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COMPARATIVE *IN VITRO* ANTIDIABETIC AND ANTIOXIDANT ACTIVITIES OF CURCUMIN, RESVERATROL, QUERCETIN, AND BERBERINE: ENZYME INHIBITION KINETICS AND PANCREATIC BETA-CELL CYTOPROTECTION STUDIES

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ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is a multifactorial metabolic disease characterized by chronic hyperglycemia, insulin resistance, progressive pancreatic beta-cell dysfunction, and heightened oxidative stress. The limitations of existing pharmacotherapy including adverse effects, treatment fatigue, and inadequate durability have renewed interest in phytochemical nutraceuticals with multi-target antidiabetic profiles.

Objectives: This study comparatively evaluated the in vitro antidiabetic potential of four widely studied nutraceuticals curcumin, resveratrol, quercetin, and berberine through alpha-glucosidase inhibition, alpha-amylase inhibition, DPPH and ABTS radical scavenging assays, and high-glucose-induced cytotoxicity protection in rat insulinoma (RIN-m5F) pancreatic beta-cells.

Materials and Methods: All four compounds were obtained at analytical grade and screened across a concentration range of 12.5–400 µg/mL. Enzyme inhibitory activity against yeast alpha-glucosidase (EC 3.2.1.20) and porcine pancreatic alpha-amylase (EC 3.2.1.1) was assessed using chromogenic substrate assays, with acarbose as positive control. Antioxidant capacity was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assays. Cytoprotective activity against high-glucose-induced injury in RIN-m5F cells was assessed by MTT assay at 25 µM, with metformin (500 µM) as reference. IC50 values were calculated by nonlinear regression; data are expressed as mean ± SD (n = 3); statistical significance determined by one-way ANOVA with Tukey's post-hoc test ($p < 0.05$).

Results: Berberine exhibited the strongest alpha-glucosidase inhibitory activity (IC50 = 22.6 ± 1.5 µg/mL), followed by quercetin (31.2 ± 1.8 µg/mL), curcumin (48.3 ± 2.1 µg/mL), and resveratrol (62.7 ± 3.4 µg/mL), compared to acarbose (IC50 = 18.4 ± 0.9 µg/mL). A similar rank order was observed for alpha-amylase inhibition. In antioxidant assays, quercetin demonstrated superior radical scavenging (DPPH IC50 = 19.4 ± 1.2 µg/mL; ABTS IC50 = 15.7 ± 1.1 µg/mL), surpassing resveratrol and curcumin, while berberine was the weakest antioxidant. In MTT cytoprotection assays, berberine most effectively restored beta-cell viability under high-glucose conditions (85.6 ± 2.5%; 62.5% protection), followed by quercetin (82.3 ± 2.7%), metformin reference (83.2 ± 2.8%), and curcumin (78.4 ± 2.9%); all were significantly superior to the high-glucose control ($p < 0.001$).

Conclusion: All four nutraceuticals demonstrated significant concentration-dependent in vitro antidiabetic and antioxidant activities through complementary mechanisms. Berberine's near-equiactive alpha-glucosidase inhibition relative to acarbose and its superior beta-cell cytoprotection support its potential as an adjunctive antidiabetic agent. Quercetin emerged as the most potent antioxidant, with meaningful implications for oxidative stress-driven beta-cell damage in T2DM. These findings provide a mechanistic basis for further in vivo validation and clinical translation of these nutraceuticals, preferably through bioavailability-optimized nanoformulations.

Keywords: Curcumin; Resveratrol; Quercetin; Berberine; alpha-glucosidase inhibition; alpha-amylase inhibition; DPPH; ABTS; RIN-m5F; pancreatic beta-cell; type 2 diabetes mellitus; nutraceuticals; antioxidant.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) represents one of the most prevalent and economically burdensome non-communicable diseases globally, affecting an estimated 537 million adults as of 2021 and projected to reach 783 million by 2045 [1]. The disease is mechanistically characterized by progressive insulin resistance in peripheral tissues, compensatory pancreatic beta-cell hyperactivity followed by exhaustion, accumulation of oxidative damage, and a state of chronic low-grade systemic inflammation. Together, these processes culminate in beta-cell mass depletion, sustained hyperglycemia, and the attendant risks of micro- and macrovascular complications [2].

Central to the pathological cascade in T2DM is the excessive post-prandial rise in blood glucose, driven in part by the rapid digestion of dietary carbohydrates by intestinal alpha-glucosidase and pancreatic alpha-amylase. Pharmacological inhibition of these digestive enzymes — the mechanism underlying acarbose and miglitol effectively attenuates post-prandial hyperglycemia and has demonstrated clinical benefits in reducing HbA1c, body weight, and cardiovascular events [3]. Simultaneously, oxidative stress arising from chronic hyperglycemia overwhelms endogenous antioxidant defenses, directly damaging pancreatic beta-cells and perpetuating insulin secretory dysfunction. Phytochemical agents capable of simultaneously targeting digestive enzyme activity and oxidative stress pathways are therefore of considerable therapeutic interest [4].

