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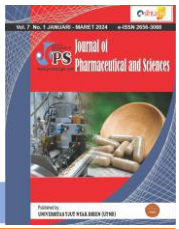

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In Vitro Evaluation of α-Amylase, α-Glucosidase Inhibitory, and Antioxidant Activities of Ethanolic Extracts from Five Indonesian Indigenous Vegetables: A Multivariate and Correlation Approach

Evaluasi Aktivitas Penghambatan α-Amilase, α-Glukosidase, dan Aktivitas Antioksidan secara *In Vitro* dari Ekstrak Etanol Lima Sayuran Indonesia: Pendekatan Multivariat dan Korelasi

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Abstract
Postprandial hyperglycemia and its associated oxidative stress are recognized as pivotal pathological hallmarks driving the progression of type 2 diabetes mellitus and its vascular complications. This study aims to evaluate and comparatively analyze the in vitro α-amylase, α-glucosidase inhibitory activities, alongside the free radical scavenging capacities, of ethanolic extracts derived from five widely consumed fresh vegetables in Indonesia: *Carica papaya, Momordica charantia, Gigantochloa atter, Diplazium esculentum*, and *Luffa acutangula*. Bioactivity assays were conducted utilizing starch-iodine, *p*-nitrophenyl-α-D-glucopyranoside, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methodologies. The experimental findings demonstrated that *D. esculentum* extract exhibited the most pronounced dual-inhibitory efficacy against α-amylase (IC_{50} 138.50 μg/mL) and α-glucosidase (IC_{50} 38.20 μg/mL). Furthermore, *D. esculentum* and *C. papaya* extracts demonstrated strong antioxidant capacities among all evaluated samples. Principal Component Analysis (PCA) and Pearson correlation analyses confirmed a strong positive correlation between free radical scavenging capacity and carbohydrate-hydrolyzing enzyme inhibition. In conclusion, *D. esculentum* emerges as a premier functional vegetable candidate, exhibiting remarkable dual-action therapeutic properties for the attenuation of postprandial hyperglycemia and mitigation of oxidative stress.

***Keywords*: Antioxidant, Diplazium esculentum, Hyperglycemia, α-Amylase, α-Glucosidase**

Abstrak
Hiperglikemia postprandial dan stres oksidatif yang menyertainya diakui sebagai patofisiologi krusial yang memicu progresivitas diabetes melitus tipe 2 beserta komplikasi vaskularnya. Penelitian ini bertujuan untuk mengevaluasi dan membandingkan aktivitas penghambatan enzim α-amilase, α-glukosidase secara in vitro, beserta kapasitas penangkapan radikal bebas, dari ekstrak etanol lima jenis sayuran segar yang umum dikonsumsi di Indonesia: pucuk daun pepaya (*Carica papaya*), buah pare (*Momordica charantia*), rebung (*Gigantochloa atter*), daun, batang muda pakis (*Diplazium esculentum*), dan buah oyong (*Luffa acutangula*) Pengujian bioaktivitas dilakukan menggunakan metode kompleks pati-iodin, *p*-nitrofenil-α-D-glukopiranosida, dan 2,2-difenil-1-pikrilhidrazil (DPPH). Hasil eksperimental menunjukkan bahwa ekstrak *D. esculentum* (pucuk pakis) memperlihatkan efikasi penghambatan ganda paling baik terhadap α-amilase (IC_{50} 138.50 μg/mL) dan α-glukosidase (IC_{50} 38.20 μg/mL). Selain itu, ekstrak *D. esculentum* dan *C. papaya* menunjukkan kapasitas antioksidan lebih baik di antara seluruh sampel yang diuji. Analisis *Principal Component Analysis* (PCA) dan *Pearson correlation* mengonfirmasi adanya sinergi positif yang signifikan antara kapasitas reduksi radikal bebas dan inhibisi enzim pencernaan karbohidrat. Kesimpulannya, *D. esculentum* teridentifikasi sebagai kandidat sayuran fungsional utama yang memiliki properti terapeutik aksi ganda yang baik untuk meredam hiperglikemia postprandial dan menekan stres oksidatif.

Kata Kunci: Antioksidan, Diplazium esculentum, Hiperglikemia, α-Amylase, α-Glucosidase.

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Introduction

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or a combination of both. This disease has become a major public health issue in Indonesia. The International Diabetes Federation estimated that approximately 20.4 million adults aged 20–79 years had diabetes in Indonesia in 2024, placing the country fifth globally for the highest number of people with diabetes. This number is projected to increase to approximately 28.6 million by 2050 [1]. This massive burden underscores the urgent need to develop effective glycemic control strategies, including the exploration of local dietary sources as bioactive compounds to support diabetes management.

