Laidlaw Research and Leadership report-Antidiabetic Effects of Phytochemicals Report

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Abstract

Phytochemicals are plant-derived bioactive compounds with potential antidiabetic effects, yet their efficacy in single and combination extracts remains underexplored.This project investigated the bioactive composition and in vitro antidiabetic properties in a range of anthocyanin-rich berries and plants. such as grape, beetroot, blueberry, hibiscus, blackcurrant, and cherry, including selected combinations. TPC was measured using Folin–Ciocalteu and Fast Blue BB assays, while betalains and anthocyanins were quantified spectrophotometrically. Enzyme inhibitory activity was assessed via IC_{50} determination.

Results revealed that grapes exhibited the highest TPC, whereas blackcurrant (whole and skin) and whole blueberry were richest in anthocyanins. Differently processed materials were used. Anthocyanin-rich extracts displayed potent α-glucosidase inhibition, while α-amylase inhibition was weaker and more variable. Combination extracts, such as grape + beetroot and blackcurrant + beetroot, showed altered TPC and inhibitory profiles, indicating potential synergistic or antagonistic interactions. Correlation analysis confirmed a strong association between phytochemical content and α-glucosidase inhibition.

These findings highlight the antidiabetic potential of phytochemical-rich extracts, with both single and multi-component formulations exhibiting bioactivity. The study underscores the importance of extract composition, processing methods, and potential interactions among phytochemicals for optimizing functional foods, nutraceuticals, and dietary interventions targeting type 2 diabetes.

Introduction

Phytochemicals are naturally occurring, bioactive, non-nutrient compounds produced by plants during secondary metabolism. While not considered essential nutrients, they have gained increasing attention due to their ability to promote human health through antioxidant, anti-inflammatory, antimicrobial, and metabolic regulatory activities (Hossain et al., 2025). In plants, phytochemicals act as pigments, defense molecules against pathogens, and protectants from environmental stressors such as ultraviolet radiation. They are widely distributed in fruits, vegetables, cereals, legumes, nuts, seeds, and medicinal herbs, and are broadly categorized into

polyphenols, carotenoids, alkaloids, glucosinolates, terpenoids, and saponins (Hossain et al., 2025).

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycemia due to impaired insulin secretion, insulin resistance, or both. It is a growing global health problem linked to obesity, sedentary lifestyle, and poor dietary habits, and it contributes significantly to cardiovascular, renal, and neurological complications (Cote et al., 2022). Although conventional therapies such as metformin, sulfonylureas, and α-glucosidase inhibitors are widely used, these treatments may have side effects and often fail to address underlying oxidative stress and inflammation, both of which play central roles in diabetic pathology.

Phytochemicals have emerged as promising agents in the prevention and management of diabetes because they target multiple mechanisms simultaneously. Polyphenols such as resveratrol (from grapes) and anthocyanins (from berries and cherries) enhance insulin sensitivity and promote glucose uptake in muscle and liver cells by upregulating GLUT4 transporters (Hossain et al., 2025). Betalains from beetroot reduce postprandial glucose levels by inhibiting digestive enzymes like α-amylase and α-glucosidase, while also protecting pancreatic β-cells from oxidative injury (Martinez et al., 2024). Hibiscus-derived flavonoids further support glycemic control by stimulating insulin secretion and enhancing glycogen storage in the liver (Cote et al., 2022). Collectively, these findings suggest that phytochemicals may provide a multi-targeted strategy to improve glycemic control and reduce diabetic complications.

In this research, the antidiabetic potential of selected phytochemical-rich sources were investigated, specifically grape, beetroot, blueberry (whole and fast-dried), hibiscus, blackcurrant (skin, whole fruit, and liquid/SPE extract), and cherry (liquid extract). Individual extracts were tested as well as combinations thereof, for example grape and beetroot or blackcurrant and beetroot. The inclusion of these mixtures was intentional, as phytochemicals can interact synergistically or antagonistically. By evaluating both single extracts and combinations, this research aimed to assess not only the individual efficacy of these phytochemical sources but also the possible interferences and complementary effects that arise when multiple compounds are present together. This approach provides a more comprehensive understanding of their potential applications in real-world dietary and therapeutic contexts.

