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# ANTIDIABETIC PROPERTIES OF ETHANOL EXTRACTS FROM *Momordica charantia* SEEDS: PHYTOCHEMICAL PROFILE AND ENZYME INHIBITION

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## ABSTRACT

***Background**:* Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, which necessitates the continuous search for effective and safe therapeutic agents. *Momordica charantia*, commonly known as bitter melon, has been traditionally used for its medicinal properties, including antidiabetic effects.

***Aim:*** This study investigates the antidiabetic properties of ethanol extracts from *M. charantia* seeds through *in vitro* approaches

***Methods:*** Dry seeds of ripe *M. charantia* were manually separated from their pericarp. The dried seeds were blended using an electric grinder and then passed through a 60-mesh sieve to obtain a uniform particle size. The *M. charantia* seeds product was defatted using n-hexane by stirring at room temperature for 1 h and soaked with n-hexane for 72 h, the mixture was filtered using sterile filter paper. The defatted seed product was dried under a hood at ambient temperature to remove the trace of the remaining n-hexane. The filtrate was then further soaked with ethanol for 72 h, the mixture was filtered using sterile filter paper, and the extract was further concentrated using a water bath set at 50 °C. The ethanol extract of *M. charantia* seeds (EEMCS) was evaluated for its potential to inhibit key enzymes involved in carbohydrate metabolism, including α-amylase and α-glucosidase, glucose uptake and glucose adsorption.

***Results:*** The phytochemical showed the detection of tannins, saponins, flavonoids, alkaloids, glycosides, phenols, phytate, and triterpene. The EEMCS showed significant inhibition of α-amylase, α-glucosidase, glucose uptake and glucose adsorption activities, with $IC_{50}$ values comparable to standards such as acarbose and metformin.

***Conclusion***: The EEMCS exhibits promising antidiabetic properties through multiple mechanisms, including enzyme inhibition and antioxidant activity.

**Keywords**: Diabetes mellitus, *Momordica charantia*, Antidiabetic properties, α-amylase and α-glucosidase inhibition, Ethanol extract, antioxidant activity, and pancreatic protection.

## 1.0 INTRODUCTION

Diabetes mellitus is considered as one of the five leading causes of death in the world (1).

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Diabetes mellitus is a major global health concerning with a projected rise in prevalence from 171 million in 2000 to 366 million in 2030 (2). It is a disorder, usually due to a combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels (hyperglycemia). Being a major degenerative disease, diabetes is found in all parts of the world and it is becoming the third most lethal disease of mankind and increasing rapidly (3). It is the most common endocrine disorder, affecting 16 million individuals in the United States and as many as 200 million individuals worldwide (4). Diabetes has been a clinical model for general medicine. Complementary and alternative medicine involves the use of herbs and other dietary supplements as alternatives to mainstream western medical treatment. A recent study has estimated that up to 30 % of patients with diabetes mellitus use complementary and alternative medicine (5).

Diabetes mellitus (DM) is a complex metabolic disorder that occurs due to the misuse in fat, proteins and carbohydrate metabolism in response to insulin deficiency or insensitivity (6). Hyperglycemia, in non-insulin dependent patients, is due to an increased starch breakdown by α-amylase and a high glucose absorption favored by the action of α-glucosidase. The inhibition of these enzymes reduce blood glucose levels and decrease hyperglycemia (7). Pharmaceutical chemical compounds such as acarbose, miglitol and voglibose, are used to maintain glucose levels efficiently, but prolonged use has been associated with several side effects (8). An alternative to avoid or minimize these side effects is to use plant extracts rich in bioactive compounds like polyphenols, which have been reported to inactivate α-amylase and α-glucosidase through non-specific enzymatic binding (9).

Plants are rich in numerous endogenous antioxidants such as polyphenols, carotenoids, ascorbic acids, tocopherol, and flavonoids (10). These valuable antioxidants, which are plant secondary metabolites whose primary functions are defensive and protective in nature against oxidative stress caused by the formation of reactive oxygen species (ROS) are widely recognized and annexed by humans as ingredients and supplements in dietary processes in the hope of maintaining health and occasioning prevention to diseases such as cancer, and coronary diseases (11). These phytochemicals are sometimes referred to as phytonutrients as they are vitamin‑based antioxidants (12).