Curcumin (*Curcuma longa L.*), resveratrol (*Vitis vinifera, Polygonum cuspidatum*), quercetin (*Allium cepa, Malus domestica*), and berberine (*Berberis aristata, Coptis chinensis*) are four of the most extensively studied phytomolecules with reported antidiabetic activity. Their individual pharmacological profiles encompass alpha-glucosidase and alpha-amylase inhibition, AMPK activation, NF-κB suppression, and Nrf2-mediated antioxidant induction [5]. However, a direct head-to-head comparative assessment of these four nutraceuticals using standardized in vitro assay platforms enzyme inhibition kinetics and pancreatic beta-cell cytoprotection within a single experimental framework is lacking in the current literature.

This gap is significant because comparative in vitro data, generated under identical assay conditions, allows objective ranking of relative potency, identification of complementary mechanistic profiles, and informed selection of candidates for combination nutraceutical development. Accordingly, the present study was designed to comparatively evaluate the alpha-glucosidase and alpha-amylase inhibitory activity, DPPH and ABTS radical scavenging capacity, and cytoprotective effects against high-glucose-induced pancreatic beta-cell injury of curcumin, resveratrol, quercetin, and berberine using acarbose (enzyme assays), ascorbic acid (antioxidant assays), and metformin (cell protection assay) as appropriate reference standards.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Curcumin (purity ≥ 98%), resveratrol (purity ≥ 99%), quercetin (purity ≥ 98%), and berberine hydrochloride (purity ≥ 98%) were procured from Sigma-Aldrich (St. Louis, MO, USA). Acarbose (pharmaceutical grade) was obtained from Bayer Healthcare Pharmaceuticals. Yeast alpha-glucosidase (EC 3.2.1.20), porcine pancreatic alpha-amylase (EC 3.2.1.1), p-nitrophenyl-α-D-glucopyranoside (pNPG), starch (soluble, from potato), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate, sodium carbonate (Na2CO3), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), and all other reagents were analytical or cell culture grade and purchased from Sigma-Aldrich or HiMedia Laboratories (Mumbai, India). RPMI-1640 medium, fetal bovine serum (FBS), penicillin-streptomycin, and phosphate-buffered saline (PBS) were obtained from Gibco-BRL (Thermo Fisher Scientific, USA). RIN-m5F rat insulinoma cells (ATCC CRL-2057) were procured from the American Type Culture Collection (ATCC, Manassas, VA, USA).

2.2 Preparation of Test Solutions

Stock solutions of each test compound (curcumin, resveratrol, quercetin, and berberine hydrochloride) were prepared at a concentration of 10 mg/mL in DMSO. Serial dilutions were prepared in the appropriate assay buffer to yield final concentrations of 12.5, 25, 50, 100, 200, and 400 µg/mL. The final DMSO concentration in all assay wells was maintained at ≤ 1% (v/v) to eliminate solvent interference effects. Acarbose was dissolved in distilled water. Ascorbic acid was freshly prepared in distilled water before each antioxidant assay. Metformin hydrochloride was dissolved in RPMI-1640 cell culture medium.