Postprandial hyperglycemia is a crucial component of glycemic control. Following carbohydrate consumption, α-amylase hydrolyzes the α-1,4-glycosidic bonds in starch, producing oligosaccharides that are subsequently degraded by α-glucosidase into absorbable glucose. Inhibiting these two enzymes can delay carbohydrate digestion and attenuate the postprandial increase in blood glucose levels [2] . This mechanism has been widely utilized in diabetes pharmacotherapy through the administration of acarbose, an α-glucosidase inhibitor that also exhibits α-amylase inhibitory activity. Despite its efficacy, the use of acarbose frequently induces gastrointestinal disturbances, such as flatulence, diarrhea, and abdominal pain, which may limit treatment tolerability in some patients [3]. These adverse effects have prompted the search for alternative carbohydrate-hydrolyzing enzyme inhibitors derived from natural sources, particularly edible plants.

Beyond glucose metabolism disorders, oxidative stress is closely linked to the progression of diabetes and its complications. Repeated glucose fluctuations can accelerate the formation of reactive oxygen species (ROS) and disrupt the balance of the endogenous antioxidant defense system. Studies on healthy individuals and patients with type 2 diabetes demonstrate that oscillating glucose levels induce greater oxidative stress and endothelial dysfunction than constant exposure to high glucose [4]. Therefore, natural products possessing dual capabilities—inhibiting carbohydrate-digesting enzymes and exhibiting antioxidant activity—emerge as promising candidates for further investigation in the development of functional foods or natural-based products. However, in vitro antioxidant activity does not directly correlate with biological effects post-consumption, thus requiring subsequent in vivo confirmation.

Various commonly consumed vegetables are known to contain bioactive compounds with potential anti-hyperglycemic properties. Protein extracts from bitter gourd (*Momordica charantia* L.) have been reported to competitively inhibit α-amylase and α-glucosidase, as well as reduce glucose responses in diabetic animal models following starch and sucrose loading [2]. The hydroalcoholic extract of edible fern leaves (*Diplazium esculentum* (Retz.) Sw.) also exhibited free radical scavenging activity and anti-hyperglycemic effects in streptozotocin-induced diabetic rats [5]. Meanwhile, papaya leaf shoots (*Carica papaya* L.) have been shown to possess higher phenolic and flavonoid contents, alongside superior DPPH radical scavenging activity, compared to other parts of the papaya plant [6]. These findings highlight the potential of vegetables as sources of enzyme inhibitors and antioxidants. However, previous studies utilized diverse extract types, plant parts, and assay models, precluding direct comparisons of bioactivity among different vegetable species. To the best of our knowledge, simultaneous comparative evaluations of these five local vegetables using standardized in vitro assay methods remain limited. Accordingly, this study was designed by integrating a multivariate Principal Component Analysis (PCA) and correlation approach to comprehensively map and differentiate their bioactivity profiles.

Based on these considerations, this study aims to compare the α-amylase and α-glucosidase inhibitory activities, as well as the antioxidant capacities, of the ethanolic extracts of five commonly consumed fresh

vegetables: papaya leaf shoots (*Carica papaya* L.), bitter gourd (*Momordica charantia* L.), bamboo shoots (*Gigantochloa atter* (Hassk.) Kurz ex Munro), edible fern leaves (*Diplazium esculentum* (Retz.) Sw.), and angled luffa (*Luffa acutangula* (L.) Roxb.). Assays were conducted using the iodine-starch method for α-amylase inhibition, the *p*-nitrophenyl-α-D-glucopyranoside substrate for α-glucosidase inhibition, and the DPPH method for antioxidant capacity. The results of this study are expected to provide a scientific rationale for selecting the most potent vegetable candidates for further studies involving active compound identification, in vivo biological testing, and functional food development.

Experimental Section

Plant materials

Fresh plant samples were collected from Salareh Aia, Palembayan District, Agam Regency, West Sumatra. The specific plant parts and materials used are presented in **Table 1**.

Table 1. Plant materials used in the study

Plant Species	Part Used
Carica papaya L.	Leaf shoots
Momordica charantia L.	Fruit
Gigantochloa atter (Hassk.) Kurz ex Munro	Bamboo shoots or young sprouts
Diplazium esculentum (Retz.) Sw.	Young leaves and stems
Luffa acutangula (L.) Roxb.	Fruit

The botanical identity of all samples was authenticated by the Herbarium of Universitas Andalas (ANDA), Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, under the identification certificate No. 016/K-ID/ANDA/I/2025.

