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α-Glucosidase Inhibitory Activity and Total Flavonoid Content of Green and Purple Kale (*Brassica oleracea* L.) Leaf Extracts

Aktivitas Inhibisi Enzim α-Glukosidase dan Kadar Flavonoid Total Ekstrak Daun Kale (*Brassica oleracea* L.) Hijau dan Ungu

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ABSTRACT

Type 2 diabetes mellitus is managed in part by delaying carbohydrate digestion through α-glucosidase inhibition. Kale (*Brassica oleracea* L.) is rich in flavonoids, but green and purple kale have not been compared under identical extraction conditions. Leaves of both varieties were extracted sequentially by reflux with *n*-hexane, ethyl acetate, and 96% ethanol. α-Glucosidase inhibition was assayed in vitro with p-nitrophenyl-α-D-glucopyranoside, and total flavonoid content by the aluminum chloride method. IC_{50} values, in that solvent order, were 82.70, 59.87, and 77.32 µg/mL for green kale and 78.35, 52.25, and 76.80 µg/mL for purple kale, against 74.63 µg/mL for acarbose. Flavonoid content followed the same pattern: 24.61–32.71 mg QE/g extract in green kale and 26.02–35.97 mg QE/g extract in purple kale. The ethyl acetate extract of purple kale was the most active and the most flavonoid-rich, indicating that semi-polar constituents drive α-glucosidase inhibition by kale leaves.

Kata kunci:
Aktivitas antidiabetes;
Brassica oleracea;
Inhibisi α-glukosidase;
Kadar flavonoid total;
Polaritas pelarut

ABSTRAK

Salah satu strategi pengelolaan diabetes melitus tipe 2 adalah memperlambat pencernaan karbohidrat melalui inhibisi enzim α-glukosidase. Kale (*Brassica oleracea* L.) kaya flavonoid, namun kale hijau dan kale ungu belum pernah dibandingkan pada kondisi ekstraksi yang sama. Daun kedua varietas diekstraksi secara bertingkat dengan metode refluks menggunakan *n*-heksana, etil asetat, dan etanol 96%. Aktivitas inhibisi α-glukosidase diuji secara in vitro dengan substrat p-nitrofenil-α-D-glukopiranosida, sedangkan kadar flavonoid total ditetapkan dengan metode aluminium klorida. Nilai IC_{50} ekstrak kale hijau berturut-turut 82,70; 59,87; dan 77,32 µg/mL, sedangkan ekstrak kale ungu 78,35; 52,25; dan 76,80 µg/mL (*n*-heksana, etil asetat, dan etanol 96%), dibandingkan akarbosa 74,63 µg/mL. Kadar flavonoid total mengikuti pola yang sama, yaitu 24,61–32,71 mg QE/g ekstrak pada kale hijau dan 26,02–35,97 mg QE/g ekstrak pada kale ungu. Ekstrak etil asetat kale ungu paling aktif sekaligus paling kaya flavonoid, sehingga senyawa semipolar diduga paling berperan dalam inhibisi α-glukosidase daun kale.

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1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia together with disturbances of carbohydrate, lipid, and protein metabolism arising from impaired insulin secretion, impaired insulin action (insulin resistance), or both (DiPiro et al., 2005). Insulin homeostasis depends on the pancreatic β-cells of the islets of Langerhans, which synthesize and secrete the hormone (Chen et al., 2017). One established approach to controlling type 2 DM is therefore to delay the intestinal digestion of dietary carbohydrate by inhibiting α-glucosidase, thereby limiting postprandial glucose excursions.

Medicinal plants have long been examined as sources of antidiabetic agents because their secondary metabolites can lower blood glucose. Flavonoids attract particular attention because they act through more than one route: they stimulate pancreatic β-cells to release insulin (Arjadi & Mustofa, 2017) and they inhibit α-glucosidase, delaying glucose absorption and blunting postprandial hyperglycemia (Proença et al., 2017). Kale (*Brassica oleracea* L.) is an unusually rich dietary source of such compounds; at least 71 naturally occurring flavonoid glycosides, predominantly quercetin, kaempferol, and isorhamnetin derivatives differing in glycosylation and acylation, have been characterized in its leaves (Schmidt et al., 2010).