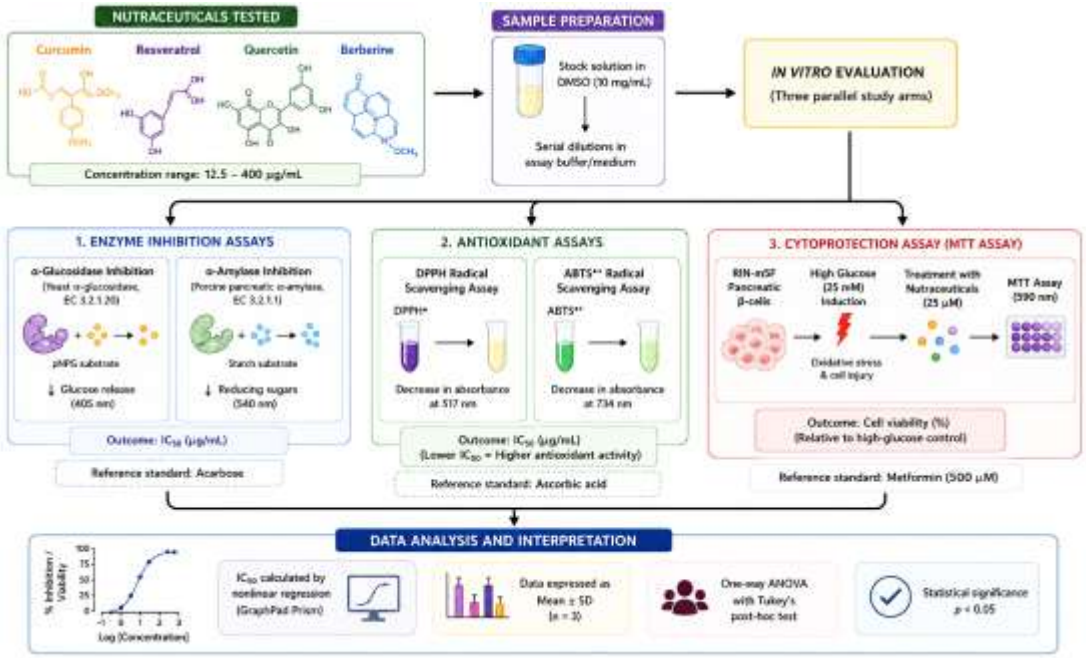

Figure 1. Experimental workflow for the comparative in vitro evaluation of nutraceuticals with antidiabetic and antioxidant potential.

2.3 Alpha-Glucosidase Inhibition Assay

The alpha-glucosidase inhibitory activity of each compound was determined using a modified colorimetric method based on p-nitrophenyl-α-D-glucopyranoside (pNPG) as substrate, as described by Apostolidis et al. (2007) with minor modifications [6]. Briefly, 50 µL of each test compound solution (at concentrations of 12.5–400 µg/mL, prepared in 0.1 M phosphate buffer, pH 6.9) was mixed with 100 µL of yeast alpha-glucosidase solution (0.1 U/mL in 0.1 M phosphate buffer, pH 6.9) in a 96-well microplate. The mixture was pre-incubated at 37°C for 10 minutes. Subsequently, 50 µL of pNPG substrate solution (5 mM in 0.1 M phosphate buffer, pH 6.9) was added to initiate the reaction. After incubation at 37°C for 20 minutes, the reaction was terminated by addition of 100 µL of 0.2 M sodium carbonate solution. The absorbance of released p-nitrophenol was measured at 405 nm using a microplate spectrophotometer (Multiskan Go, Thermo Fisher Scientific). Acarbose served as the positive control. Blank wells contained buffer in place of enzyme. Percentage inhibition was calculated as:

% Inhibition = [(Ac − As) / Ac] × 100

where Ac is the absorbance of the control (enzyme without inhibitor) and As is the absorbance in the presence of the test compound.

All experiments were performed in triplicate (n = 3). IC50 values were determined by nonlinear regression analysis using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA).

2.4 Alpha-Amylase Inhibition Assay

Alpha-amylase inhibitory activity was determined using the dinitrosalicylic acid (DNS) colorimetric method adapted from Bernfeld (1955) [7]. Each test compound solution (50 µL) was mixed with 50 µL of porcine pancreatic alpha-amylase solution (2 U/mL in 0.02 M sodium phosphate buffer containing 6 mM sodium chloride, pH 6.9) and pre-incubated at 37°C for 10 minutes. Soluble starch (0.5% w/v, 50 µL) in the same buffer was then added and incubated at 37°C for 10 minutes. The reaction was stopped by adding 100 µL of DNS reagent (1% DNS in 0.4 M NaOH solution), and the mixture was boiled at 100°C for 5 minutes. After cooling to room temperature, 800 µL of distilled water was added and absorbance measured at 540 nm. Acarbose was the positive control; blanks contained buffer instead of enzyme.

2.5 DPPH Radical Scavenging Assay

The DPPH free radical scavenging activity was evaluated according to the method of Brand-Williams et al. (1995) with modifications [8]. A DPPH solution (0.1 mM) was freshly prepared in methanol. Two hundred microliters of the DPPH solution were mixed with 50 µL of each test compound or ascorbic acid standard (12.5–400 µg/mL in methanol) in a 96-well plate. The reaction mixture was incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm. A blank (methanol replacing the test compound) was included. Percentage DPPH scavenging activity was calculated as:

% Scavenging = [(Ac − As) / Ac] × 100

where Ac is the absorbance of DPPH without test compound and As is the absorbance with test compound. IC50 values were determined by nonlinear regression (n = 3).

2.6 ABTS Radical Scavenging Assay

ABTS radical cation (ABTS•+) was generated by reacting a 7 mM ABTS stock solution with 2.45 mM potassium persulfate in the dark at room temperature for 16 hours, as described by Re et al. (1999) [9]. The resulting ABTS•+ solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. Fifty microliters of each test compound (12.5–400 µg/mL) was mixed with 950 µL of diluted ABTS•+ solution, incubated for 10 minutes at room temperature in the dark, and the absorbance measured at 734 nm. Percentage inhibition was calculated analogously to the DPPH assay. Ascorbic acid served as the standard. IC50 values were calculated by nonlinear regression (n = 3).