Chemicals and apparatus

The primary chemicals used included 96% ethanol (food grade), analytical grade methanol, dimethyl sulfoxide (DMSO), sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, sodium carbonate, hydrochloric acid, potassium iodide, iodine, and starch (all purchased from Merck). The enzymes utilized were α-amylase from *Bacillus licheniformis* and α-glucosidase from *Saccharomyces cerevisiae* (Sigma-Aldrich). The substrate for the α-glucosidase assay was *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG), while 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used for the antioxidant assay. Acarbose (Sigma-Aldrich) served as the positive control in both enzyme assays, and ascorbic acid (Sigma-Aldrich) was used as the reference standard for the antioxidant assay. The main apparatus included an analytical balance (Sartorius), rotary evaporator (Büchi R-210), micropipettes (Eppendorf), 96-well microplates, 96-well deep-well plates, ultrasonicator (Bransonic), vortex mixer (BiONEX), water bath, and a microplate reader (Allsheng FlexA-200).

Sample preparation and extraction

Fresh samples were chopped to increase the surface area for solvent contact. A weighed amount of each fresh sample was macerated separately using 96% ethanol at a ratio of 1:10 (w/v) for 24 hours at room temperature with intermittent manual stirring every 6 hours for 5 minutes. The macerate was then filtered, and the residue was rinsed with a small volume of ethanol. The combined filtrates were concentrated using a rotary evaporator at 60°C to yield a thick extract. The extract yield percentage for each sample was calculated as the weight of the obtained extract divided by the initial weight of the sample multiplied by 100%.

α-Amylase inhibitory activity assay

The α-amylase inhibitory activity was evaluated using the starch-iodine complex method, modified from Sudha et al. [7]. A 20 mM phosphate buffer (pH 6.9) containing approximately 6 mM NaCl was used as the reaction medium. A 0.25% starch solution was prepared in the phosphate buffer and heated until clear. The iodine reagent consisted of 5 mM I_2 and 5 mM KI. Ten milligrams of each extract were dissolved in DMSO to a volume of 1 mL to obtain a stock solution of 10,000 μg/mL. This solution was further subsequently diluted using phosphate buffer (pH 6.9) to ensure that the final concentration of DMSO in the reaction mixture did not exceed 1% (v/v). The concentration series of the extracts used in the assay were 15.625, 31.25, 62.5, 125,

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250, 500, and 1,000 μg/mL. Acarbose solutions were prepared similarly as a reference standard. The reaction mixture consisted of 20 μL phosphate buffer, 20 μL extract or acarbose solution, and 20 μL α-amylase solution (1 U/mL). The mixture was incubated at 37°C for 10 minutes. Subsequently, 20 μL of the 0.25% starch solution was added, and the mixture was re-incubated at 37°C for 15 minutes. The reaction was terminated by adding 20 μL of 1 M HCl, followed by the addition of 100 μL of iodine reagent. Absorbance was measured at a wavelength of 620 nm using a microplate reader. Each test was accompanied by a blank and blank control (the blank was prepared without the extract, while the blank control lacked both the extract and enzyme, both containing DMSO at the working concentration), sample control, solvent control, and positive control. The sample control was prepared without the enzyme to correct for the intrinsic absorbance of the extracts.

α-Glucosidase inhibitory activity assay

The α-glucosidase inhibitory activity was assessed using pNPG as the substrate, following a modified method by Maryam et al. [8]. The α-glucosidase solution was prepared in phosphate buffer (pH 6.9) at a concentration of 10 U/mL. A 5 mM pNPG solution served as the substrate, and 200 mM Na_2CO_3 was used to terminate the reaction. Ten milligrams of each extract were dissolved in DMSO to a volume of 1 mL to obtain a stock solution of 10,000 μg/mL. This solution was further subsequently diluted using phosphate buffer (pH 6.9) to ensure that the final concentration of DMSO in the reaction mixture did not exceed 1% (v/v). The concentration series of the extracts used in the assay were 15.625, 31.25, 62.5, 125, 250, 500, and 1,000 μg/mL. Acarbose was used as the reference standard at concentrations ranging from 1.953 to 125 μg/mL. In a microplate, 30 μL phosphate buffer, 30 μL extract or acarbose solution, and 20 μL α-glucosidase solution (10 U/mL) were mixed and incubated at 37°C for 5 minutes. Following this, 20 μL of 5 mM pNPG solution was added, and the mixture was further incubated at 37°C for 15 minutes. The reaction was stopped by adding 100 μL of 200 mM Na_2CO_3. The absorbance of the resulting *p*-nitrophenol was measured at 405 nm. Each test was accompanied by a blank and blank control (the blank was prepared without the extract, while the blank control lacked both the extract and enzyme, both containing DMSO at the working concentration), sample control, solvent control, and positive control. The sample control was prepared without the enzyme to correct for the intrinsic absorbance of the extracts.