Kale has consequently emerged as a candidate functional food for type 2 DM. Its reported benefits are attributed largely to polyphenols, which have been associated with improved insulin sensitivity, better regulation of glucose metabolism, and amelioration of metabolic syndrome (Šamec et al., 2019). Das et al. (2022) reported that silver nanoparticles biosynthesized with kale extract (BroL-AgNPs) inhibited α-glucosidase with an IC_{50} of 2.29 µg/mL, indicating that kale constituents can act on this enzyme at low concentrations when suitably formulated.

Direct comparisons of green and purple kale under identical extraction conditions nevertheless remain scarce. Whether varietal pigmentation influences extraction yield, total flavonoid content, and α-glucosidase inhibition has not been examined systematically, and the relationship between solvent polarity, the profile of extracted constituents, and the resulting activity is correspondingly unclear. Because pigmentation reflects underlying differences in secondary metabolite composition, green and purple kale were expected to differ in both flavonoid content and inhibitory potency. This study therefore compared green and purple kale leaves extracted sequentially with solvents of increasing polarity, and evaluated the extraction yield, total flavonoid content, and in vitro α-glucosidase inhibitory activity of the resulting extracts.

2. METHODS

2.1. Study Design

A two-factor experimental design was used to examine the effects of kale variety (green, purple) and extraction solvent (*n*-hexane, ethyl acetate, 96% ethanol) on extraction yield, total flavonoid content, and in vitro α-glucosidase inhibitory activity. Each variety–solvent combination was prepared as three independent extractions (n = 3).

2.2. Plant Material and Authentication

Fresh leaves of green and purple kale (*Brassica oleracea* L.) were collected from Manoko, Bandung Regency, West Java, Indonesia. Botanical identity was confirmed at the Herbarium of the School of Life Sciences and Technology (SITH), Institut Teknologi Bandung, Indonesia Number: 8272/IT1.C11.2/TA.00/2024. Leaves were washed under running water to remove soil, dust, and other adhering material, Drying was carried out using an oven at a temperature of 45–50°C, and ground to a powder (simplicia). The powdered material was characterized for loss on drying, total ash, acid-insoluble ash, water-soluble extractive value, and ethanol-soluble extractive value.

2.3. Chemicals and Reagents

α-Glucosidase from yeast (Saccharomyces cerevisiae, Sigma-Aldrich catalog G5003-10UN, 9.66 UN/mg), p-nitrophenyl-α-D-glucopyranoside (pNPG), phosphate buffer (pH 6.8), acarbose, and quercetin were all obtained from Merck.

2.4. Sequential Reflux Extraction

Powdered simplicia was extracted sequentially by reflux with solvents of increasing polarity: *n*-hexane (non-polar), then ethyl acetate (semi-polar), and finally 96% ethanol (polar), simplicia-to-solvent ratio 1:10 (w/v) at 70 °C for 2 h per reflux cycle. Each solvent step was repeated three times; filtrates were combined and concentrated under reduced pressure in a rotary vacuum evaporator (Buchi R-100). Extraction yield was expressed as a percentage of the dry weight of the simplicia.

2.5. Phytochemical Screening and Thin-Layer Chromatography

Standard qualitative tests were used to detect alkaloids (Mayer's, Dragendorff's, and Wagner's reagents), flavonoids, tannins (1% $FeCl_3$ and 1% gelatin), saponins, steroids/triterpenoids, and quinones. Thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck) developed with *n*-hexane–ethyl acetate (7:3, v/v). Developed plates were examined under visible light and under UV illumination at 254 and 366 nm, and then sprayed with 10% H_2SO_4, 5% $AlCl_3$, or 10% $FeCl_3$. Retardation factor (R_f) values were calculated for the major zones.