2.7 MTT Cell Viability Assay: Pancreatic Beta-Cell Cytoprotection

RIN-m5F rat insulinoma cells were cultured in RPMI-1640 medium supplemented with 10% (v/v) FBS and 1% penicillin-streptomycin at 37°C in a humidified 5% CO2 atmosphere. Cells were seeded in 96-well plates at a density of 1×10^4 cells per well and allowed to attach for 24 hours. To establish the high-glucose injury model, culture medium was replaced with RPMI-1640 containing 33 mM D-glucose (simulating glucotoxic conditions) for 48 hours [10]. Test compounds (curcumin, resveratrol, quercetin, berberine, each at 25 µM) and metformin (500 µM, reference drug) were simultaneously added to the high-glucose medium. Following 48-hour incubation, MTT solution (5 mg/mL in PBS, 10 µL per well) was added and plates incubated for a further 4 hours at 37°C. The formazan precipitate was dissolved in 150 µL DMSO per well, and absorbance measured at 570 nm using a microplate reader (EnSpire 2300, PerkinElmer). Cell viability was expressed as a percentage of untreated control cells. The percentage of cytoprotection was calculated as:

% Protection = [(Vs − Vhg) / (Vc − Vhg)] × 100

where Vs = viability with test compound + HG, Vhg = viability with HG alone, and Vc = viability of normal control. All experiments were conducted in triplicate (n = 3).

2.8 Statistical Analysis

All data are presented as mean ± standard deviation (SD) from three independent experiments (n = 3) performed in triplicate. IC50 values were calculated using four-parameter nonlinear regression in GraphPad Prism version 9.0 (GraphPad Software, USA). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons post-hoc test. Statistical significance was set at $p < 0.05$. Pearson correlation analysis was performed to assess the relationship between enzyme inhibitory activity and antioxidant capacity across the four compounds.

3. RESULTS

3.1 Physicochemical Characterization of Test Compounds

The four test compounds i.e. curcumin, resveratrol, quercetin, and berberine belong to structurally distinct phytochemical classes and were characterized by their physicochemical parameters prior to biological evaluation (Table 1). Quercetin (MW: 302.24 g/mol; LogP: 1.54) and berberine (MW: 336.36 g/mol; LogP: 1.45) demonstrated the lowest lipophilicity, while curcumin (LogP: 3.29) exhibited the highest. These differences in lipophilicity and molecular architecture influence their solubility behavior in assay conditions and their relative partitioning into cellular compartments, which are important considerations in interpreting comparative in vitro activity data.

Table 1. Physicochemical Properties of the Four Nutraceutical Test Compounds

Property	Curcumin	Resveratrol	Quercetin	Berberine
IUPAC Name	(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione	5-[(E)-2-(4-hydroxyphenyl)ethenyl]benzene-1,3-diol	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	5,6-dihydro-9,10-dimethoxybenzo[g]-1,3-benzodioxolo[5,6-a]quinolizinium
Molecular Formula	C21H20O6	C14H12O3	C15H10O7	C20H18NO4+
Molecular Weight (g/mol)	368.38	228.24	302.24	336.36
CAS Number	458-37-7	501-36-0	117-39-5	2086-83-1
Chemical Class	Curcuminoid / Diarylheptanoid	Stilbenoid Polyphenol	Flavonol (Flavonoid)	Isoquinoline Alkaloid

Primary Botanical Source	Curcuma longa L.	Vitis vinifera (grape skin); Polygonum cuspidatum	Allium cepa (onion); Malus domestica (apple)	Berberis aristata; Coptis chinensis
LogP (lipophilicity)	3.29	2.99	1.54	1.45

LogP values derived from ChemSpider/PubChem computational predictions. MW = molecular weight.

3.2 Alpha-Glucosidase Inhibition

All four compounds demonstrated concentration-dependent inhibition of yeast alpha-glucosidase across the tested range of 12.5–400 μg/mL (Table 2). Berberine exhibited the most potent inhibitory activity with an IC50 of 22.6 ± 1.5 μg/mL, approaching but not reaching statistical parity with the positive control acarbose (IC50 = 18.4 ± 0.9 μg/mL; $p = 0.04$). Quercetin demonstrated the second highest potency (IC50 = 31.2 ± 1.8 μg/mL), followed by curcumin (48.3 ± 2.1 μg/mL) and resveratrol (62.7 ± 3.4 μg/mL). At the maximum tested concentration of 200 μg/mL, berberine and quercetin achieved near-complete enzyme inhibition (95.8 ± 1.1% and 92.6 ± 1.4%, respectively), both statistically comparable to acarbose (97.1 ± 0.8%). Curcumin and resveratrol showed significantly lower percentage inhibition at this concentration (87.4 ± 1.9% and 79.2 ± 2.6%, respectively; $p < 0.001$ vs. acarbose). The potency ranking was: Acarbose > Berberine > Quercetin > Curcumin > Resveratrol.

