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# *Corchorus olitorius* Aqueous Lyophilized Leaves Scavenge Radicals and Inhibit Diabetes-related Enzymes Uncompetitive

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**Abstract:** *Corchorus olitorius* L. is extensively consumed in Nigeria and is believed to reduce glucose absorption from glucose-rich complementary meals. This study investigated the radical scavenging and diabetes-related enzyme inhibitory properties of *C. olitorius* aqueous lyophilized leaves (CO). Quantitative phytochemical analysis, radical scavenging assessments, reducing power, antioxidant capacity, and carbohydrate metabolizing enzyme inhibitory activity were all determined using standard biochemical methods. Phytochemical analysis revealed the presence of flavonoids (513.95 ± 36.88 mg RE/g), tannins (345.64 ± 12.66 mg TAE/g), phenols (10.24 ± 0.23 mg GAE/g), β-carotene (1.12 ± 0.28 mg CE/g) and lycopene (0.58 ± 0.10 mg CE/g). CO scavenged 2, 2-diphenyl-2-picrylhydrazyl radical, exhibited reducing power and antioxidant capacity at the highest concentration compared to the reference standards. α-amylase and α-glucosidase enzyme inhibitory activities were also observed; however, they were lower ($p<0.05$) than acarbose. Both α-amylase and α-glucosidase were inhibited uncompetitively by CO, reducing their $V_{max}$ and $K_m$. Conclusively, *C. olitorius* can be utilized in managing postprandial hyperglycemia when consumed with glucose-rich complementary meals.

**Keywords:** *Corchorus olitorius*; radical scavenging; α-amylase; α-glucosidase; uncompetitive; postprandial hyperglycemia; $V_{max}$; $K_m$.



## 1. Introduction

Diabetes mellitus (DM) is a serious endocrinal disorder that poses severe health complications when not properly managed [1]. Globally, DM affects roughly 9 % of the world's population; this disease has reached epidemic proportions and is one of the most prevalent non-communicable diseases. In 2019, the incidence of DM resulted in 4.7 million deaths; by 2030, it is anticipated to rank as the seventh cause of death due to associated complications [1,2]. Major complications associated with poorly managed DM states are cardiovascular diseases, retinopathy, nephropathy, and death. DM is characterized mainly by

hyperglycemia, whereby a persistent hyperglycemic state produces oxidative stress by activating the auto-oxidation of glucose, protein glycation, and polyol pathways [3,4].

Most of the human diet comprises carbohydrates, usually simple sugars and starch. The recommended carbohydrate intake daily to meet the body's energy requirements is estimated to be between 40 and 45 %. Hydrolysis of starch yields monosaccharides, which are then catabolized to generate energy or stored as glycogen in the liver when excess [5,6]. It is important to note that the breakdown of starch after consuming a carbohydrate meal can result in an elevated postprandial glucose concentration, which can also cause hyperglycemia. Reactive oxygen species (ROS) generation is proportionally associated with chronic hyperglycemia [7]. As a result of ROS oxidation of cellular components, cellular functions are compromised. Naturally, all aerobic organisms have a built-in antioxidant defense system that inhibits oxidative damage, but ROS often overwhelms the body's natural defense mechanism. Thus, dietary consumption of antioxidants is advised to help combat it. Naturally occurring antioxidants act as singlet oxygen quenchers, pro-oxidant metal complexes, reduction agents, and free-radical scavengers [8]. Additionally, they can increase plasma antioxidants' capacity, protect against free radicals and lipid oxidative rancidity in foods, and slow the advancement of many chronic diseases [9].

A common approach used in managing hyperglycemia by diabetic patients is fasting, though its efficacy is limited [1]. Diabetes management can be achieved using a variety of therapy modalities, one of which involves slowing down the absorption of glucose in the intestines by blocking enzymes responsible for the hydrolysis of carbohydrates, such as amylase and glucosidases [10]. Pancreatic juice and saliva contain α-amylase, which converts soluble starch molecules into simple, ingestible sugars. In contrast, α-glucosidase is located at the border brush of the small intestine, where it catalyzes glycosidic bonds, releasing glucose of either an oligo- or polysaccharide from the non-reducing end. Voglibose, miglitol, and acarbose are current prescription medications for inhibiting α -glucosidase and α -amylase, but there have been incidences of side effects that have been recorded [2,11].