Free radicals are generated via several endogenous metabolic mechanisms and actions such as enzymatic activities of NADPH oxidase, xanthine oxidase, and peroxidases in the cells. Once formed, they participate in several reactions yielding various ROS such as hydrogen peroxide, OH radical, hypochlorous acid, and so on (13). When an antioxidant destroys a free radical, this antioxidant itself becomes oxidized. Therefore, the antioxidant sources must be constantly restored in the body. Thus, while in one system an antioxidant is effective against free radicals; in other systems the same antioxidant could become ineffective (14).

Medicinal plants and its products continue to be an important therapeutic aid for alleviating the ailments of mankind (15). Herbs for

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diabetes treatment are not new. The World Health Organization (WHO) has listed 21, 000 plants, which are used for medicinal purposes around the world. Among them, 150 species are used commercially on a fairly large scale (16).

*Momordica charantia*, also known as bitter melon, karela, balsam pear, or bitter gourd, is a popular plant used for the treating of diabetes-related conditions amongst the indigenous populations of Asia, South America, India, the Caribbean and East Africa (17). Its fruit has a distinguishing bitter taste, which is more pronounced as ripens, hence, the name bitter melon or bitter gourd. Biochemical and animal model experiments have produced abundant data and hypotheses accounting for the anti-diabetic effects of *M. charantia*. (18).

## 2.0 MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.2 Equipment and Instruments

Electric grinder (Jiangman China), maceration setup, Water bath (Infitek, Hong Kong), weighing balance (Gromy China), Filterpaper (Whatman, United Kingdom), Conical flasks (Glassco, India)

#### 2.1.3 Chemicals and Reagents

Ethanol (analytical grade), n-hexane, distilled water, dilute tetraoxosulphate (IV) acid ($H_2SO_4$), sodium hydroxide (NaOH), zinc chips, Meyer's reagent, Dragendoff's reagent, ammonia, chloroform ($CHCl_3$), nutrient agar, ethyl acetate, acetone, aqueous hydrochloric acid (1 % HCl), ethanol, benzene, chloroform, diethyl ether and acetone were products of Schar Lab S.L., Barcelona, Spain. Acetic acid, ethanol and ethyl acetate were products of Loba Chemie Pvt Ltd., Mumbai, India. Streptozotocin (Elab Science, Texas, USA) and the assay kits (Randox Laboratories Ltd., Antrim, UK). All other reagents used were of analytical grade.

### 2.2 Methods

#### 2.2.1 Sample Collection

The seeds of *M. charantia* were collected at Oja-Tuntun, Ilorin, Kwara State, Nigeria.

#### 2.2.2 Sample Preparation

Dry seeds of ripe *M. charantia* were manually separated from pericarp, blended using an electric grinder and then passed through a 60-mesh sieve to obtain a uniform particle size. The *M. charantia* seeds product was defatted using n-hexane by stirring at room temperature for 1 h and soaked with n-hexane for 72 h, the mixture was filtered using sterile Whatman product filter paper grade 1. The defatted seed product was dried under a hood at ambient temperature to remove the trace of the remaining n-hexane. The filtride was then soaked with ethanol for 72 h, the mixture was filtered using sterile Whatman product filter paper grade 1. The extract was further concentrated using a water bath set at 50 °C.

#### 2.2.3. Phytochemical Screening

##### 2.2.3.1 Qualitative Phytochemical Screening

Phytochemical analysis of the ethanol extract of *M. charantia* seeds (EEMCS) was carried out using the method described by Odebiyi &

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Sofowora (19) for the detection of saponins, tannins, phenolics, alkaloids, steroids, triterpenes, tannins, glycosides and flavonoids.