The objectives of this research were threefold. First, to quantify the total phenolic content (TPC) of the extracts using both the Folin–Ciocalteu assay and the Fast Blue BB method, as phenolics are closely associated with antioxidant and antidiabetic activities (Singleton et al., 1999; Medina, 2011). Second, to determine the anthocyanin content of the extracts, given that anthocyanins are strongly implicated in improving insulin sensitivity and reducing postprandial hyperglycemia (Khoo et al., 2017). Third, to evaluate the inhibitory activity of the extracts against α-amylase and α-glucosidase, key enzymes in carbohydrate digestion whose inhibition delays glucose

absorption and blunts postprandial glucose spikes (Tundis et al., 2010; Sales et al., 2012). By combining phytochemical quantification with functional enzyme assays, this study sought to connect composition with bioactivity, while also assessing the impact of combining different extracts.

The research was guided by the following questions: (1) Do grape, beetroot, blueberry, hibiscus, blackcurrant, and cherry extracts differ in their phenolic and anthocyanin contents? (2) Do these extracts inhibit α-amylase and α-glucosidase activity? (3) Do combinations of extracts display synergistic or antagonistic effects compared to single extracts? (4) Is there a relationship between phytochemical composition and enzyme inhibition? Based on existing literature, it was hypothesized that extracts rich in phenolics and anthocyanins, such as blackcurrant, blueberry, and hibiscus, would show the strongest inhibitory effects. Furthermore, mixtures of extracts were expected to display enhanced activity due to synergistic interactions between different phytochemical classes. Lastly, it was predicted that a positive correlation would exist between phytochemical content and enzyme inhibitory potential, supporting the idea that phytochemical richness directly contributes to antidiabetic efficacy.

Methods

Folin–Ciocalteu Assay for Total Polyphenols

The total polyphenol content (TPC) of plant extracts was measured using the Folin–Ciocalteu colorimetric assay, adapted from Singleton et al. (1999). This method is based on the reduction of the Folin–Ciocalteu reagent, a mixture of phosphotungstic and phosphomolybdic acids, by phenolic compounds to form a blue-colored phosphotungstic-phosphomolybdenum complex. The intensity of the blue color is proportional to the concentration of polyphenols present. Standards were prepared via serial dilution of gallic acid (7.8125–500 µg/mL). Sample or standard (3 × 10 µL) was pipetted into 96-well plates, followed by 40 µL Folin reagent and 150 µL sodium carbonate solution. Plates were incubated at room temperature in the dark for 30 minutes, and absorbance was read at 765 nm using a Tecan Spark plate reader. Blank-corrected absorbances were used to construct a standard curve and calculate TPC (mg GAE/g sample).

Fast Blue BB Assay for Total Phenolics

Total phenolics were also quantified using the Fast Blue BB (FBBB) diazonium salt method, which forms stable azo complexes with phenolic hydroxyl groups under alkaline conditions, measurable at 420 nm (Medina, 2011; Hinojosa-Nogueira et al., 2017; Pico et al., 2020). Standards were prepared via serial dilution of gallic acid (10 mg/mL). Sample or standard (200 µL) was mixed with 20 µL Fast Blue BB and 20 µL NaOH in 96-well plates. The reaction was incubated at room temperature in the dark for 2 hours, and absorbance measured at 420 nm.

Blank-corrected absorbances were used to construct gallic acid standard curves, allowing calculation of phenolic content.

Quantification of Betalains and Anthocyanins

Betalains, including betacyanins and betaxanthins, were extracted from powdered samples using 20% methanol and quantified according to Wruss et al. (2015) and Stintzing et al. (2003, 2005). One gram of powder was mixed with 20 mL 20% methanol, vortexed for 2–3 minutes, incubated at room temperature for 15 minutes, and centrifuged at 3000 × g for 5 minutes. The supernatant was filtered and measured immediately, with excess stored at –20 °C. Absorbances were recorded at 536 nm (betacyanins) and 485 nm (betaxanthins), with background subtraction at 650 nm. Concentrations were calculated using:

$$\text{Betacyanin/Betaxanthin (mg/L)} = (A \times DF \times MW \times 1000) \div (\varepsilon \times i)$$

where A is the corrected absorbance, DF the dilution factor, MW the molecular weight (550 g/mol for betacyanins, 339 g/mol for betaxanthins), ε the molar extinction coefficient (60,000 L mol^{-1} cm^{-1} for betacyanins, 48,000 L mol^{-1} cm^{-1} for betaxanthins), and i the path length in cm.