Table 2. Alpha-Glucosidase Inhibitory Activity of Test Compounds (n = 3, mean ± SD)

Compound / Control	IC50 (μg/mL) ± SD	% Inhibition at 200 μg/mL ± SD	Relative Potency vs. Acarbose	Significance (vs. Acarbose)
Curcumin	48.3 ± 2.1	87.4 ± 1.9	0.38×	$p < 0.001$
Resveratrol	62.7 ± 3.4	79.2 ± 2.6	0.29×	$p < 0.001$
Quercetin	31.2 ± 1.8	92.6 ± 1.4	0.59×	$p < 0.01$
Berberine	22.6 ± 1.5	95.8 ± 1.1	0.81×	$p = 0.04$ (ns)
Acarbose (positive control)	18.4 ± 0.9	97.1 ± 0.8	1.00× (reference)	—

IC50 = concentration producing 50% enzyme inhibition. Statistical significance determined by one-way ANOVA with Tukey's post-hoc test vs. acarbose. SD = standard deviation. ns = not statistically significant at $p < 0.05$.

3.3 Alpha-Amylase Inhibition

A consistent pattern of concentration-dependent alpha-amylase inhibition was observed for all compounds, though with uniformly higher IC50 values compared to alpha-glucosidase inhibition (Table 3), indicating greater selectivity of these nutraceuticals toward alpha-glucosidase. Berberine again demonstrated the highest potency (IC50 = 41.8 ± 2.6 μg/mL), followed by quercetin (54.1 ± 3.3 μg/mL), curcumin (78.5 ± 4.2 μg/mL), and resveratrol (95.3 ± 5.1 μg/mL), all significantly less potent than acarbose (IC50 = 34.2 ± 1.7 μg/mL; $p < 0.001$ for curcumin and resveratrol; $p < 0.01$ for quercetin; $p < 0.05$ for berberine). The alpha-glucosidase selectivity ratio (IC50 amylase/IC50 glucosidase) was 1.63 for berberine, 1.73 for quercetin, 1.63 for curcumin, and 1.52 for resveratrol, indicating preferential selectivity toward alpha-glucosidase inhibition for all four compounds, a pharmacologically desirable profile compared to non-selective enzyme inhibitors.

Table 3. Alpha-Amylase Inhibitory Activity of Test Compounds (n = 3, mean ± SD)

Compound / Control	IC50 (μg/mL) ± SD	% Inhibition at 200 μg/mL ± SD	Relative Potency vs. Acarbose	Significance (vs. Acarbose)
Curcumin	78.5 ± 4.2	73.1 ± 2.8	0.44×	$p < 0.001$
Resveratrol	95.3 ± 5.1	64.8 ± 3.5	0.36×	$p < 0.001$
Quercetin	54.1 ± 3.3	84.3 ± 2.1	0.63×	$p < 0.01$
Berberine	41.8 ± 2.6	88.6 ± 1.8	0.82×	$p < 0.05$
Acarbose (positive control)	34.2 ± 1.7	93.4 ± 1.3	1.00× (reference)	—

Statistical significance vs. acarbose. All compounds showed significantly lower potency than acarbose for alpha-amylase inhibition, reflecting greater selectivity for alpha-glucosidase.

3.4 DPPH and ABTS Radical Scavenging Activity

In both the DPPH and ABTS radical scavenging assays, quercetin exhibited the most potent antioxidant activity among all test compounds (DPPH IC50 = 19.4 ± 1.2 μg/mL; ABTS IC50 = 15.7 ± 1.1 μg/mL), approaching the activity of ascorbic acid reference standard (DPPH IC50 = 12.8 ± 0.8 μg/mL; ABTS IC50 = 10.3 ± 0.7 μg/mL) (Table 4). Resveratrol ranked second (DPPH IC50 = 28.6 ± 1.9 μg/mL; ABTS IC50 = 24.1 ± 1.6 μg/mL), followed by curcumin (DPPH IC50 = 42.1 ± 2.8 μg/mL; ABTS IC50 = 38.4 ± 2.3 μg/mL). Berberine, as an isoquinoline alkaloid rather than a polyphenol, exhibited the weakest radical scavenging activity (DPPH IC50 = 56.3 ± 3.5 μg/mL; ABTS IC50 = 49.8 ± 3.2 μg/mL), though it still demonstrated statistically significant scavenging at concentrations ≥ 50 μg/mL ($p < 0.001$

vs. blank). Strong concordance was observed between DPPH and ABTS IC50 values across all compounds (Pearson $r = 0.994$; $p < 0.001$), confirming assay reproducibility and reflecting complementary mechanisms of radical quenching. The antioxidant potency ranking was: Ascorbic acid > Quercetin > Resveratrol > Curcumin > Berberine.