According to the WHO, nearly 80 % of people worldwide currently utilize herbal medicines for various parts of primary healthcare. Traditional herbal remedies, culinary spices, and herbs have influenced the foundation of human healthcare in treating and managing chronic illnesses [9,12–14]. *Corchorus olitorius* L., commonly called "Jews mallow", is a vegetable plant that belongs to the Malvaceae family, originally known as Tiliaceae. It is a tall, annual plant that grows well in various soils [15,16]. *C. olitorius* is a popular vegetable in Nigeria, Egypt, Africa, Southern Asia, and the Middle East [17]. In Western Nigeria, the leaf of *C. olitorius* is extensively utilized as a vegetable in the preparation of 'Ewedu', a stew-based diet of mucilaginous texture [15]. Herbal tea can be obtained from the preparation of the leaves, while the seeds are widely employed as a flavoring agent. Previous research on *C. olitorius* revealed the existence of phytocomponents with a range of pharmacological functions, including antioxidants like vitamin E, glutathione, β-carotene, vitamin C, phenols and α-tocopherol; minerals, fatty acids, and mucilaginous polysaccharides [16,18,19]. There is a school of thought among herbal practitioners in Nigeria that taking *C. olitorius* reduces the absorption of glucose from glucose-rich complementary meals such as cassava flakes, yam flour, and wheat flour. To scientifically evaluate this hypothesis, this study investigated the phytochemical content, radical scavenging, and carbohydrate metabolizing enzyme inhibitory activity of *C. olitorius* lyophilized leaves.

## 2. Materials and Methods

*2.1. Chemicals and reagents.*

*α*-Glucosidase, *α*-amylase, para-nitrophenyl-glucopyranoside (ρNPG), gallic acid, tannic acid, rutin, and soluble amylose were obtained from Solarbio Life Sciences, Beijing, China. Acarbose was acquired from GlaxoSmithKline, Nigeria. Hydrogen peroxide, 2, 2-diphenyl-2-picrylhydrazyl, ascorbic acid, quercetin, and para-nitrophenyl-glucopyranoside (*ρ*NPG) were obtained from Sigma-Aldrich, USA. Dimethyl sulfoxide, sodium carbonate, and Folin-Ciocalteu reagent were procured from Qualikem, Mumbai, India. Other sourced reagents and chemicals utilized in the research were of analytical grade.

*2.2. Collection, identification and sample preparations.*

Fresh leaves of *C. olitorius* were acquired in October 2021 from Evergreen Farms, Ota, Ogun State, Nigeria (8.2681°N, 4.3267 °E). The plant sample was identified, and the voucher (CO/CUBio/315) number was assigned. *C. olitorius* leaves were shade-dried for ten days and crushed to powdered form. The crushed leaf sample (692 g) was immersed in distilled water (10 L) for 1 hour and allowed to boil for 10 minutes at 100$^{o}$C. The mixture was then filtered with a muslin cloth to obtain a freeze-dried filtrate using a lyophilizer to get *C. olitorius* aqueous lyophilized leaves sample (CO).

*2.3. Phytochemical evaluation.*

2.3.1 Total Phenolic Content (TPC) Assessment.

The TPC of CO was evaluated following the Folin-Ciocalteu method described by Iheagwam *et al*. [20]. TPC was expressed as mg equivalents of gallic acid per g of sample (GAE/g).

2.3.2. Total Flavonoid Content (TFC) Assessment.

The TFC of CO was assessed using the aluminum chloride assay method, as outlined by Iheagwam *et al*. [20]. TFC was expressed as mg equivalents of rutin per g of sample (RE/g).

2.3.3. Total Tannin Content (TTC) Assessment.

The TTC of CO was measured by the modified Folin-Ciocalteu method, as explained by Iheagwam *et al*. [20]. TTC was expressed as mg equivalents of tannic acid per gram of sample (TAE/g).

2.3.4. β-Carotene and lycopene assessment.