Antioxidant activity assay

The antioxidant activity was evaluated using the DPPH radical scavenging method, adapted from Ali et al. [9]. Ten milligrams of the extract were dissolved in analytical grade methanol up to 10 mL to produce a 1,000 μg /mL stock solution. The solution was serially diluted to a range of 0.488 to 1,000 μg/mL. Ascorbic acid served as the positive control across the same concentration range. A 500 μM DPPH solution was prepared in methanol. In a 96-well microplate, 100 μL of the extract or ascorbic acid solution was mixed with 100 μL of the DPPH solution. The mixture was incubated at 37°C for 30 minutes in the dark. The blank consisted of 100 μL methanol and 100 μL DPPH solution. Absorbance was recorded at 515 nm, and each measurement was performed in triplicate.

Data analysis

The inhibition percentages of α-amylase and α-glucosidase were calculated using the absorbance values corrected against the respective sample controls:

$$A_{corrected} = A_{sample} - A_{sample\_control} \quad (1)$$

$$Inhibtioni\ \alpha\text{-}amylase\ /\ \alpha\text{-}glukosidase\ (\%) = \frac{A_{blank} - A_{corrected}}{A_{blank}} \times 100 \quad (2)$$

The DPPH radical scavenging percentage was calculated using the following equation:

$$Inhibtion\ \ DPPH\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (3)$$

The half-maximal inhibitory concentration (IC_{50}) values for α-amylase, α-glucosidase, and DPPH were determined via linear regression, plotting the logarithmic concentration on the x-axis against the probit value

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of the inhibition percentage on the y-axis. The data are also presented as the relative potency of the test extracts compared to the reference standards, calculated using the equation:

$$Potency\,(\alpha\text{-}amylase\,/\,\alpha\text{-}glukosidase\,/\,DPPH)\,(\%) = \frac{IC50\ Standard}{IC50\ Extract} \times 100 \qquad (4)$$

The potency data were statistically analyzed using One-Way ANOVA, Pearson correlation analysis, and Principal Component Analysis (PCA) via SPSS software 22nd version.

Results and Discussion

Extraction

The single-cycle maceration of the five fresh vegetable samples using 96% ethanol yielded varying extract percentages, ranging from 0.24% to 1.70% (**Table 2**). Among the tested samples, *D. esculentum* provided the highest extraction yield (1.70%), followed by *L. acutangula* (1.33%) and *C. papaya* (1.21%). The high yield in *D. esculentum* indicates a favorable accumulation of ethanol-soluble polar secondary metabolites, such as phenolics and flavonoids. Conversely, *G. atter* exhibited the lowest yield (0.24%), which is primarily attributed to its high moisture and water matrix content in fresh sprouts that limits the partitioning of ethanol-soluble extractives.

Table 2. Extract yield percentages of the ethanolic extracts from the sample

Plant Species	Extract Yield (%)
Carica papaya L.	1.21
Momordica charantia L.	0.79
Gigantochloa atter (Hassk.) Kurz ex Munro	0.24
Diplazium esculentum (Retz.) Sw.	1.70
Luffa acutangula (L.) Roxb.	1.33

In vitro bioactivity assays

This study evaluated the potential of five fresh vegetables commonly consumed in Indonesia as bioactive agents for postprandial hyperglycemia control and oxidative stress suppression. The evaluated parameters included the inhibitory activities against carbohydrate-digesting enzymes (α-amylase and α-glucosidase) and free radical scavenging capacity (DPPH). The IC_{50} values and the relative potency percentages of the extracts compared to positive controls (acarbose and ascorbic acid) are summarized in **Table 3**, and their statistical distributions are visualized in **Figure 1**.

Table 3. IC_{50} values and relative potency percentages of extracts in α-amylase, α-glucosidase, and DPPH inhibition assays.