α-Amylase and α-Glucosidase Inhibition Assays

α-Amylase activity was measured using a direct colorimetric assay with 2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) as substrate, which releases 2-chloro-4-nitrophenol upon hydrolysis, detectable at 405 nm (Deen et al., 1988). Extracts, inhibitors, or acarbose (positive control) were preincubated with 1 U/mL porcine pancreatic α-amylase in 50 mM phosphate buffer (pH 6.9) containing 200 mM NaCl and 5 mM $CaCl_2$. The reaction was initiated by adding 2 mM CNPG3 substrate and incubated at 37 °C for 10 minutes. Absorbance was recorded at 405 nm in 1-minute intervals. α-Glucosidase inhibition was measured similarly using p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate. Percent inhibition was calculated relative to 100% activity controls, and IC_{50} values were determined from dose-response curves. Lineweaver-Burk plots were constructed to evaluate enzyme kinetics and inhibition mechanisms.

Summary of Assays :Table 1

Assay	Principle	Sample/Standard	Reagents & Buffers	Wavelength (nm)	Calculation / Notes
Folin–Ciocalteu (Total Polyphenols)	Reduction of Folin–Ciocalteu reagent by phenolics forms blue complex	Extracts and gallic acid standard (7.8125–500 µg/mL)	25% Folin reagent, 4% Na_2CO_3, water	765	TPC (mg GAE/g) from standard curve, blank-corrected
Fast Blue BB (Total Phenolics)	Phenolic hydroxyls form stable azo complexes under alkaline conditions	Extracts and gallic acid standard (10 mg/mL)	0.1% Fast Blue BB, 5% NaOH, 0.4 M HCl	420	TPC (mg GAE/g) from standard curve, blank-corrected
Betalains (Betacyanins & Betaxanthins)	Colorimetric quantification based on absorbance of extracted pigments	Powder extracts (1 g in 20 mL 20% MeOH)	McIlvaine's citrate-phosphate buffer (pH 6.5)	536 (betacyanins), 485 (betaxanthins), 650 (background)	Concentration (mg/L)=A×DF×MW ×1000ε×iConcentration (mg/L)=ε×iA×DF× MW×1000
α-Amylase Inhibition (CNPG3)	Hydrolysis of CNPG3 releases 2-chloro-4-nitrophenol	Extracts, inhibitors, acarbose (positive control)	50 mM phosphate buffer (pH 6.9) + 200 mM NaCl + 5 mM $CaCl_2$	405	% Inhibition = (Control – Sample)/Control×100(Control – Sample)/Control×100; IC_{50} from dose-response
α-Glucosidase Inhibition (pNPG)	Hydrolysis of pNPG releases p-nitrophenol	Extracts, inhibitors, acarbose	50 mM phosphate buffer (pH 6.9)	405	% Inhibition = (Control – Sample)/Control×100(Control – Sample)/Control×100; IC_{50} from dose-response