The β-Carotene and lycopene contents of CO were probed by the spectrophotometric method described by Iheagwam *et al*. [20]. β-carotene and lycopene contents were calculated according to the formula in equations 1 and 2, respectively, and values expressed as µg equivalents of carotene per g of sample (µg CAE/g):

$$\beta\text{-carotene} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453} \quad (1)$$

$$\text{Lycopene} = 0.0458A_{663} + 0.372A_{505} + + 0.0806A_{453} \quad (2)$$

*2.4. Antioxidant assays.*

2.4.1. Radical scavenging activity.

The scavenging activity of CO against 2, 2-diphenyl-2-picrylhydrazyl (DPPH) and hydrogen peroxide ($H_2O_2$) radicals was assessed following the methods described by Iheagwam *et al*. [8]. Ascorbic acid (AA), quercetin (QUER), and gallic acid (GA) were used as standards, while dimethyl sulfoxide (DMSO) was used as a control. The scavenging activity was expressed as the percentage using the formula shown in Equation 3:

$$\text{Radical Scavenged (\%)} = 100 \times \left(\frac{A_c - A_s}{A_c}\right) \quad (3)$$

Where $A_c$ and $A_s$ = absorbance of the control and the test sample, respectively.

2.4.2. Antioxidant activity.

Ferric reducing antioxidant power (FRAP) and total antioxidant capacity (TAC) were carried out according to the methods described by Iheagwam *et al*. [8]. Ascorbic acid (AA), quercetin (QUER), and gallic acid (GA) were used as standards, while dimethyl sulfoxide (DMSO) was used as a control.

*2.5. Glucose metabolizing enzyme inhibitory assays.*

2.5.1. α-Glucosidase inhibitory activity and mode of inhibition.

α-Glucosidase inhibitory activity and mode of inhibition of CO were evaluated according to the method described by Iheagwam *et al*. [10]. Graded CO or acarbose concentrations (0.0625-1 mg/mL, 250 µL) were incubated with 500 µL of enzyme solution (1 U/mL) at 37°C for 15 min. After that, 250 µL of ρ-Nitrophenyl-α-D-glucopyranoside (ρNPG) solution (5 mM) was added to the mixture and incubated at 37°C for 20 min. The absorbance of the released p-nitrophenol was measured at 405 nm. All solutions were prepared in 0.1 M phosphate buffer (pH 6.8). Inhibitory activities were calculated as shown in Equation 4:

$$\text{\% Inhibition} = 100 \times \left(\frac{A_c - A_s}{A_c}\right) \quad (4)$$

Where $A_c$ = absorbance of the control and $A_s$ = absorbance of the test sample.

For the mode of inhibition, CO (250 µL, 1 mg/mL) was incubated with 500 µL of enzyme solution for 10 min at 25°C as inhibitor samples, while 500 µL of α-glucosidase was preincubated with 250 µL of phosphate buffer (pH 6.8) in another set of tubes as non-inhibitor samples. Varying ρNPG concentrations (250 µL, 0.15-5 mM) were pipetted to both inhibitor and non-inhibitor mixtures to start the reaction. The mixture was allowed to proceed, as emphasized above. The amount of reducing sugars released was determined spectrophotometrically using a p-nitrophenol standard curve. Reaction rates were calculated, and double reciprocal plots of enzyme kinetics were constructed following the Lineweaver-Burk method to study the inhibition and kinetic parameters.

2.5.2. α-Amylase inhibitory activity and mode of inhibition.

α-Amylase inhibitory activity and mode of inhibition of CO were evaluated according to the method described by Iheagwam *et al*. [10]. Graded CO or acarbose concentrations (0.0625-1 mg/mL, 250 µL) were incubated with 500 µL of enzyme solution (2 U/mL) at 37°C

for 20 min. After that, 250 µL of starch solution (1%) was added to the mixture and incubated at 37°C for an hour. DNS color reagent (1 mL) was added and boiled for 10 min. The absorbance of the mixture was measured at 540 nm. All solutions were prepared in 0.1 M phosphate buffer (pH 6.8). Inhibitory activities were calculated as shown in Equation 5:

$$\% \text{ Inhibition} = 100 \times \left(\frac{A_c - A_s}{A_c}\right) \quad (5)$$

Where $A_c$ = absorbance of the control and $A_s$ = absorbance of the test sample.