Test Sample	α-Amylase Inhibitory Activity		α-Glucosidase Inhibitory Activity		DPPH Inhibitory Activity	
	IC_{50} (μg/mL)	Potency (%)[a]	IC_{50} (μg/mL)	Potency (%)[a]	IC_{50} (μg/mL)	Potency (%)[b]
C. papaya	217.84 ± 28.12	2.73 ± 0.35**	633.56 ± 49.21	0.76 ± 0.06*	224.21 ± 3.65	4.81 ± 0.08***
M. charantia	365.15 ± 54.86	1.65 ± 0.25*	125.60 ± 13.32	3.85 ± 0.37**	1515.71 ± 188.36	0.73 ± 0.09**
G. atter	730.07 ± 52.66	0.80 ± 0.06*	325.02 ± 49.17	1.54 ± 0.26*	2738.41 ± 317.71	0.41 ± 0.05*
D. esculentum	138.50 ± 4.07	4.17 ± 0.13***	38.20 ± 1.22	12.43 ± 0.40***	205.21 ± 4.15	5.26 ± 0.10****
L. acutangula	435.42 ± 45.59	1.35 ± 0.13*	275.03 ± 104.99	2.20 ± 0.63*	6456.11 ± 886.22	0.17 ± 0.02*
Acarbose	5.76 ± 0.02	-	4.74 ± 2.16	-	n.d	n.d
Ascorbic Acid	n.d	n.d	n.d	n.d	10.79 ± 0.31	-

[a] = potency data compared to acarbose; [b] = potency data compared to ascorbic acid; n = 3. n.d = not determined

** = significant differences based on Tukey's B post-hoc test at a 95% confidence level.*

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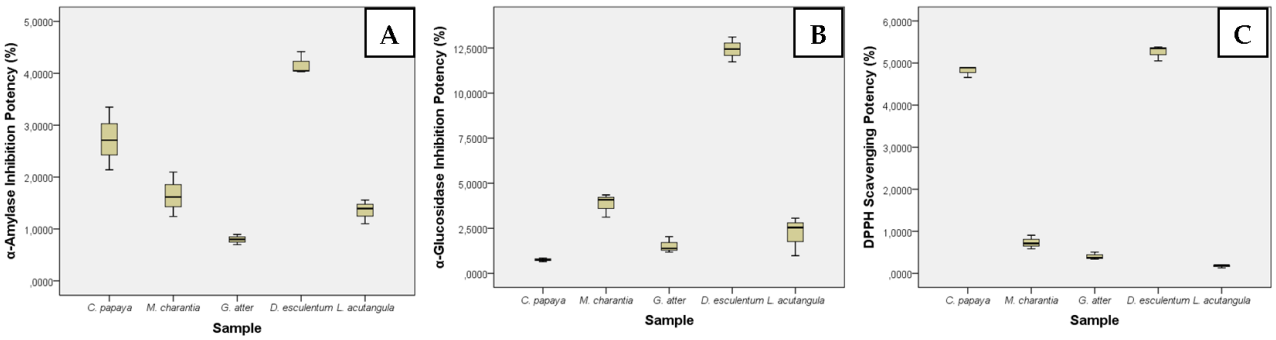

Figure 1. Boxplot graphs of the extracts relative potencies against the reference standards (a) α-amylase inhibition; (b) α-glucosidase inhibition; (c) DPPH inhibition.

α-Amylase and α-Glucosidase inhibitory activities

The inhibition of carbohydrate-digesting enzymes, namely α-amylase in saliva and the pancreas, and α-glucosidase in the brush border of the small intestine, is a primary pharmacological mechanism to delay glucose absorption and minimize postprandial blood glucose fluctuations [2]. The assay results (Table 2) indicated that all vegetable extracts exhibited *in vitro* inhibitory activities with varying profiles. *D. esculentum extract* demonstrated the most potent inhibitory activity against both enzymes, recording IC_{50} values of 138.50 ± 4.07 μg/mL for α-amylase and 38.20 ± 1.22 μg/mL for α-glucosidase. This efficacy equates to relative potencies of 4.17% and 12.43%, respectively, compared to the commercial drug acarbose.

One-Way Analysis of Variance (ANOVA) followed by Tukey's B post-hoc test at a 95% significance level confirmed that the inhibitory potency of the fern shoot extract was significantly different ($p < 0.05$) and occupied the highest subset, distinct from the other vegetables. The dominant activity of *D. esculentum* aligns with previous studies establishing it as a rich source of phenolic and flavonoid compounds with distinct anti-hyperglycemic properties [5]. Flavonoid derivatives are believed to interact non-covalently via hydrogen bonds and van der Waals forces with the amino acid residues in the active site pocket of the α-glucosidase enzyme, inducing structural conformational changes that effectively block substrate access [10].