To test for the presence of alkaloids in the extracts, exactly 1 ml of 1% hydrochloric acid (HCl) was added to 3 ml of the extract in a test tube. The mixture was then heated for 20 min, allowed to cool, and subsequently filtered. The filtrate obtained was subjected to a test by adding 2 drops of Wagner's reagent to 1 ml of the filtrate. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

The presence of tannins was tested by adding 1 ml of freshly prepared 10 % potassium hydroxide (KOH) to 1 ml of the extract. The appearance of a dirty white precipitate was indicative of the presence of tannins.

To detect phenolics, 2 drops of 5 % ferric chloride ($FeCl_3$) were added to 1 ml of the extract in a test tube. The formation of a greenish precipitate confirmed the presence of phenolics.

For the detection of glycosides, 10 ml of 50 % sulfuric acid ($H_2SO_4$) was added to 1 ml of the extract, and the mixture was heated in boiling water for 15 min. Subsequently, 10 ml of Fehling's solution was added, and the mixture was boiled. The presence of glycosides was confirmed by the appearance of a brick-red precipitate.

The frothing test was employed to test for saponins. In this test, 2 ml of the EEMCS was placed in a test tube and vigorously shaken for 2 min. The presence of frothing was indicative of saponins.

To test for flavonoids, 1 ml of 10 % sodium hydroxide (NaOH) was added to 3 ml of the EEMCS. The development of yellow coloration indicated the presence of flavonoids.

Finally, the presence of steroids was tested by adding 5 drops of concentrated sulfuric acid ($H_2SO_4$) to 1 ml of the extract. The formation of red coloration was a positive indication of steroids in the EEMCS.

### 2.2.3.2. Quantitative Phytochemical Screening

**Determination of Total Phenolic Content**

Folin-Ciocalteu reagent was used for analysis of total phenolics content (20). Briefly, 0.5 ml of the EEMCS was mixed with 0.5 ml of Folin-Ciocalteu reagent. The solution was kept at 25 $^{o}$C for 5-8 min before adding 2 ml of sodium carbonate solution 7.5 % and adjusting the volume to 8 ml with water. After 2 h, the absorbance was measured at 725 nm. Gallic acid was used as standard for the calibration curve. Total phenolic content was expressed as mg gallic acid equivalents per gram of sample (mg/g).

**Determination of Tannins:** Tannin content was determined following the method of Ajiboye *et al*. (21). Specifically, 50 ml of distilled water was added to 500 mg of the sample in a 500 ml flask, which was then shaken for 1 h. The mixture was filtered into a 50 ml volumetric flask. Then, 5 ml of the filtrate was pipetted into a test tube and mixed with 2 ml of 10-fold diluted 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance of the sample was measured at 605 nm after 10 min using a spectrophotometer.

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**Determination of Total Flavonoid Content**

The total flavonoid content was measured by a colorimetric assay (22). One hundred microliters of EEMCS was added to 4 ml of distilled water. Then, 0.3 ml of 5 % sodium nitrite was added. After 5 min, 0.3 ml of 10 % aluminium chloride was added. In 6 min, 2 ml of 1 M sodium hydroxide was added to the mixture. Immediately, the mixture was diluted by the addition of 3.3 ml distilled water and mixed thoroughly. The absorbance was determined at 510 nm versus a blank. Catechin was used as standard for the calibration curve. The total flavonoids content of the extract was expressed as mg catechin equivalents per gram of sample (mg/g).

**Determination of Saponin Content**

Saponin content was determined according to the method described by Oloyede (23). A 0.5 g portion of the sample was boiled in 20 ml of 1 N HCl for 4 h. After cooling, the mixture was filtered, and 50 ml of petroleum ether was added to the filtrate. The ether layer was evaporated to dryness, and 5 ml of acetone ethanol was added to the residue. Subsequently, 0.4 ml of each solution was mixed with 6 ml of ferrous sulfate reagent followed by 2 ml of concentrated $H_2SO_4$. After 10 min, the absorbance was read at 490 nm, and a calibration curve using standard saponin was prepared.