Results

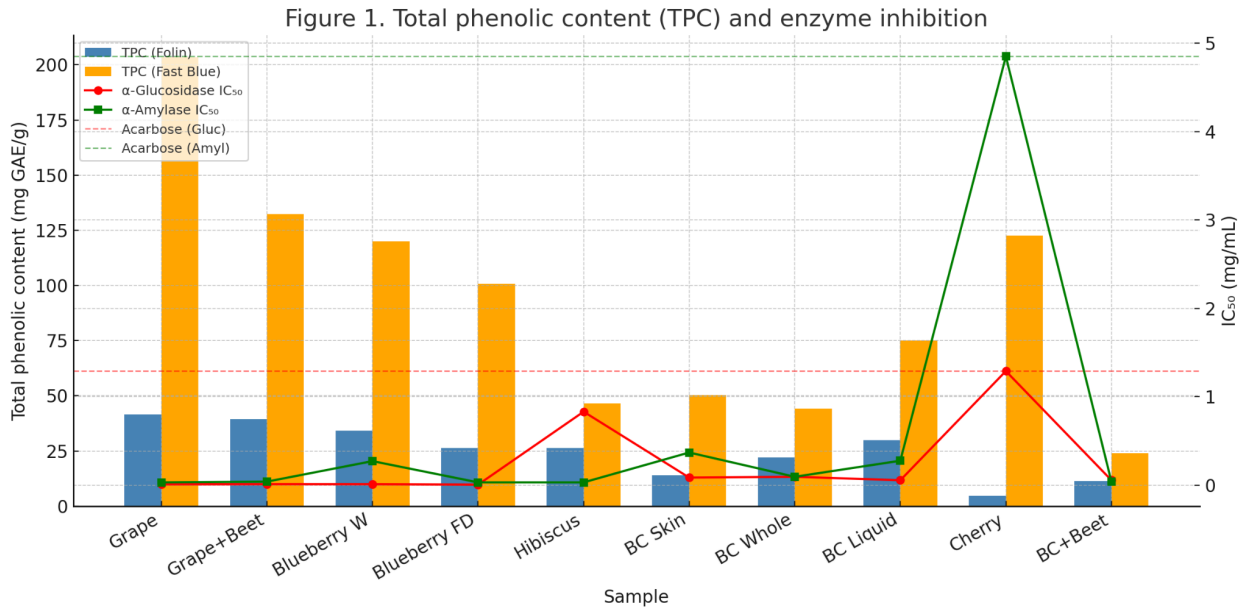

Figure 1. Figure 1. Total phenolic content (TPC) and enzyme inhibition activity of fruit extracts. The bars indicate TPC measured using the Folin–Ciocalteu (blue) and Fast Blue BB (orange) assays on the left y-axis, while the lines show IC_{50} values for α-glucosidase (red) and α-amylase (green) inhibition on the right y-axis. All IC_{50} values were calculated from dose–response curves using serial dilutions, ensuring that comparisons across extracts are consistent and reliable. The dashed lines represent the IC_{50} values of the pharmaceutical inhibitor acarbose (1.29 mM for α-glucosidase and 4.85 mM for α-amylase) for reference. Lower IC_{50} values correspond to stronger enzyme inhibition.

Grape extract (GP) exhibited the highest TPC in both assays and very low IC_{50} values for both α-glucosidase and α-amylase, indicating potent inhibitory activity. Whole blueberry (BB_W) and blackcurrant (BC_Skin and BC_Whole) also displayed strong α-glucosidase inhibition despite having lower TPC than grape, suggesting that specific phenolic compounds in these fruits possess particularly high bioactivity. Hibiscus extract, in contrast, showed low TPC and only weak enzyme inhibition. Overall, all fruit extracts demonstrated IC_{50} values considerably lower than those of acarbose, highlighting their strong intrinsic activity. Across the samples, α-amylase inhibition was generally weaker and more variable compared to α-glucosidase inhibition, which aligns with previous reports indicating that α-amylase is less susceptible to inhibition by phenolic compounds.