Conversely, *C. papaya* extract displayed a specific inhibition pattern; it ranked second highest in α-amylase inhibition (IC_{50} 217.84 ± 28.12 μg/mL; potency 2.73%) but showed the weakest activity against α-glucosidase (IC_{50} 633.56 ± 49.21 μg/mL; potency 0.76%). This suggests that the phytochemical constituents in papaya leaves possess a more specific binding affinity towards the molecular architecture of α-amylase than that of α-glucosidase [11]. *M. charantia* extract, highly popular in traditional diabetes medicine, ranked intermediate with a potency of 3.85% against α-glucosidase, confirming its competitive nature in oligosaccharide hydrolysis [2]. Meanwhile, *G. atter* and *L. acutangula* extract exhibited the lowest enzymatic bioactivities among the tested samples.

In this study, the α-amylase inhibitory activity was evaluated using the spectrophotometric starch-iodine method, which remains widely adopted due to its suitability for high-throughput comparative screening [7]. Nevertheless, inherent methodological limitations associated with this assay must be critically acknowledged. As documented in previous literature, plant extracts or bioactive compounds possessing high antioxidant and polyphenolic contents can interact directly with the iodine-potassium iodide reagent, causing a decolorization effect on the starch-iodine complex independently of enzymatic activity. This non-enzymatic reaction mimics free radical reduction (such as in DPPH assays), which can confound absorbance measurements and potentially lead to inaccuracies in apparent inhibition outcomes [12]. Although sample controls were implemented in this study to correct for the intrinsic background absorbance of the extracts, such controls do not fully eliminate dynamic reagent-level interactions. Consequently, future investigations are strongly recommended to corroborate these findings using alternative analytical approaches—such as the 3,5-dinitrosalicylic acid (DNS) method targeting reducing sugars or chromatography-based sugar quantification techniques-to ensure interference-free profiling of α-amylase inhibition.

Antioxidant Activity and Bioactivity Relationships

Rapid spikes in glucose levels frequently trigger the formation of reactive oxygen species (ROS), leading to oxidative stress and vascular endothelial cell dysfunction [4]. Consequently, an agent's ability to scavenge free radicals serves as a vital indicator in preventing diabetic vascular complications.

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Based on the DPPH radical scavenging assay (Table 2), *D. esculentum* and *C. papaya* extract led with the highest antioxidant capacities, exhibiting IC_{50} values of 224.21 ± 3.65 μg/mL (potency 4.81%) and 205.21 ± 4.15 μg/mL (potency 5.26%), respectively. Tukey's B statistical test for DPPH revealed that both *D. esculentum* and *C. papaya* extract belonged to the same homogeneous subset, indicating no statistically significant difference ($p > 0.05$) in their high antioxidant activities. The robust radical-reducing capacity of *C. papaya* extract correlates with the high accumulation of phenolic secretions concentrated in the shoot area [6].

Conversely, the other three extracts (M. charantia, G. atter, and L. acutangula) were classified into subsets with significantly lower antioxidant powers, recording IC_{50} values of 1515.71 ± 188.36 µg/mL (potency 0.73%), 2738.41 ± 317.71 µg/mL (potency 0.41%), and 6456.11 ± 886.22 µg/mL (potency 0.17%), respectively. This low activity yield in fresh vegetable fruits/sprouts is generally influenced by their high water matrix content, which dilutes the extraction ratio of bioactive polyphenol components [13]. Overall, the *D. esculentum* extract stood out for demonstrating a dual-action therapeutic effect: acting as a highly potent carbohydrate-digesting enzyme inhibitor (α-amylase IC_{50} 138.50 μg/mL; α-glucosidase IC_{50} 38.20 μg/mL) and a competitive antioxidant agent. Such comprehensive therapy is highly ideal in modern pharmacotherapy. Specifically, its strong α-glucosidase inhibition coupled with moderate α-amylase inhibition makes *D. esculentum* an optimal candidate for preventing excessive sugar absorption without triggering the fermentation of undigested carbohydrates in the colon, which frequently causes gastrointestinal side effects in patients [3,10].

Correlation Analysis and Principal Component Analysis (PCA)

Pearson correlation analysis (**Figure 2**) demonstrated a strong and significant positive linear relationship between α-amylase and α-glucosidase inhibitory activities ($r = 0.7574$; $p < 0.01$), as well as between α-amylase inhibition and DPPH radical scavenging capacity ($r = 0.8867$; $p < 0.001$). The positive correlation between α-glucosidase and DPPH was moderate ($r = 0.5218$; $p < 0.05$). The upward-sloping regression lines across all three graphs confirm that extracts with high antioxidant capacity tend to possess robust enzyme inhibitory power. The positive correlation suggests that extracts with higher antioxidant capacity tend to also exhibit stronger enzyme inhibitory activity, possibly due to the dual function of polyphenolic compounds. Based on established phytochemical profiles of these species, the observed enzyme inhibitory and antioxidant activities are strongly presumed to be mediated by secondary metabolites, particularly phenolic and flavonoid derivatives. Previous investigations on *D. esculentum* and *C. papaya* have highlighted their rich polyphenolic compositions and robust antioxidant capacities [5,6], whose functional hydroxyl groups are well-documented to participate in free radical scavenging and hydrogen bonding interactions with the active sites of carbohydrate-hydrolyzing enzymes [10,14]. Nonetheless, quantitative phytochemical assays—such as total phenolic content (TPC) and total flavonoid content (TFC)—as well as advanced chromatographic identification methods like LC-MS/MS, remain essential for our subsequent investigations to precisely profile and isolate the active constituents.