**Determination of Total Alkaloid Content**

Total alkaloid content was determined using the 1,10-phenanthroline method as described by Singh *et al*. (24). A 100 mg sample was extracted in 10 ml of 80 % ethanol, centrifuged, and the supernatant used for estimation. The reaction mixture contained 1 ml of plant EEMCS, 1 ml of 0.025 M $FeCl_3$ in 0.5 M HCl, and 1 ml of 0.05 M 1, 10-phenanthroline in ethanol. The mixture was incubated at 70 ± 2 °C for 30 min, and the absorbance of the red-colored complex was measured at 510 nm against a reagent blank. The alkaloid content was determined using a standard curve of quinine.

**Determination of Cyanogenic Glycoside**

Cyanogenic glycoside was determined using the alkaline picrate method as described by Olopade & Onwuka (25). The ground sample (5.0 g) was dissolved in 50 ml of distilled water and allowed to extract overnight, followed by filtration. Various concentrations of KCN solution were prepared to create a standard curve. To 1 ml of the sample filtrate or cyanide standard solution, 4 ml of alkaline picrate solution (1 g picric acid and 5 g $Na_2CO_3$ in 200 ml distilled water) was added and incubated in a water bath for 15 min. Absorbance was measured at 490 nm against a blank containing only distilled water and alkaline picrate solution.

**Determination of Steroid Content**

The steroid content was determined using a modified method where 1 ml of test EEMCS was transferred into a 10 ml volumetric flask. To this, 2 ml of 4N sulfuric acid, 2 ml of 0.5 % w/v iron (III) chloride, and 0.5 ml of potassium hexacyanoferrate (III) solution (0.5 % w/v) were added. The mixture was heated in a water bath at 70 ± 20 °C for 30 min, occasionally shaking, and diluted to the mark with distilled water. Absorbance was measured at 780 nm against a reagent blank.

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**Determination of Terpenoid Content**
Terpenoid content was determined by the method described by Indumathi *et al*. (26). Dried EEMCS (100 mg) was taken and soaked in 9 ml of ethanol for 24 h. The EEMCS after filtration, was extracted with 10ml of petroleum ether using separating funnel. The ether extract was separated into pre-weighed glass vials and waited for its complete drying. Ether was evaporated and the weight of total terpenoids content was measured.

### 2.2.4 *In Vitro* Studies

#### 2.2.4.1 Determination of Antidiabetic (Enzyme Inhibition) Properties α-Amylase inhibitory activity:

α-Amylase inhibitory activity of EEMCS and fractions was carried out according to the standard method with minor modification (27). In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6.8), 10 µl α–amylase (2 U/ml), and 20 µl of varying concentrations of EEMCS and fractions (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) were preincubated at 37 °C for 20 min. Then, the 20 µl of 1 % soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and incubated further at 37 °C for 30 min; 100 µl of the DNS color reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using Multiplate Reader (Multiska Thermo Scientific, version 1.00.40). Acarbose at various concentrations (0.1–0.5 mg/ml) was used as a standard. Without test (EEMCS and fractions) substance was set up in parallel as control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

$$\text{Inhibitory activity (\%)} = \frac{1 - \text{As}}{\text{Ac}} \times 100$$

Where, As is the absorbance in the presence of test substance and Ac is the absorbance of control.

#### 2.2.4.2. α-Glucosidase inhibitory activity:

α-glucosidase inhibitory activity of EEMCS and fractions was carried out according to the standard method with minor modification (28). In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6. 8), 10 µl alpha glucosidase (1 U/ml), and 20 µl of varying concentrations of EEMCS and fractions (0.1, 0.2, 0.3, 0.4, and 0.5 mg/ml) were preincubated at 37 °C for 15 min. Then, 20 µl P-NPG (5 mM) was added as a substrate and incubated further at 37 °C for 20 min. The reaction was stopped by adding 50 µl $Na_2CO_3$ (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Multiplate Reader. Acarbose at various concentrations (0.1–0.5 mg/ml) was included as a standard. Without test substance was set up in parallel as a control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

$$\text{Inhibitory activity (\%)} = \frac{1 - \text{As}}{\text{Ac}} \times 100$$

Where, As is the absorbance in the presence of test substance and Ac is the absorbance of control.