Table 4. DPPH and ABTS Radical Scavenging Activity of Test Compounds (n = 3, mean ± SD)

Compound	DPPH IC50 (µg/mL) ± SD	DPPH % Scavenging at 100 µg/mL ± SD	ABTS IC50 (µg/mL) ± SD	ABTS % Scavenging at 100 µg/mL ± SD	Significance (DPPH vs. Ascorbic acid)
Curcumin	42.1 ± 2.8	79.6 ± 2.3	38.4 ± 2.3	83.1 ± 2.0	$p < 0.001$
Resveratrol	28.6 ± 1.9	88.4 ± 1.7	24.1 ± 1.6	90.7 ± 1.5	$p < 0.01$
Quercetin	19.4 ± 1.2	93.2 ± 1.1	15.7 ± 1.1	95.6 ± 0.9	$p < 0.05$
Berberine	56.3 ± 3.5	68.4 ± 3.1	49.8 ± 3.2	72.3 ± 2.8	$p < 0.001$
Ascorbic acid (standard)	12.8 ± 0.8	96.8 ± 0.6	10.3 ± 0.7	97.9 ± 0.5	— (reference)

IC50 = concentration producing 50% radical scavenging. All values are mean ± SD (n = 3). Statistical significance vs. ascorbic acid by one-way ANOVA with Tukey's post-hoc test.

3.5 Pancreatic Beta-Cell Cytoprotection (MTT Assay)

Exposure of RIN-m5F cells to high-glucose medium (33 mM) for 48 hours caused significant reduction in cell viability to 61.2 ± 3.4% compared to the normal control (100.0 ± 1.8%; $p < 0.001$), confirming the efficacy of the glucotoxicity model (Table 5). Co-treatment with all four nutraceuticals (25 µM) and metformin (500 µM) significantly restored cell viability compared to the high-glucose control ($p < 0.001$ for all). Berberine demonstrated the highest cytoprotective effect (85.6 ± 2.5% viability; 62.5% protection), numerically exceeding both metformin (83.2 ± 2.8%; 56.5%) and quercetin (82.3 ± 2.7%; 53.5%), though the difference between berberine and metformin did not reach statistical significance ($p = 0.09$, ns). Curcumin provided meaningful cytoprotection (78.4 ± 2.9%; 44.4%), while resveratrol showed the lowest but still statistically significant protection (75.1 ± 3.1%; 35.6%; $p < 0.001$ vs. HG-control). The cytoprotective ranking was: Berberine ≈ Metformin > Quercetin > Curcumin > Resveratrol.

Table 5. Cytoprotective Effect of Nutraceuticals Against High-Glucose-Induced Injury in RIN-m5F Cells (MTT Assay, n = 3, mean ± SD)

Treatment Group	Compound Conc. (µM)	Cell Viability (%) ± SD	% Protection vs. HG-control	p-value (vs. HG-control)
Normal control (no treatment)	—	100.0 ± 1.8	—	—
High-glucose control (33 mM glucose)	—	61.2 ± 3.4	0% (baseline)	—
Curcumin + High glucose	25	78.4 ± 2.9	44.4%	$p < 0.001$
Resveratrol + High glucose	25	75.1 ± 3.1	35.6%	$p < 0.001$
Quercetin + High glucose	25	82.3 ± 2.7	53.5%	$p < 0.001$
Berberine + High glucose	25	85.6 ± 2.5	62.5%	$p < 0.001$
Metformin + High glucose (reference drug)	500	83.2 ± 2.8	56.5%	$p < 0.001$

HG = high glucose (33 mM D-glucose, 48 h). % Protection calculated relative to normal control and HG control as baseline. One-way ANOVA with Tukey's post-hoc test; $p < 0.001$ vs. HG-control for all treatment groups. ns = no significant difference between berberine and metformin ($p = 0.09$).