For the mode of inhibition, CO (250 µL, 1 mg/mL) was preincubated with 500 µL of enzyme solution for 10 min at 25°C as inhibitor samples, while 500 µL of α-glucosidase was preincubated with 250 µL of phosphate buffer (pH 6.8) in another set of tubes as non-inhibitor samples. Varying starch concentrations (250 µL, 0.15-5 mg/mL) were added to inhibitor and non-inhibitor mixtures to start the reaction. The mixture was allowed to proceed, as emphasized above. The amount of reducing sugars released was determined spectrophotometrically using a standard maltose curve. Reaction rates were calculated, and double reciprocal plots of enzyme kinetics were constructed following the Lineweaver-Burk method to study the inhibition and kinetic parameters.

### *2.6. Statistical analysis.*

All data was cleaned on Microsoft Excel v2021 and presented as mean±SD. Differences in mean values were analyzed on IBM SPSS v25 using one-way ANOVA. The statistical difference among groups was evaluated at a 95% confidence interval using Duncan multiple range post hoc analysis.

## 3. Results and Discussion

### *3.1. Quantitative phytochemical analysis.*

From Table 1, the TFC (513.95 ± 36.88 mg RE/g) and TTC (345.64 ± 12.66 mg TAE/g) were present in CO in high quantities, followed by TPC (10.24 ± 0.23 mg GAE/g). β-carotene and lycopene contents were the least present phytochemicals in CO, with respective values of 1.12 ± 0.28 and 0.58 ± 0.10 mg CE/g. For millennia, people have opted for medicinal plants as an alternate choice of medicine in treating and managing diverse diseases. According to research, the therapeutic properties exhibited by these medicinal plants are attributed to both phytochemicals and non-phytochemical components that the plants contain. The high concentration of flavonoid and tannin in *C. olitorius* leaf and the corresponding low lycopene and β-carotene contents result from the use of water as the solvent. The latter two are lipid-soluble compounds, hence their inability to be extracted effectively by a polar solvent, while the former two are made up of majorly polar compounds hence their high extraction. Our findings have been corroborated by previous studies [21,22].

**Table 1.** Quantitative phytochemical assessment of *C. olitorius* lyophilized leave.

| **Phytochemical** | **Values** |
|---|---|
| Total Phenol Content (mg GAE/g) | 10.24 ± 0.23 |
| Total Flavonoid Content (mg RE/g) | 513.95 ± 36.88 |
| Total Tannin Content (mg TAE/g) | 345.64 ± 12.66 |
| Lycopene (mg CE/g) | 0.58 ± 0.10 |
| β-carotene (mg CE/g) | 1.11 ± 0.28 |

Values are represented as Mean ± SD of triplicate determination

These phytochemicals have been previously reported to exert antioxidant activity and, thus, may be important in curbing systemic hyperglycemia-induced oxidative stress, protecting cells from free-radical damage [7,23,24].

*3.2. Antioxidant assessment.*

*C. olitorius* aqueous lyophilized leaves exhibited a significantly ($p<0.05$) lower DPPH radical scavenging activity compared to AA, QUER, and GA at all tested concentrations. Interestingly, no significant difference ($p>0.05$) was observed between CO and the standards at the highest concentration of 1 mg/mL (Figure 1). Figure 2 shows a dose-dependent H2O2 radical scavenging activity for CO and the standards. However, CO exhibited a significantly ($p<0.05$) lower $H_2O_2$ radical scavenging activity compared to AA, QUER, and GA at all test concentrations. Figure 3 illustrates the ferric-reducing antioxidant power of CO. The result shows that CO was significantly ($p<0.05$) lower than the standards at varying low concentrations but showed no significant ($p>0.05$) difference at concentrations of 0.5 and 1 mg/mL in comparison with AA, QUER, and GA. CO's total antioxidant capacity was significantly ($p<0.05$) lower than AA and QUER at the varying low concentrations but showed no significant difference at the highest concentration. In contrast, CO was not significantly ($p>0.05$) different from GA at low concentrations (0.0625 – 0.5 mg/mL), but it was significantly ($p<0.05$) higher at 1 mg/mL (Figure 4). Free radicals are highly reactive and unstable oxygen- and nitrogen-containing species that gain stability by extracting electrons from other molecules. Unstable chemical species are harmed by free radicals because of their high level of reactivity. Elevated levels of free radicals result from a deficient endogenous antioxidant defense system, damage to cell structures, exposure to external oxidants, and chronic diseases such as diabetes mellitus [9,25]. The leaves of *C. olitorius* exhibited radical scavenging activity, reducing power and antioxidant capacity, especially at the highest concentration. This can be ascribed to the large concentration of flavonoids and tannins. These phytocompounds are known to exist as phenolic compounds and thus can donate protons, scavenge radical oxygen species, and bind metals [9]. On this basis, they exhibit their reducing potential and scavenge free radicals [26].