2.6. Determination of Total Flavonoid Content

Total flavonoid content was determined by the aluminum chloride colorimetric (Chang) method as described previously (Budiana et al., 2022). Extract (10 mg) was dissolved in ethanol to a final volume of 10 mL (1000 µg/mL). One millilitre of this solution was mixed with 1 mL of 10% $AlCl_3$ and 1 mL of 1 M sodium acetate and left for 60 min at room temperature (25–30 °C). Absorbance was read on a UV-Vis spectrophotometer (Thermoscientific spectrophotometer, Multiscan skyhigh, Singapore.) at the wavelength of maximum absorbance (405 nm). Quercetin was the

calibration standard the concentrations ranging from 20-80 µg/mL, r^2 0,9902, and results were expressed as mg quercetin equivalent (QE) per gram of extract.

2.7. α-Glucosidase Inhibitory Assay

α-Glucosidase inhibition was measured in vitro with yeast α-glucosidase and pNPG as substrate, following Gaurav et al. (2020) with minor modifications. Reaction mixtures were assembled in 96-well microplates as set out in Table 1. Plates were incubated at 37 ˚C for 15 min after addition of the enzyme; pNPG was then added and incubation continued at 37 ˚C for a further 15 min. Reactions were terminated with 0.2 M Na_2CO_3, and the p-nitrophenol released was measured at 405 nm on a microplate reader (Thermoscientific spectrophotometer, Multiscan skyhigh, Singapore). Acarbose served as the positive control; the enzyme control contained buffer in place of the test sample. Absorbance readings were corrected against the corresponding substrate blanks, and inhibition was calculated as $[(A_{control} - A_{sample})/A_{control}] \times 100$. IC_{50} values were obtained by linear regression of percentage inhibition against extract concentration. The concentration series of acarbose 20, 40, 60, 80, 100µg/mL. r^2= 0,974. All samples were tested at concentrations of 50, 60, 70, 80, and 90 µg/mL, with a coefficient of determination (R^2) of 0.9884. The regression analysis used was % inhibition versus concentration (µg/mL).

All volumes are in µL. Buffer, phosphate buffer (pH 6.8); Enzyme, yeast α-glucosidase; pNPG, p-nitrophenyl-α-D-glucopyranoside as substrate; Na_2CO_3, 0.2 M sodium carbonate as stopping reagent. Reagents were added in the order listed from left to right; plates were incubated at 37 ˚C for 10 min after addition of the enzyme and for a further 10 min after addition of the substrate, and the reaction was then stopped with Na_2CO_3. Absorbance was measured at 405 nm.

2.8. Statistical analysis

Results are reported as mean ± standard deviation of three independent extractions (n = 3). Total flavonoid content and IC_{50} were each analyzed by two-way analysis of variance (ANOVA) with kale variety and extraction solvent as fixed factors, followed by Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$; effect sizes are reported as partial η^2. The six extracts and acarbose were additionally compared by one-way ANOVA with Tukey HSD so that the positive control could be ranked against the extracts. The relationship between total flavonoid content and IC_{50} was examined by Pearson correlation, calculated separately for each variety across its three solvent extracts. Differences were considered statistically significant at $p < 0.05$. Statistical analyses were performed using the reported group means, standard deviations, and replicate numbers.

Table 1. Composition of the reaction mixtures used in the α-glucosidase inhibitory assay

Reaction mixture	Standard or sample	Buffer	Enzyme	pNPG	Na_2CO_3	Total
Enzyme control	–	100	30	30	90	250
Control blank	–	130	–	30	90	250
Standard (acarbose)	100	–	30	30	90	250
Sample	100	–	30	30	90	250
Sample blank	100	30	–	30	90	250