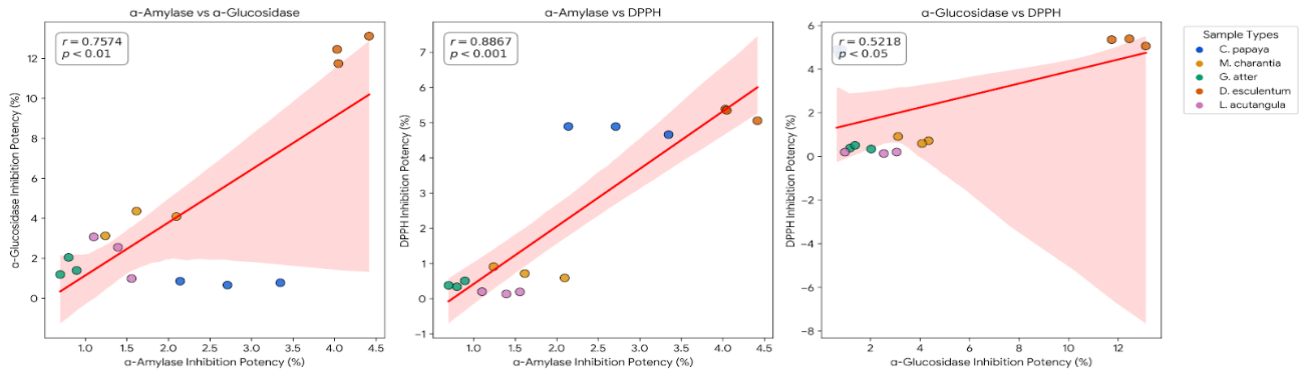

Figure 2. Linear regression plots of Pearson correlation among the relative potencies of α-amylase inhibition, α-glucosidase inhibition, and DPPH free radical scavenging of the five vegetable extracts. The red shaded area indicates the 95% confidence interval.

To reduce data dimensionality and group the vegetable extracts based on their bioactivity profile similarities, Principal Component Analysis (PCA) was applied. This extraction yielded two principal components (PC1 and PC2) that cumulatively explained 98.09% of the total variance (PC1: 81.80%; PC2:

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16.29%) (**Figure 3**). The PC1 axis aligns with the bioactivity spectrum, where coordinate locations with high positive scores represent superior enzyme inhibitory power and antioxidant capacity [15].

Further elucidate contribution of each bioactivity parameter, the eigenvalues, total variance percentages, and component loading factors derived from the PCA are summarized in **Table 4**. PC1 exhibited an eigenvalue of 2.454, accounting for 81.80% of the total variance, with α-glucosidase (loading = 0.942) and α-amylase (loading = 0.812) demonstrating the highest positive loadings. Conversely, PC2 accounted for 16.29% of the total variance (eigenvalue = 0.489), which was predominantly dominated by the DPPH antioxidant activity parameter (loading = 0.878). These loading distributions statistically confirm that PC1 primarily represents the carbohydrate-hydrolyzing enzyme inhibitory potency of the extracts, whereas PC2 captures their free radical scavenging capacity, providing a robust multivariate justification for the observed sample clustering.

Table 4. Eigenvalues, variance percentages, and factor loadings of variables in Principal Component Analysis (PCA).

Principal Component (PC)	Eigenvalue	Variance (%)	Cumulative Variance (%)	Factor Loadings		
				α-Amylase	*α*-Glucosidase	DPPH
PC1	2.454	81.80	81.80	0.812	0.942	0.655
PC2	0.489	16.29	98.09	0.235	-0.450	0.878

