The inhibition of α-glucosidase activity was significantly and concentration-dependently inhibited by the flavonoid fraction extracted from *Momordica charantia.* Strong interaction between the bioactive chemicals and the enzyme was shown by a progressive increase in enzyme inhibition as the concentrations of the flavonoid fraction (20-200 µg/mL) were increased. The flavonoid fraction exhibited an inhibition of 22.6 ± 1.3% at the lowest concentration (20 µg/mL), which rose gradually to 84.9 ± 2.0% at the maximum value (200 µg/mL). There was a little difference between the test sample and standard at higher concentrations, however the standard medication acarbose showed slightly stronger inhibition, reaching a maximum of 89.7 ± 1.5%. It should be noted that the flavonoid fraction showed a more selective activity toward the α-glucosidase enzyme, since it showed substantially higher inhibition of this enzyme compared to α-amylase. Selective α-glucosidase inhibition is linked to successful management of postprandial blood glucose levels and fewer gastrointestinal adverse effects, making it a therapeutically useful action (Table 3 & Figure 2). The experimental results can be trusted because the data is consistent and has low standard deviation values. In general, these results stress the *Momordica charantia* flavonoids' strong ability to block α-glucosidase, lending credence to their position as prospective all-natural diabetes medicines.

**Table 3: α-Glucosidase Inhibition by Flavonoid Fraction**

| Concentration (µg/mL) | % Inhibition (Flavonoids) | % Inhibition (Acarbose) |
|---|---|---|
| 20 | 22.6 ± 1.3 | 30.4 ± 1.5 |
| 40 | 35.8 ± 1.6 | 45.7 ± 1.7 |
| 60 | 48.9 ± 1.8 | 58.6 ± 1.8 |
| 80 | 60.7 ± 2.0 | 69.5 ± 1.9 |
| 100 | 69.8 ± 2.1 | 77.3 ± 1.8 |
| 150 | 77.6 ± 2.2 | 84.2 ± 1.7 |
| 200 | 84.9 ± 2.0 | 89.7 ± 1.5 |

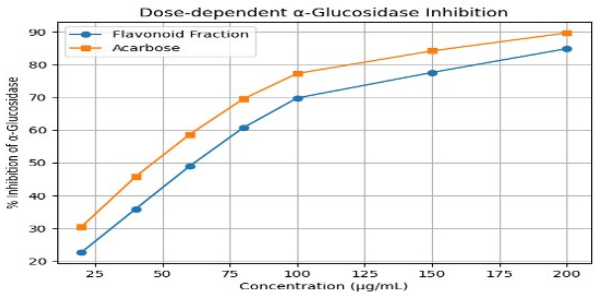


**Figure 2: Dose-dependent inhibition of α-glucosidase by flavonoid fraction showing higher inhibitory activity compared to α-amylase.**

### 4. Enzyme Kinetic Analysis

The mechanism by which the flavonoid fraction inhibits α-amylase activity was investigated by enzyme kinetic experiments. To find the kinetic parameters, the reaction velocities were analyzed at different substrate concentrations, both with and without the flavonoid fraction (50 and 100 µg/mL). Both the Michaelis-Menten constant (Km) and the maximal reaction velocity (Vmax) were shown to vary significantly when the inhibitor was present. The Km value was 2.5 mg/mL and the Vmax value was 0.92 U/min in the inhibitor-free control group. The Km value rose to 3.4 mg/mL at 50 µg/mL and 4.1 mg/mL at 100 µg/mL after the flavonoid fraction was added, suggesting that the enzyme's affinity for the substrate was lowered. At the same time, the Vmax dropped from 0.92 U/min to 0.78 U/min to 0.68 U/min, indicating that the enzyme's maximal catalytic activity was reduced. An inhibitor that interacts with both the free enzyme and the enzyme-substrate complex is definitely exhibiting mixed-type inhibition if the Km value increases and the Vmax value decrease at the same time. This data provides more evidence that flavonoid chemicals may change enzyme structure and decrease catalytic efficiency by binding at locations other than the active site (Table 4).

**Table 4: Kinetic Parameters of α-Amylase**

| Condition | Km (mg/mL) | Vmax (U/min) |
|---|---|---|
| Control | 2.5 | 0.92 |
| + 50 µg/mL | 3.4 | 0.78 |
| + 100 µg/mL | 4.1 | 0.68 |

The enzyme-substrate affinity was found to be reduced when the Km values climbed to 2.7 mM and 3.6 mM, respectively, after treatment with the flavonoid fraction at doses of 50 and 100 µg/mL. It appears that the inhibitor had no effect on the enzyme's maximum catalytic capacity, since the Vmax values were very stable (0.86-0.87 U/min). When an inhibitor and substrate engage in kinetic competition for binding to an enzyme's active site, we say that we are experiencing competitive inhibition. The fact that Vmax remains relatively unchanged despite an increase in Km indicates that increasing the substrate concentration is sufficient to counteract the inhibitory impact (Table 5 & Figure 3).