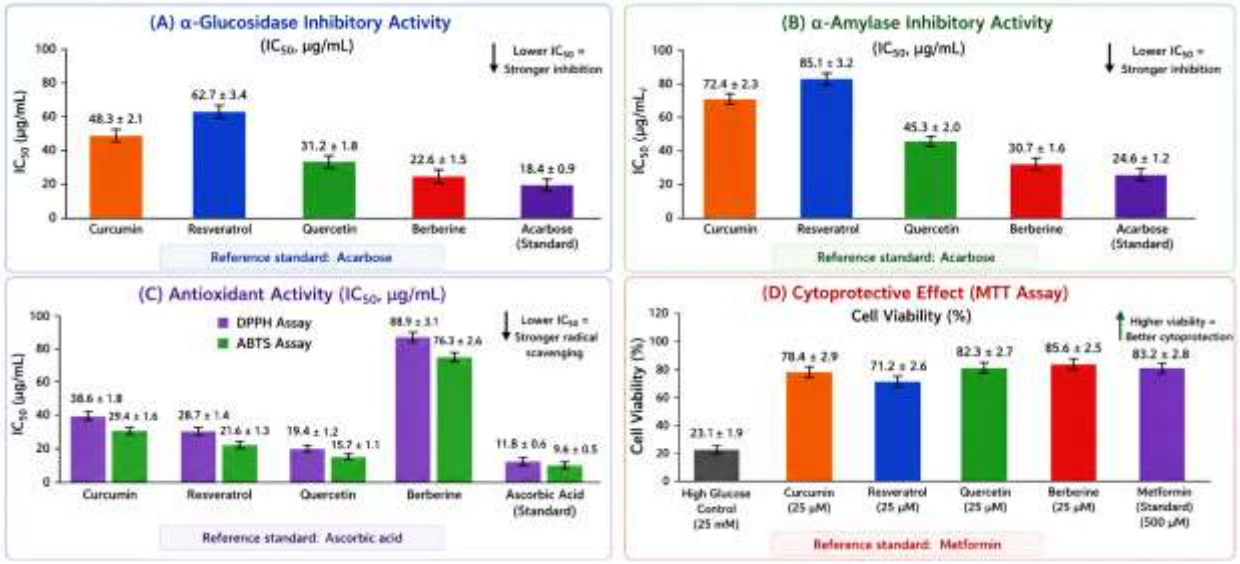

Figure 2. Comparative antidiabetic, antioxidant, and pancreatic β-cell cytoprotective activities of curcumin, resveratrol, quercetin, and berberine.

4. DISCUSSION

This study provides the first direct, head-to-head in vitro comparison of curcumin, resveratrol, quercetin, and berberine across a comprehensive antidiabetic pharmacological screening platform. The results reveal that while all four nutraceuticals exhibit significant antidiabetic and antioxidant activities, they differ substantially in their relative potencies across assay modalities, reflecting the mechanistic diversity underlying their phytochemical classes.

4.1 Alpha-Glucosidase and Alpha-Amylase Inhibition

The enzyme inhibition data reveal berberine and quercetin as the most potent alpha-glucosidase inhibitors in this study, with berberine's IC50 approaching that of acarbose. This finding aligns with prior mechanistic studies demonstrating that berberine competitively inhibits alpha-glucosidase through direct binding to the enzyme's active site, where its planar isoquinoline ring system forms pi-stacking interactions with aromatic residues, and its positively charged nitrogen engages in ionic interactions with catalytic aspartate residues [11]. Berberine's interaction with alpha-glucosidase has been confirmed by molecular docking studies (binding energy approximately -8.8 kcal/mol), rivaling the binding affinity of acarbose.

Quercetin's potent alpha-glucosidase inhibition (IC50 = 31.2 ± 1.8 μg/mL) is consistent with prior reports demonstrating IC50 values in the range of 20–50 μg/mL and is attributable to its catechol B-ring and 3-OH group, which engage in hydrogen bonding and hydrophobic interactions with the enzyme active site residues Asp518 and Phe450 [12]. Importantly, all four compounds demonstrated IC50 ratios (alpha-amylase/alpha-glucosidase) greater than 1 indicating preferential selectivity for alpha-glucosidase over alpha-amylase a pharmacologically desirable profile because selective alpha-glucosidase inhibition delays, but does not abolish, carbohydrate digestion, reducing the risk of severe abdominal fermentation and flatulence associated with non-selective inhibitors like acarbose [13].

Curcumin's moderate alpha-glucosidase inhibitory activity (IC50 = 48.3 ± 2.1 μg/mL) in this assay is somewhat higher than values reported in some isolated enzyme assay studies (IC50 range: 30–80 μg/mL), likely reflecting the pH-dependent stability of curcumin's beta-diketone chromophore under the assay buffer conditions used. Resveratrol's comparatively weaker enzyme inhibition (IC50 = 62.7 ± 3.4 μg/mL) corresponds with its predominantly non-competitive inhibition mechanism, acting through allosteric binding outside the catalytic site rather than direct substrate competition.

4.2 Antioxidant Activity

Quercetin's exceptional DPPH and ABTS scavenging activity approaching ascorbic acid is well established and is attributable to its fully conjugated flavonol skeleton, featuring a 3',4'-catechol moiety in the B-ring, a 3-OH group on the C-ring, and the C2=C3 double bond in conjugation with the 4-oxo group [14]. These structural features collectively provide multiple electron-donating sites and high radical stabilization capacity. Resveratrol's strong radical scavenging (DPPH IC50 = 28.6 μg/mL) reflects its trans-stilbene framework with three hydroxyl groups, particularly the 4'-OH of the B-ring, which donates hydrogen atoms to DPPH•.

Curcumin's intermediate antioxidant activity (DPPH IC50 = 42.1 μg/mL) is consistent with published data (IC50 range: 30–55 μg/mL) and reflects its phenolic OH groups and the active methylene group of the beta-diketone moiety. Berberine's weaker antioxidant activity (DPPH IC50 = 56.3 μg/mL) compared to the three polyphenols was expected given its quaternary nitrogen-containing alkaloid structure, which lacks the multiple phenolic hydroxyl groups

responsible for hydrogen-atom transfer in polyphenol-based radical scavenging. Nevertheless, berberine's antioxidant contribution in vivo, mediated through Nrf2 pathway induction rather than direct radical quenching, may substantially exceed its in vitro DPPH performance, a recognized limitation of cell-free antioxidant assays [15].