**2.2.4.3. Glucose uptake capacity:** This assay was performed according to the well-

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defined method of Cirillo (29). Commercial baker's yeast was dissolved in distilled water to prepare 1 % suspension. The suspension was kept overnight at room temperature (25 °C). On the next days, yeast cells suspension was centrifuged at 4200 rpm for 5 min. The process was repeated by the addition of distilled water to the pallet until a clear supernatant was obtained. Exactly 10 parts of the clear supernatant fluids were mixed with 90 parts of distilled water to get a 10 % v/v suspension of the yeast cells. 7.125 – 1,000 µg/ml of EEMCS was mixed with dimethyl sulfoxide (DMSO) till dissolution. The mixture was then supplemented with various concentrations (5, 10, and 25 mM) of 1 ml of glucose solution and incubated for 10 min at 37 °C. To initiate the reaction, 100 µl of yeast suspension was poured in the mixture of glucose and EEMCS, vortexed, and incubated for another 60 min at 37 °C. After incubation, the tubes were centrifuged for 5 min at 3800 rpm and glucose was estimated by using a spectrophotometer at 520 nm. Absorbance for the respective control was also recorded on the same wavelength. The percent increase in uptake was calculated by the formula:

$$\text{Inhibitory activity (\%)} = \frac{A_C - A_S}{A_C} \times 100$$

Where, Ac is the absorbance of the control and As is the absorbance of the sample. Control is the solution having all reagents except the test sample. Metformin was used as a standard drug.

**2.2.4.4. Glucose adsorption assay:** The glucose adsorption capacity of the EEMCS was determined by the method of Ou *et al.* (30). Approximately, 1 gram of EEMCS was added to 100 ml of glucose solution of five different concentrations (5, 10, 15, 20, and 30 mM). Each of these mixtures was mixed well, stirred, and incubated in a shaker water bath at 37 °C for 6 h, respectively. After incubation, the mixture was centrifuged at 4800 rpm for 20 min and finally the glucose content was determined in the supernatant by using glucose oxidase peroxidase diagnostic kit. The amount of bound glucose was determined by the given formula: Here, 1 represents the glucose concentration of the original solution, while 6 represent the glucose concentration after 6 h.

$$Glucose\ Bound = \frac{G1 - G6}{Weight\ of\ sample} \times Volume\ of\ Sample$$

Here, G1 represents the glucose concentration of the original solution, while G6 represents the glucose concentration after 6 h.

**2.2.5. Data Analysis**

Data analysis was conducted using SPSS software (version 25.0). Data were expressed as mean ± standard error of mean and analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test for multiple comparisons. A p-value < 0.05 was considered statistically significant. $IC_{50}$ values were calculated using GraphPad Prism 8 version 8.0.2 (Graph pad software, Inc., La Jolla, CA, USA) statistical software.

## 3.0 RESULTS

### 3.1 Qualitative and Quantitative Phytochemicals Screening of *Momordica charantia*

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The qualitative phytochemical analysis of *M. charantia* seeds revealed the presence of a variety of bioactive compounds, as summarized in Table 1 below. The detected phytochemicals include tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids, phytates, and triterpenes. Each of these compounds is known for its potential health benefits, which contribute to the medicinal properties of *M. charantia*.

The Quantitative data reveal that glycosides and steroids had the highest concentrations, with values of 30.83 ± 30.73 and 23.72 ± 23.76 mg/100g, respectively. Flavonoids, saponins, and alkaloids also exhibit significant concentrations, highlighting the seeds' potential as a source of these bioactive compounds. Phenols, tannins, and triterpenes were found in moderate amounts, while terpenoids had the lowest concentration.