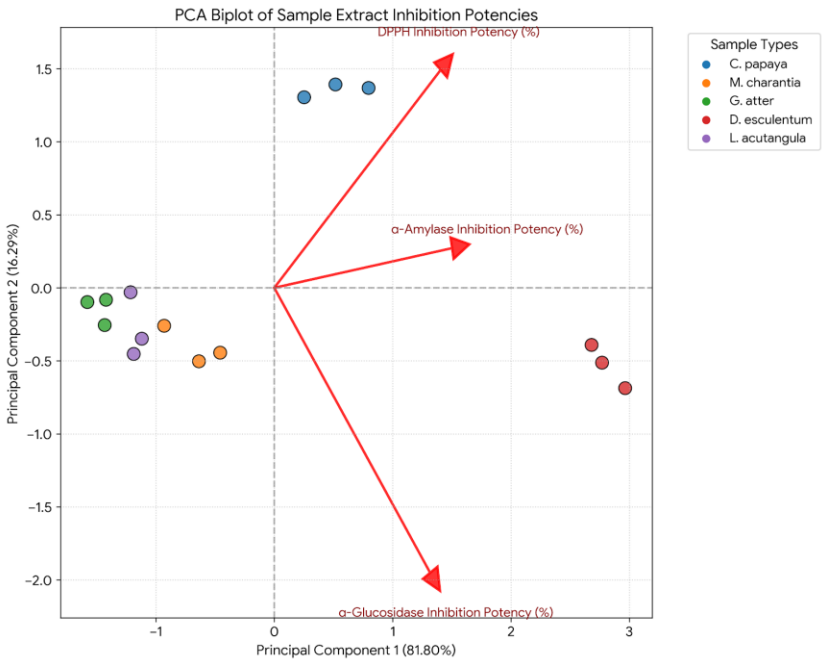

Figure 3. Principal Component Analysis (PCA) biplot visualizing the clustering of the five vegetable extracts based on their bioactivity profiles (relative potencies of *α*-amylase inhibition, *α*-glucosidase inhibition, and DPPH radical scavenging capacity).

The spatial visualization of the PCA biplot demonstrated highly specific clustering among the test samples. *D. esculentum* was distributed solitarily in the right area (positive PC1) parallel to the *α*-glucosidase and *α*-amylase activity vectors, confirming its comprehensive activity as a dual enzyme inhibitor. Conversely, the papaya leaf shoot (*C. papaya*) clustered in the direction of the DPPH vector, underscoring its dominant biological affinity as an oxidative stress-reducing agent. Bitter gourd (*M. charantia*) positioned itself in the central/origin area (moderate), while bamboo shoots (*G. atter*) and angled luffa (*L. acutangula*) clustered tightly at the far-left quadrant boundary (extreme negative PC1), indicating their weak bioactivity across all test parameters [15].

This multivariate mapping provides a rational foundation for future phytopharmaceutical selection. This study recommends *D. esculentum* as the most strategic raw material candidate for functional foods

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controlling postprandial glucose spikes, whereas *C. papaya* isolates hold greater potential to be specifically formulated as diabetic oxidative stress defense supplements [16].

Study Limitations and Future Directions

Despite the promising findings regarding the bioactivity of these indigenous vegetables, several limitations of this study must be acknowledged. First, the *in vitro* assays utilized microbial-derived enzymes (*Bacillus licheniformis* and *Saccharomyces cerevisiae*). Although the use of these enzymes serves as a standard model for preliminary screening, they possess structural and kinetic differences compared to mammalian or human digestive enzymes, which could potentially lead to variations in binding affinities and physiological inhibitory profiles. Second, the present study evaluated crude ethanolic extracts without subsequent fractionation processes. Consequently, the specific bioactive compounds responsible for the observed enzyme inhibitory and antioxidant activities remain definitively unidentified. Third, these purely *in vitro* enzymatic assays cannot fully represent the complexity of the human physiological environment, including gastrointestinal digestion, systemic bioavailability, and potential interactions with the gut microbiota. Finally, cytotoxicity and safety profiles were not evaluated within the scope of this initial comparative screening. Therefore, future research focusing on the use of mammalian enzyme models, bioassay-guided fractionation, *in vivo* pharmacological testing, and comprehensive toxicity evaluations is highly warranted to safely and effectively advance the development of these plant extracts into functional food products.

Conclusions

The evaluation of ethanolic extracts from five fresh vegetables revealed significant variations in their potential to inhibit carbohydrate metabolism enzymes and neutralize free radicals. *D. esculentum* were identified as the premier functional candidate, exhibiting significantly ($p < 0.05$) α-amylase and α-glucosidase inhibitory efficacy compared to the other vegetables, bolstered by a high antioxidant capacity (comparable to the activity of *C. papaya*). These data provide a scientific basis for future investigations—including in vivo efficacy evaluations, toxicity assessments, and the isolation and identification of active compounds—to support the prospective development of functional foods derived from local vegetable sources.

Conflict of Interest

The authors declare that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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