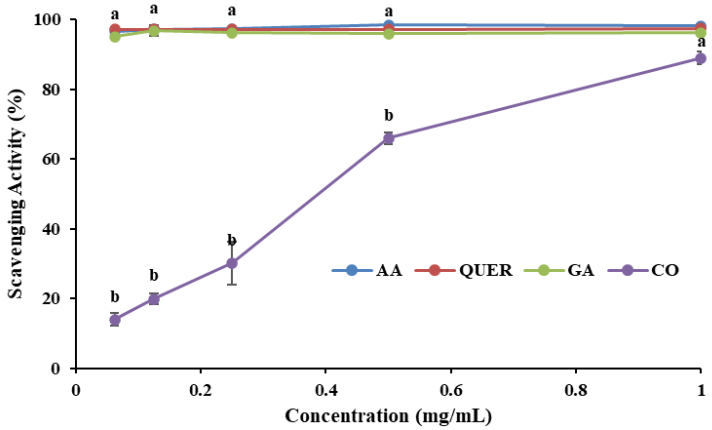


**Figure 1**. DPPH radical scavenging ability of *C. olitorius* aqueous lyophilized leaves. Data are represented as Mean ± SD (n=3). AA: Ascorbic acid, QUER: Quercetin, GA: Gallic acid, CO: *C. olitorius.*
Different superscripts per concentration = $p<0.05$
Same superscripts per concentration = $p>0.05$

These polyphenols serve as hydrogen donors, reduction agents, free radical scavengers, metal chelators, and singlet oxygen quenchers; hence, it is possible to link the radical

scavenging activity of the aqueous leave extracts of *C. olitorius* in the current study to their high concentration [27]. The findings from this study corroborate with previous reports of Oboh *et al*. [28] and Sadat *et al*. [22].

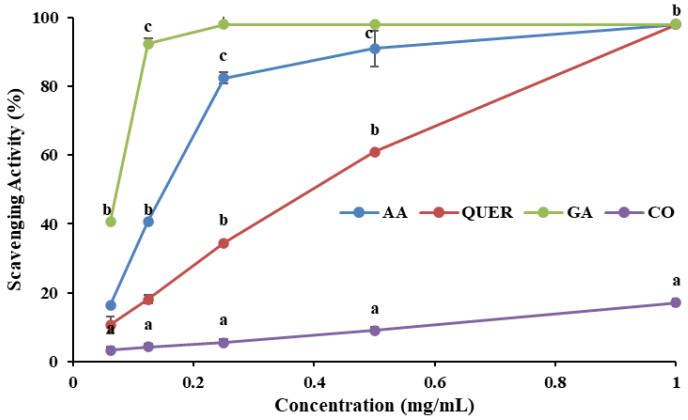


**Figure 2**. $H_2O_2$ radical scavenging ability of *C. olitorius* aqueous lyophilized leaves. Points are expressed as means ± SD (n=3). AA: Ascorbic acid, QUER: Quercetin, GA: Gallic acid, CO: *C. olitorius.*
Different superscripts per concentration = p<0.05
Same superscripts per concentration = p>0.05.