**Table 1: Results of Qualitative and Quantitative Phytochemicals screening of *Momordica charantia***

| Phytochemical Constituents | Qualitative analysis | Quantitative analysis (mg/100g) |
|---|---|---|
| Alkaloid | + | 5.97 ± 0.01 |
| Flavonoid | + | 10.01 ± 0.87 |
| Glycoside | + | 30.83 ± 0.03 |
| Phenol | + | 1.41 ± 0.03 |
| Steroid | + | 23.72 ± 0.05 |
| Saponin | + | 13.69 ± 0.12 |
| Phytate | + | 3.76 ± 0.05 |
| Tannin | + | 8.35 ± 0.01 |
| Terpenoid | + | 0.02 ± 0.00 |
| Triterpene | + | 6.90 ± 0.07 |

Key: + - Detected.

### 3.2 *In Vitro* Enzyme Inhibition Studies

#### 3.2.1 α-Amylase Inhibition

The EEMCS showed dose-dependent inhibition of α-amylase activity. The $IC_{50}$ value, representing the concentration needed to inhibit 50 % of the enzyme activity, was found to be 24.47±1.36 µg/ml. This value is significantly higher than the $IC_{50}$ value of the standard, which was recorded at 10.96±0.79 µg/ml. This indicates that *M. charantia* has inhibitory activity against α-amylase (Figure 1).

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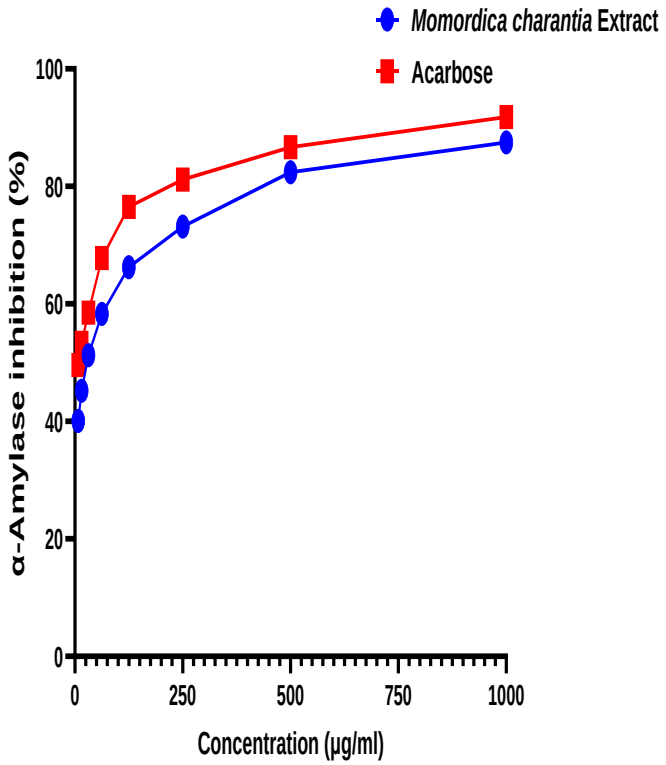


**Figure 1: Percentage inhibition of α-amylase activity of *Momordica charantia* seed and Acarbose**

### 3.2.2 α-Glucosidase Inhibition

Similarly, the EEMCS exhibited dose-dependent inhibition of α-glucosidase activity, with an $IC_{50}$ value of 10.10±0.70 µg/ml. Compared to the $IC_{50}$ value of standard, which was 8.12±0.58 µg/ml, *M. charantia* is less potent in inhibiting α-glucosidase (Figure 2).