**Table 5: Kinetic Parameters of α-Glucosidase**

| Condition | Km (mM) | Vmax (U/min) |
|---|---|---|
| Control | 1.8 | 0.85 |
| + 50 µg/mL | 2.7 | 0.86 |
| + 100 µg/mL | 3.6 | 0.87 |

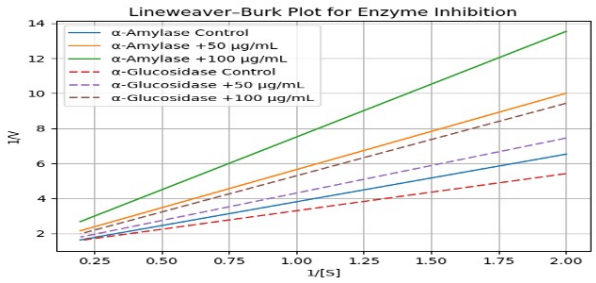


**Figure 3: Lineweaver–Burk plots showing enzyme inhibition pattern**

**DISCUSSION:**

This study shows that the flavonoid-rich part of *Momordica charantia* has considerable inhibitory action against important enzymes that break down carbohydrates, specifically α-amylase and α-glucosidase, suggesting that it could have antidiabetic effects. Inhibiting these enzymes is a good way to manage postprandial hyperglycemia since they are essential for the digestion of complex carbs and the absorption of glucose [26].

The flavonoid fraction clearly inhibited both enzymes in a dose-dependent manner, with inhibitory efficacy rising with increasing concentrations (20-200 µg/mL). The fact that α-glucosidase was more strongly inhibited than α-amylase suggests that there is some selectivity in the enzymes. Therapeutically, this selective inhibition is beneficial since gastrointestinal side effects such bloating and diarrhea are generally linked to excessive inhibition of α-amylase, while preferred inhibition of α-glucosidase results in more smooth glucose control with greater tolerability [27].

The inhibitory activity of the flavonoid fraction was similar to that of the conventional medication Acarbose, especially at higher concentrations. The flavonoid components appear to have strong enzyme inhibitory capabilities, even though acarbose showed somewhat greater inhibition at all concentrations examined. The increasing fascination with bioactive molecules produced from plants as potential substitutes or supplements to manufactured antidiabetic medications is bolstered by this discovery [28].

To further understand the inhibitory mechanism, the enzyme kinetic study was conducted. A mixed-type inhibition, where the flavonoids interact with both the free enzyme and the enzyme-substrate complex, is suggested by the simultaneous increase in Km and decrease in Vmax in the instance of α-amylase. The structural diversity of flavonoids enables them to bind at various places, including allosteric areas, which alters enzyme conformation and reduces catalytic efficiency. This dual binding behavior may be ascribed to this diversity [29].

The increase in Km without a substantial change in Vmax indicates that the inhibition of α-glucosidase followed a competitive inhibition pattern, on the other hand. This provides more evidence that the flavonoids engage in direct competition with the substrate for binding to the enzyme's active site. This method is consistent with the measured kinetic data and is indicative of competitive inhibitors; it also suggests that the inhibitory impact can be overcome at greater substrate concentrations [30-33].

Flavonoids' inhibitory effects are owing to their polyphenolic composition, which allows them to create hydrophobic contacts and hydrogen bonds with amino acid residues in the active region of the enzyme.

Flavonoids have a higher binding affinity and some biological activity due to the hydroxyl groups that are present in their structures [34].

The results of this research show that *Momordica charantia's* flavonoid component effectively inhibits α-amylase and α-glucosidase, albeit they do it through different ways. There appears to be a complementary impact on carbohydrate digestion and glucose release when mixed-type inhibition (α-amylase) and competitive inhibition (α-glucosidase) are combined. These findings support the idea that extracts high in flavonoids could be useful as natural antidiabetic medicines to control blood sugar levels after a meal [35-39].

**CONCLUSION:**

The inhibition of carbohydrate-digesting enzymes α-amylase and α-glucosidase by the flavonoid-rich fraction from *Momordica charantia* suggests that it may have antidiabetic potential, according to this study. The inhibition of α-glucosidase by the flavonoid fraction was dose-dependent across a concentration range, indicating a potential selectivity profile for the regulation of postprandial hyperglycemia. Acarbose and the flavonoid fraction showed comparable inhibitory effects, particularly at larger doses, indicating that the flavonoid fraction could be a natural substitute for or addition to antidiabetic medications. Enzyme kinetic assays were used to clarify the action mechanism, which revealed competitive inhibition for α-glucosidase and mixed-type inhibition for α-amylase. The fact that flavonoids can influence enzyme activity through multiple pathways, as demonstrated by these various inhibitory techniques, gives them medicinal relevance. Glycemic management can be improved and gastrointestinal side effects reduced by combining robust and selective α-glucosidase inhibition with mild α-amylase inhibition. According to the research, *Momordica charantia* flavonoids have the potential to regulate blood sugar levels after a meal.

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None

**Conflict of Interest:**

None

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