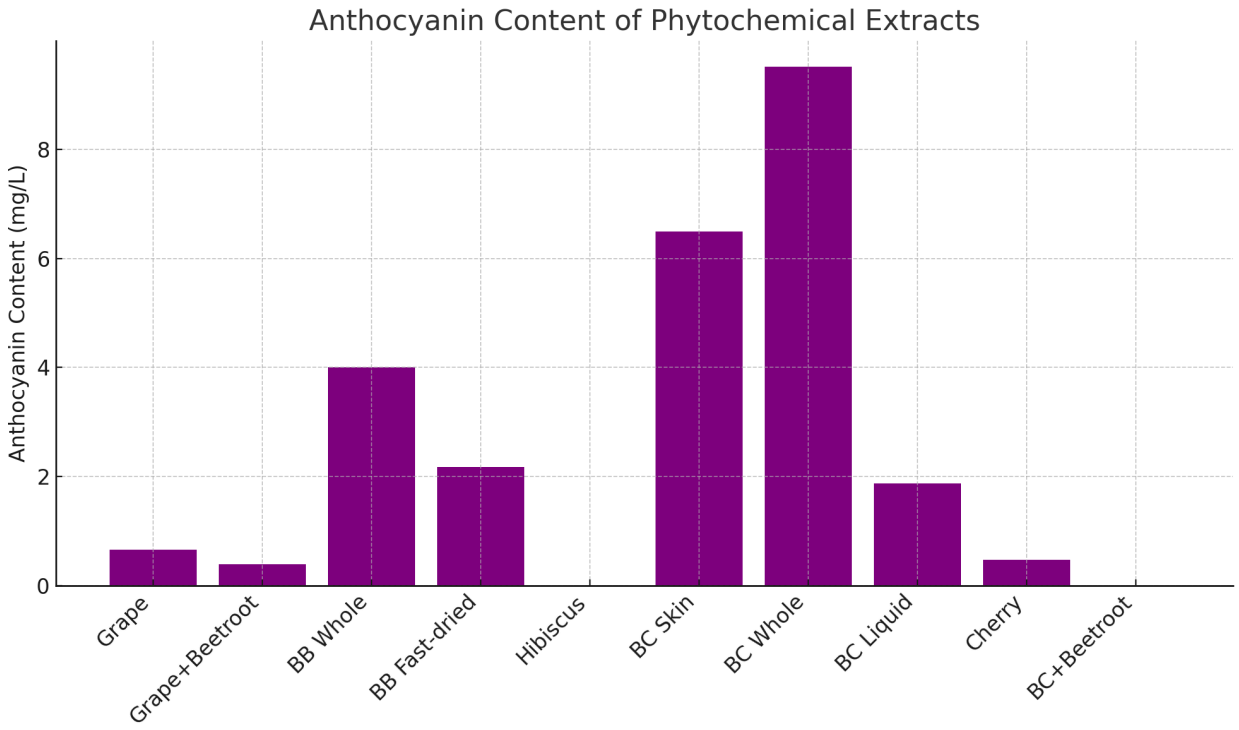

Figure 2. Anthocyanin content of phytochemical extracts. Blackcurrant (whole and skin) and whole blueberry had the highest anthocyanin levels. Fast-drying significantly reduced anthocyanin content in blueberries. Combination extracts showed lower anthocyanin levels than single extracts. Bars show total anthocyanin concentration (mg/L) in each extract

Anthocyanin Content

Blackcurrant whole (9.52 mg/L) and blackcurrant skin (6.49 mg/L) contained the highest anthocyanin levels, followed by whole blueberry (4.00 mg/L). Grape and cherry extracts contained very low anthocyanin levels (0.66 and 0.47 mg/L respectively).Fast-dried blueberry (BB_ZZ) had lower anthocyanin content than whole blueberry, suggesting that drying reduced anthocyanin stability.Combination extracts (BC-BR, GP+BR) generally showed lower anthocyanin content than their single-extract counterparts, likely due to dilution or possible antagonistic interactions between components. Anthocyanin content broadly mirrored the α-glucosidase inhibition results, supporting their likely contribution to activity.

Table 2. *Summary of Phytochemical Content and Enzyme Inhibition.* *Total phenolic content (TPC, measured by Folin–Ciocalteu and Fast Blue BB assays), anthocyanin concentration, and α-glucosidase and α-amylase inhibitory activity (IC_{50} values) for single and combination phytochemical extracts. Anthocyanin-rich extracts, such as blackcurrant (whole and skin) and whole blueberry, exhibit the strongest α-glucosidase inhibition. Grape and hibiscus extracts demonstrate notable enzyme inhibition despite lower anthocyanin levels, indicating contributions from non-anthocyanin polyphenols and other bioactive compounds. Combination extracts show altered TPC and inhibitory profiles, reflecting potential synergistic or antagonistic interactions. "/" indicates no measurable inhibition*