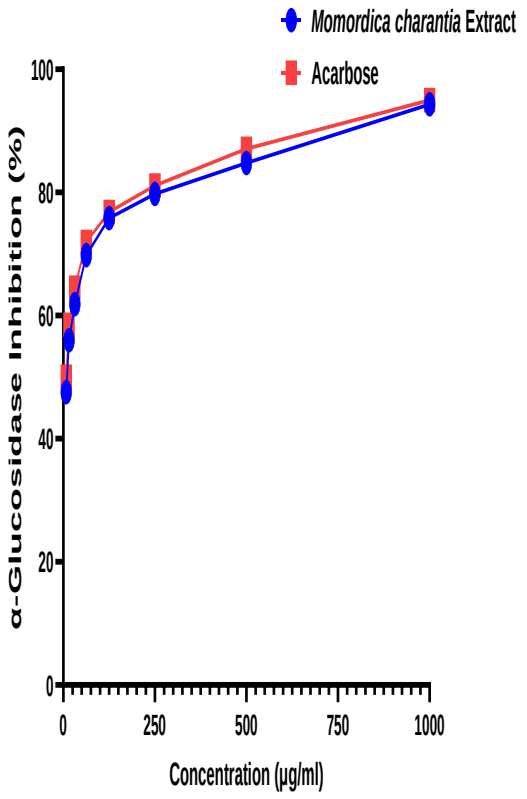


**Figure 2: Percentage inhibition of α-glucosidase activity by *Momordica charantia* seed and Acarbose.**

### 3.2.3 Glucose Uptake Inhibition

The glucose uptake inhibition assay revealed that the EEMCS inhibited glucose uptake in a dose-dependent manner. The $IC_{50}$ value for this activity was 8.85±0.61 µg/ml, which is slightly higher than the $IC_{50}$ value of standard, recorded at 6.98±0.64 µg/ml. This suggests that *M. charantia* is also effective in inhibiting glucose uptake (Figure 3).

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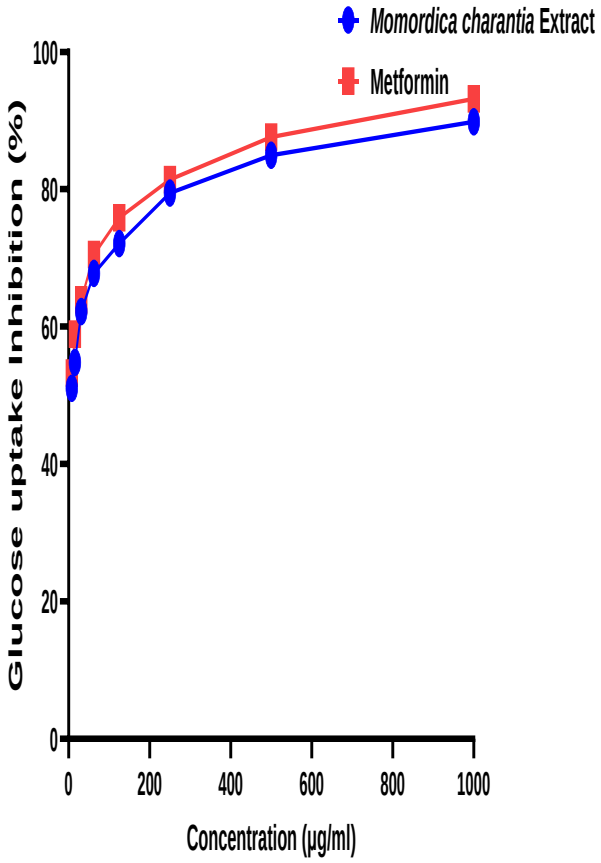


**Figure 3: Percentage inhibition of glucose uptake by *Momordica charantia* seed and Metformin**

### 3.2.4 Glucose Adsorption Inhibition

In the glucose adsorption assay, the EEMCS demonstrated dose-dependent inhibition with an $IC_{50}$ value of 555.60±224.40 µg/ml. This is significantly lower than the $IC_{50}$ value of metformin, which was 596.70±223.50 µg/ml, indicating that *M. charantia* has a higher potency in glucose adsorption activity compared to standard (Figure 4).