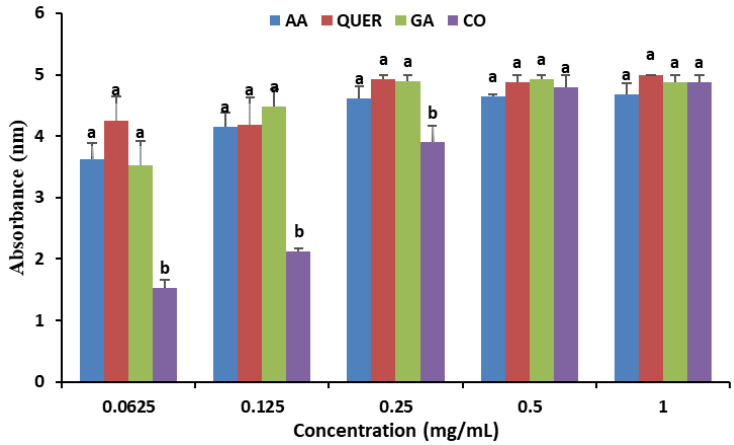


**Figure 3**. Ferric reducing antioxidant power of *C. olitorius* aqueous lyophilized leaves. Bars are expressed as means ± SD (n=3). AA: Ascorbic acid, QUER: Quercetin, GA: Gallic acid, CO: *C. olitorius*.
Different superscripts per concentration = p<0.05
Same superscripts per concentration = p>0.05.

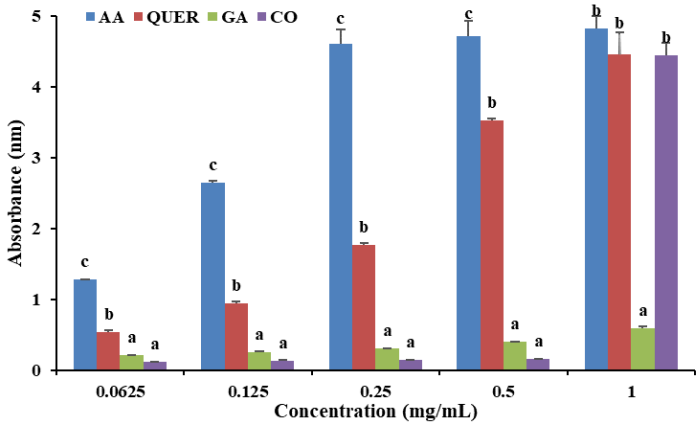


**Figure 4**. Total antioxidant capacity of *C. olitorius* aqueous lyophilized leaves. Bars are expressed as means ± SD (n=3). AA: Ascorbic acid, QUER: Quercetin, GA: Gallic acid, CO: *C. olitorius*.
Different superscripts per concentration = p<0.05
Same superscripts per concentration = p>0.05.

*3.3. Glucose metabolizing enzyme inhibitory assessment.*

In Figures 5 and 6, CO possessed a significantly ($p<0.05$) lower inhibitory action on *α*-amylase and *α*-glucosidase at all concentrations compared to acarbose. α-glucosidase and α-amylase are digestive enzymes that play a major role in carbohydrate metabolism. Thus, one of the most effective methods for managing diabetes mellitus is the regulation of blood glucose, which is achieved by inhibiting these two enzymes [8]. According to reports from some studies, plants with carbohydrate metabolizing enzyme inhibition activity have promising anti-diabetic characteristics primarily due to the bioactive constituent of their parts [29–31]. Diverse polyphenolic compounds present in medicinal and food plants can interact with proteins and suppress carbohydrate-hydrolyzing enzymes to elicit their anti-diabetic effects [32]. Therefore, the presence of polar flavonoids and phenolic compounds in *C. olitorius* could be the principal factors responsible for α-glucosidase and α-amylase inhibitory effects. The potential of plants high in phenolic compounds to lower postprandial blood glucose levels by inhibiting α-amylase and α-glucosidase is well-documented, corroborating the findings of this study [33].