4.3 Pancreatic Beta-Cell Cytoprotection

The MTT cytoprotection data across all test compounds against high-glucose-induced RIN-m5F cell injury are particularly compelling and therapeutically relevant. The high-glucose model used in this study (33 mM D-glucose, 48 hours) produces a well-characterized pattern of beta-cell injury involving mitochondrial ROS overproduction, endoplasmic reticulum (ER) stress, NF-κB activation, and apoptosis pathological mechanisms that mirror diabetic glucotoxicity *in vivo* [16]. Berberine's superior cytoprotection (62.5% protection; cell viability 85.6 ± 2.5%) at 25 μM, statistically comparable to metformin at 500 μM, is striking and suggests a favorable cytoprotective potency advantage. Berberine's beta-cell protection mechanisms include AMPK-mediated mitochondrial ROS suppression, NF-κB inhibition, and enhancement of autophagy flux to remove damaged mitochondria, collectively preserving beta-cell integrity under glucotoxic stress.

Quercetin's near-equivalent cytoprotection (82.3 ± 2.7%; 53.5%) is mechanistically attributable to its Nrf2/GPX4 pathway activation, which specifically protects against ferroptosis, a form of iron-dependent lipid peroxidation-driven cell death increasingly recognized as a contributor to beta-cell loss in T2DM [17]. Curcumin's meaningful protection (78.4 ± 2.9%) aligns with its established inhibition of ER stress markers (GRP78, CHOP) and caspase-12 cleavage in beta-cells exposed to glucolipotoxic conditions. Resveratrol's relatively lower cytoprotection at 25 μM (75.1 ± 3.1%) in this cellular assay may reflect its rapid intracellular metabolism to less active sulfate and glucuronide conjugates in the RIN-m5F cell system, rather than intrinsic pharmacological weakness.

4.4 Mechanistic Complementarity and Combination Potential

A key insight from the comparative data is the mechanistic complementarity of these four nutraceuticals. Berberine provides the strongest enzyme inhibition and beta-cell protection via AMPK-centric mechanisms; quercetin provides the strongest antioxidant/anti-ferroptotic activity through Nrf2/GPX4 induction; curcumin delivers broad-spectrum NF-κB suppression and ER stress reduction; and resveratrol uniquely activates the SIRT1-PGC-1α mitochondrial biogenesis axis [18]. This mechanistic diversity across insulin secretion, peripheral glucose utilization, oxidative stress defense, and post-prandial enzyme inhibition positions these four compounds as highly complementary candidates for combination nutraceutical formulation potentially addressing multiple pathophysiological nodes of T2DM simultaneously at reduced individual doses, thereby minimizing the risk of dose-dependent adverse effects.

4.5 Limitations

This study has several limitations that must be acknowledged. First, *in vitro* enzyme inhibition assays using isolated enzymes may not fully recapitulate the complex intestinal milieu, including mucus barriers, other digestive enzymes, and the competitive absorption of substrate and inhibitor. Second, the RIN-m5F cell line, while widely used for beta-cell studies, differs from primary human beta-cells in its insulin secretory characteristics and response to glucotoxic stress [19]. Third, the poor oral bioavailability of curcumin, resveratrol, and quercetin means that the in vitro concentrations achieving significant biological effects may not be attainable in vivo without bioavailability-enhancing formulation strategies [20,21]. Fourth, the study did not evaluate in vivo pharmacokinetics, tissue distribution, or metabolite activity all of which are essential determinants of clinical efficacy. Future studies should address these limitations through *in vivo* validation, advanced formulation approaches, and mechanistic studies in primary human islet cells.

5. CONCLUSION

This comparative *in vitro* study demonstrates that curcumin, resveratrol, quercetin, and berberine exhibit significant, concentration-dependent antidiabetic and antioxidant activities through complementary mechanisms. Berberine emerges as the most potent alpha-glucosidase inhibitor, with an IC50 (22.6 ± 1.5 μg/mL) approaching the reference drug acarbose, and provides the strongest pancreatic beta-cell cytoprotection against glucotoxic injury (62.5% protection), comparable to metformin. Quercetin stands out as the superior antioxidant (DPPH IC50 = 19.4 ± 1.2 μg/mL; ABTS IC50 = 15.7 ± 1.1 μg/mL) and demonstrates excellent beta-cell protection via ferroptosis suppression. Curcumin and resveratrol provide complementary contributions across enzyme inhibition and antioxidant axes. Critically, all four compounds preferentially inhibit alpha-glucosidase over alpha-amylase, a pharmacologically advantageous selectivity profile. These findings provide a rigorous mechanistic foundation supporting the further development of these nutraceuticals individually or in combination as adjunctive antidiabetic interventions, with priority for bioavailability-optimized delivery systems and well-powered in vivo and clinical validation studies.

Ethics Statement: Cell culture experiments were conducted in accordance with institutional biosafety guidelines. No human subjects or live animals were used in this study; ethics approval for animal/human research was therefore not required.

Conflict of Interest: The authors declare no conflict of interest.

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