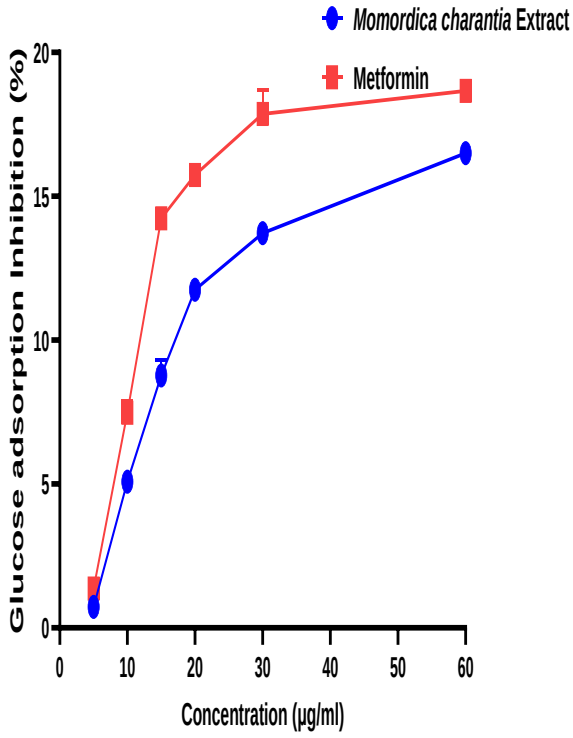


**Figure 4: Percentage inhibition of glucose adsorption by *Momordica charantia* seeds and Metformin**

## 4.0 DISCUSSION

Quantitative analysis reveals that *M. charantia* seeds contain moderate levels of phenols, tannins, and triterpenes, with lower concentrations of terpenoids, which, despite their lower abundance, may still contribute to the seeds' therapeutic potential. Specifically, phenols and tannins have been identified as key bioactive compounds due to their pronounced antioxidant and anti-inflammatory properties, which play a pivotal role in the prevention and management of chronic conditions such as cancer and cardiovascular diseases (31, 32).

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The phytochemical profile, as determined by both qualitative and quantitative assays, underscores *M. charantia* seeds as a rich source of bioactive compounds with notable health benefits. These findings corroborate existing literature that promotes the traditional and contemporary medical applications of *M. charantia* (33, 34). Further investigation into the specific pathways through which these compounds act could illuminate their exact mechanisms of action, and research into the bioavailability and pharmacokinetics of these constituents could enhance understanding of their therapeutic potential for future pharmaceutical applications.

The seed extracts' α-amylase and α-glucosidase inhibitory activities, which are critical for modulating postprandial hyperglycemia, showed significantly higher $IC_{50}$ values compared to the standard drug acarbose, indicating a comparatively lower inhibitory efficacy. This observation is consistent with previous studies on related medicinal plants that exhibited moderate inhibitory effects on these enzymes, supporting the trend observed in *M. charantia* (35, 36). Although *M. charantia* seeds demonstrated a glucose uptake inhibition capacity, it was found to be less potent than metformin. Metformin primarily enhances glucose uptake via AMP-activated protein kinase (AMPK) activation, which improves insulin sensitivity and regulates glucose metabolism (37). In contrast, the higher $IC_{50}$ value for *M. charantia* suggests that it may exert its effects through alternative, potentially less efficient mechanisms (38).

Furthermore, the proteinase inhibition assay indicated that *M. charantia* seeds possess modest anti-inflammatory properties. However, their efficacy was significantly lower than that of metformin, a result that aligns with findings by Adaramoye (35), which documented similar moderate anti-inflammatory effects in other species of the genus Momordica (17). The relatively low inhibitory potency of *M. charantia* seeds implies a limited but contributory role in inflammation reduction, suggesting that it may be more beneficial as an adjunctive rather than a primary anti-inflammatory agent in therapeutic settings.

## 5.0 CONCLUSION AND RECOMMENDATION

The findings from this study underscore the potential of EEMCS as a therapeutic agent for managing diabetes and its complications. These results contribute to the growing body of evidence supporting the use of *M. charantia* in traditional medicine and provide a scientific basis for its inclusion in modern diabetes treatment protocols. Further research is warranted to fully understand the mechanisms by which EEMCS exerts its protective effects and to explore its potential applications in clinical settings.

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