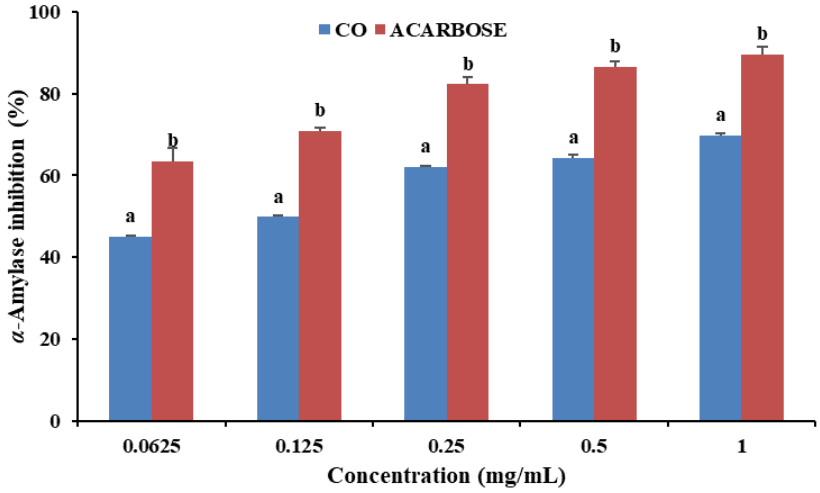


**Figure 5.** Inhibitory activity of *C. olitorius* aqueous lyophilized leaves on *α*-amylase activity. Bars are expressed as means ± SD (n=3). CO: *C. olitorius.*
Different superscripts per concentration = $p<0.05$
Same superscripts per concentration = $p>0.05$.

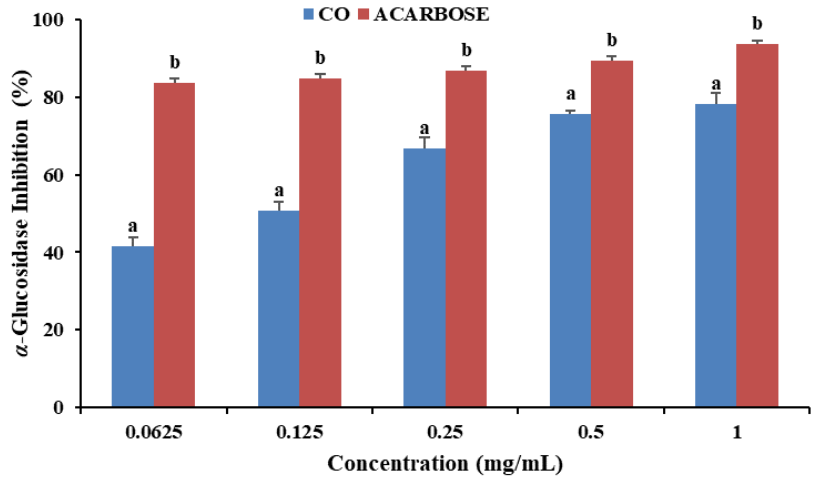


**Figure 6**. Inhibitory activity of *C. olitorius* aqueous lyophilized leaves on α-glucosidase activity. Bars are expressed as means ± SD (n=3). CO: *C. olitorius.*
Different superscripts per concentration = $p<0.05$
Same superscripts per concentration = $p>0.05$

Figures 7 and 8 show that CO elicited an uncompetitive mode of inhibition on *α*-amylase and *α*-glucosidase activities considering the parallel lines of the Lineweaver-Burk plots and the activity lines intersection which is close to the y-axis on the Michaelis-Menten plots. The $V_{max}$ of *α*-amylase (285.71 mM/min) and *α*-glucosidase (500 mM/min) were reduced by CO (111.11 and 156.25 mM/min), while the respective $K_m$ values (1.54 mg/mL and 1.18 mM) were also decreased by CO (0.69 mg/mL and 0.44 mM) (Table 2). The decrease in $K_m$ and $V_{max}$ suggests that the active compounds in *C. olitorius* aqueous lyophilized leaves bind only to the enzyme-substrate complex at an allosteric site after the carbohydrate binds to α-amylase and α-glucosidase in the active site [8]. The uncompetitive inhibitory effect of *C. olitorius* aqueous lyophilized leaves is most effective when the substrate concentration is high; thus, the inhibitory effect can only be reversed by decreasing the enzyme-substrate complex. This inhibition cannot be reversed by substrate concentration increase, benefiting diabetics in postprandial glucose mobilization truncation irrespective of the amount of carbohydrate ingested during the fed state and controlling feedback regulation [34,35]. The use of numerous vegetables in T2DM management as α-amylase and α-glucosidase inhibitors has been documented. This activity has been attributed to their phytoprinciples un-competitively inhibiting these enzymes' activities, corroborating our findings [36–38].