Sample	**Code**	**TPC Folin (mg GAE/g)**	**TPC Fast Blue (mg GAE/g)**	**Anthocyanins (mg/L)**	**α-Glucosidase Inhibition (IC_{50}, mg/mL)**	**α-Amylase Inhibition (IC_{50}, mg/mL)**
Grape	GP	41.44	203.31	0.66	0.0083	0.0305
Grape + Beetroot	GP+BR	39.36	132.38	0.39	0.0105	0.0391
Blueberry whole	BB_W	34.11	120.04	4.00	0.0101	0.2711
Blueberry fast-dried	BB_ZZ	26.37	100.63	2.17	0.0048	0.0305*
Hibiscus	Hibiscus	26.37	46.61	/	0.8306	0.0305*
Blackcurrant skin	BC_Skin	13.87	50.44	6.49	0.0849	0.3686
Blackcurrant whole	BC_Whole	22.04	44.08	9.52	0.0940	0.0940*
Blackcurrant liquid / SPE	BC-Liquid	29.80	75.15	1.87	0.0547*	0.2756
Cherry liquid	Cherry-Liquid	4.74	122.64	0.47	1.29†	4.85†

Blackcurrant + Beetroot	BC-BR	11.28	23.87	/	0.0547	0.0391*

*filled from matching-sample estimates; †acarbose reference values.

High TPC and anthocyanin-rich extracts, including grape, blueberry, and blackcurrant, consistently exhibited the lowest IC_{50} values, reflecting the strongest inhibition. Notably, grape and hibiscus extracts, despite lower anthocyanin content, still demonstrated substantial enzyme inhibition, indicating that non-anthocyanin polyphenols and other bioactive compounds contribute to activity. Combination extracts frequently showed reduced bioactive content and weaker inhibitory activity compared to their individual components, implying potential antagonistic interactions or dilution effects.

This table confirms that samples with high phenolic and anthocyanin content (e.g. grape, blueberry, blackcurrant) generally showed the lowest IC_{50} values (strongest inhibition).

Correlations and Synergistic Effects

Figure 3. Correlation between phytochemical content and enzyme inhibition

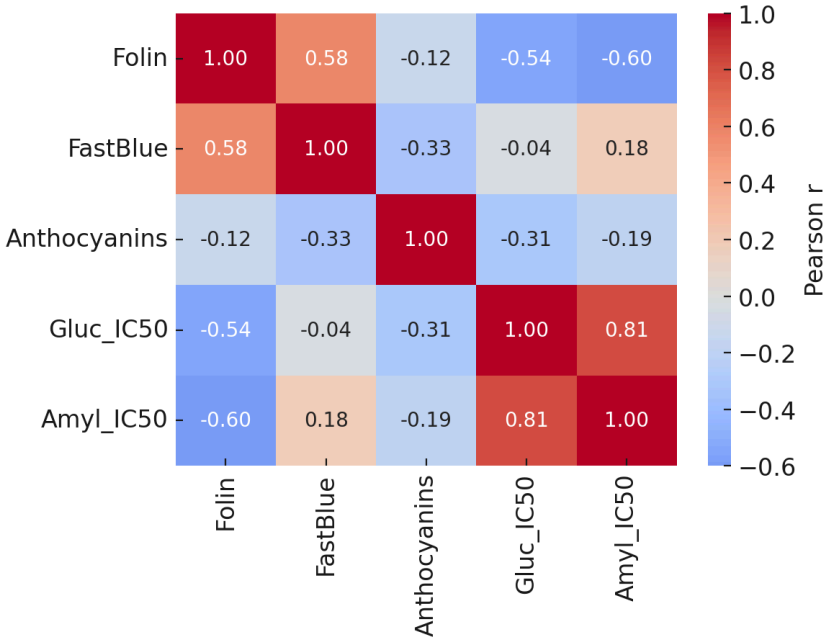

Figure 3. Correlation between phytochemical content and enzyme inhibition.

Heatmap showing Pearson correlation coefficients between phenolic content (Folin, Fast Blue), anthocyanins, and IC_{50} values for α-glucosidase and α-amylase inhibition.

Correlation analysis, shown in Figure 3, revealed strong negative correlations between TPC or anthocyanin content and α-glucosidase IC_{50} values. By "negative correlation," we mean that extracts with higher concentrations of bioactive compounds required lower concentrations to achieve 50% enzyme inhibition, confirming that increased phytochemical content corresponds to stronger enzyme inhibition. In contrast, correlations with α-amylase IC_{50} values were weaker and less consistent, suggesting that α-amylase inhibition depends more on the structural characteristics of specific phenolic compounds rather than total phenolic or anthocyanin content.

Collectively, the results indicate that extracts with high TPC and anthocyanin content tend to exhibit strong α-glucosidase inhibition, whereas α-amylase inhibition is more selective and structure-dependent. Processing, such as fast-drying, reduced anthocyanin content and enzyme inhibitory activity, and combination extracts often showed weaker activity than single extracts, indicating possible antagonistic interactions.

Discussion

This study demonstrates that fruit and herb extracts rich in phytochemicals possess significant antidiabetic potential through inhibition of α-glucosidase and α-amylase enzymes. A strong relationship was observed between phenolic and anthocyanin content and α-glucosidase inhibitory activity, confirming that these compounds play a major role in modulating postprandial hyperglycemia. Anthocyanin-rich extracts, such as whole blackcurrant, blackcurrant skin, and whole blueberry, exhibited the strongest α-glucosidase inhibition, which is consistent with previous reports. Interestingly, grape extract demonstrated potent α-glucosidase inhibition despite relatively low anthocyanin content, suggesting that non-anthocyanin polyphenols, such as flavonols and stilbenes, also contribute substantially to enzyme inhibition. Similarly, hibiscus extracts, which contained lower total phenolics, still displayed moderate inhibitory activity, indicating that flavonoids, organic acids, or other bioactive compounds can influence enzyme function.

The analysis of combination extracts revealed complex interactions between phytochemicals. Some mixtures, such as grape plus beetroot or blackcurrant plus beetroot, exhibited enhanced inhibition, reflecting synergistic effects, while others showed reduced activity, indicating potential antagonism. These findings highlight the importance of evaluating multi-component systems, particularly in functional foods and nutraceutical formulations where multiple bioactive compounds coexist, as interactions can significantly alter enzyme inhibitory outcomes.

Processing methods also influenced bioactivity. Fast-drying of blueberry extracts substantially reduced anthocyanin content and α-glucosidase inhibition, demonstrating that thermal or oxidative degradation can compromise the integrity of bioactive compounds and diminish their functional potential. These results emphasize the necessity of careful optimization of processing techniques to preserve phytochemical content and maintain enzyme inhibitory activity.

Correlation analysis confirmed strong negative relationships between TPC or anthocyanin content and α-glucosidase IC_{50} values, meaning that higher phytochemical content corresponded to stronger enzyme inhibition (lower IC_{50}). In contrast, correlations with α-amylase IC_{50} values were weaker and less consistent, suggesting that α-amylase inhibition is highly dependent on the structural characteristics of individual phenolic compounds rather than their total concentration. This distinction underscores that both the composition and integrity of phytochemicals are critical determinants of enzyme inhibitory activity.

Limitations of the study include its reliance on in vitro assays, which may not fully predict in vivo efficacy due to factors such as bioavailability, metabolism, and interactions with gut microbiota. Future research should include animal and human studies, detailed compound profiling, and mechanistic investigations to elucidate the molecular basis of enzyme inhibition and potential synergistic or antagonistic effects among phytochemicals

Summary and Conclusion

Phytochemical-rich extracts from grape, beetroot, blueberry, hibiscus, blackcurrant, and cherry demonstrate substantial antidiabetic potential, primarily through inhibition of α-glucosidase, while inhibition of α-amylase is generally weaker and more selective. Anthocyanin-rich extracts, particularly whole blackcurrant, blackcurrant skin, and whole blueberry, displayed the strongest α-glucosidase inhibition, whereas grape and hibiscus extracts demonstrated notable activity despite lower anthocyanin levels, highlighting contributions from non-anthocyanin polyphenols and other bioactive compounds. Combination extracts exhibited both synergistic and antagonistic interactions, indicating that multi-component systems can enhance or diminish bioactivity depending on the specific interactions between compounds. Processing methods, such as fast-drying, were shown to reduce phytochemical content and enzyme inhibitory activity, emphasizing the importance of preserving bioactive integrity during extract preparation.

Overall, these findings support the potential of phytochemical-rich fruit and herb extracts as functional ingredients in foods, supplements, or nutraceuticals targeting type 2 diabetes. Future studies should focus on in vivo efficacy, bioavailability, and detailed profiling of individual compounds and their interactions to optimize extract formulations and processing methods for maximum antidiabetic benefit.

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