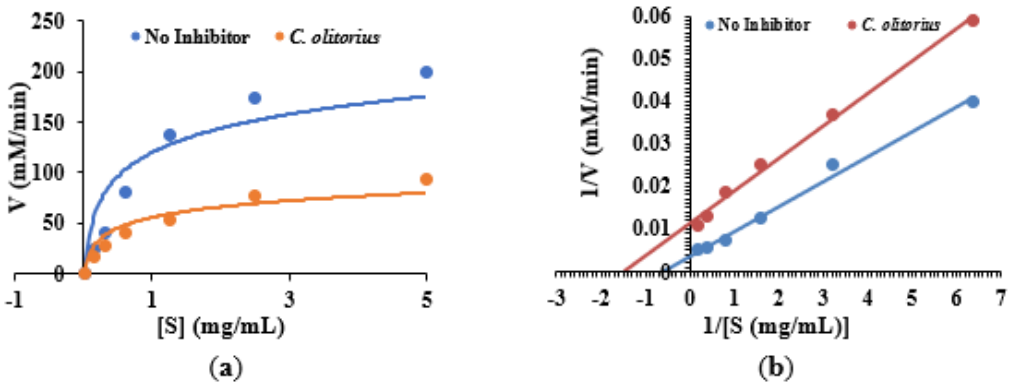


**Figure 7**. (**a**) Michaelis-Menten and (**b**) Lineweaver-Burk plots of *C. olitorius* aqueous lyophilized leaves mode of inhibition on α-amylase activity.

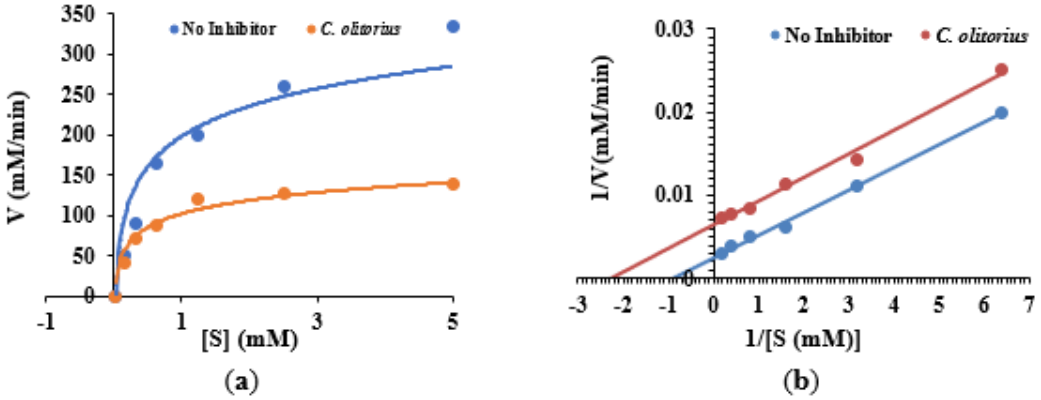


**Figure 8**. (**a**) Michaelis-Menten and (**b**) Lineweaver-Burk plots of *C. olitorius* aqueous lyophilized leaves mode of inhibition on α-glucosidase activity.

**Table 2.** Effect of *C. olitorius* aqueous lyophilized leaves on *α*-amylase and α-glucosidase kinetic parameters.

| | | $V_{max}$ (mM/min) | $K_m$ |
|---|---|---|---|
| ***α*-amylase** | No Inhibitor | 285.71 | 1.54[a] |
| | *C. olitorius* | 90.91 | 0.69[a] |
| **α-glucosidase** | No Inhibitor | 500.00 | 1.18[b] |
| | *C. olitorius* | 156.25 | 0.44[b] |

[a]: mg/mL, [b]: mM

## 4. Conclusions

The results from this study provide evidence that *C. olitorius* leaves contain potent phytochemicals, possess free radical scavenging activity, and have inhibitory effects on key carbohydrate metabolic enzymes. Overall, these findings contribute to the body of knowledge on the pharmacological qualities of *C. olitorius*, justifying its ethnomedicinal practices and usage in reducing the absorption of glucose from glucose-rich complementary meals. Thus, *C. olitorius* can be utilized as a natural source of anti-diabetic agents in managing postprandial hyperglycemia. It can also be a natural pleiotropic nutraceutical due to the phytoagents that positively impact myriad health outcomes. Further studies are underway to clarify the molecular processes that underlie the reported effects and evaluate the therapeutic effectiveness of *C. olitorius*.

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## Conflicts of Interest

The authors declare no conflict of interest.

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