3. RESULTS AND DISCUSSION

3.1. Characterization of the simplicia

Characterization of the dried leaf material was undertaken to document its identity and quality before extraction, since physicochemical standardization underpins the reproducibility of any subsequent pharmacological work (Amlia & Hazar, 2022). Farmakope Herbal Indonesia specifies quality limits monograph by monograph rather than as general thresholds for a plant part, and it contains no monograph for kale; the values in Table 2 are therefore reported descriptively and compared with the limits set for two leaf drugs that do have monographs, guava leaf and *Vernonia amygdalina* leaf (Kementerian Kesehatan Republik Indonesia, 2017). Total ash was 9.6% in green kale and 9.2% in purple kale, below the not-more-than 11.0–11.5% specified for those two leaf drugs and consistent with a normal mineral content rather than gross inorganic contamination. Loss on drying, 7.31% and 7.62%, likewise fell below the not-more-than 10% specified in both monographs, so the material retained little moisture and can be expected to resist microbial spoilage and hydrolytic degradation in storage. Acid-insoluble ash — the fraction attributable to soil, sand, and silica — was 1.06% and 0.93% respectively, which is higher than the not-more-than 0.7–0.8% set for the same two leaf drugs. Those limits are species-specific and do not formally apply to kale, but the comparison is worth making because acid-insoluble ash is the parameter most sensitive to post-harvest handling, and it suggests that a small amount of adhering mineral debris survived washing. Purple kale gave slightly higher water-soluble (42.67% versus 41.33%) and ethanol-soluble (17.33% versus 14.67%) extractive values — an early indication that it holds a larger pool of solvent-extractable constituents.

3.2. Phytochemical screening

Qualitative screening detected alkaloids, flavonoids, and tannins in both varieties (Table 3). Saponins were found only in purple kale, and neither variety gave a positive reaction for steroids/triterpenoids or quinones. These differences suggest that genotype and leaf pigmentation are accompanied by differences in secondary metabolite composition.

Table 2. Characterization of green and purple kale leaf simplicia

Test parameter	Green kale leaves (%)	Purple kale leaves (%)
Total ash	9.6 ± 0.2	9.2 ± 0.2
Acid-insoluble ash	1.06 ± 0.31	0.93 ± 0.12
Loss on drying	7.31 ± 0.25	7.62 ± 0.34
Water-soluble extractive value	41.33 ± 1.15	42.67 ± 1.15
Ethanol-soluble extractive value	14.67 ± 1.52	17.33 ± 0.58

Alkaloid detection relies on reaction between the basic nitrogen of the alkaloid and heavy-metal or polyhalide ions (Mathe et al., 2024). Mayer's reagent precipitates basic alkaloids as a tetraiodomercurate(II) complex, mercury being capable of precipitating alkaloid bases (Oktapiya et al., 2022). Wagner's reagent forms an alkaloid–polyiodide precipitate, and the hydrochloric acid added beforehand converts the free bases into salts, partitioning them into the acidic phase and separating them from other cell constituents (Elfira et al., 2024). Dragendorff's reagent yields a brick-red to orange-brown precipitate with bismuth-based ions (Citra & Marpaung, 2024). Both varieties reacted positively with Dragendorff's and Wagner's reagents but not with Mayer's. Discordance of this kind between alkaloid reagents applied to the same extract is well documented: screening 26 plant extracts with Mayer's, Dragendorff's, and Wagner's reagents in parallel, Godlewska et al. (2022) found that the reagents differ appreciably in sensitivity and that several extracts gave a Dragendorff's-positive but Mayer's-negative result. The pattern observed here therefore reflects the differing precipitating ion and detection threshold of the three reagents rather than an absence of alkaloids. The screening was qualitative, so no inference about alkaloid concentration can be drawn from it.

Both varieties gave the orange-red color in the amyl alcohol layer that indicates flavonoids, confirming these compounds as major metabolites of kale leaves and agreeing with Lučić et al. (2023) and with Schmidt et al. (2010), who characterized kaempferol and quercetin glycosides as the dominant flavonoids of the species.

For tannins, both varieties produced a dark green color with 1% $FeCl_3$, consistent with polyphenols, but neither precipitated gelatin. Tannins were therefore either present at low concentration or predominantly condensed rather than hydrolyzable, condensed tannins having a lower protein-precipitating capacity (Sunani & Hendriani, 2023).

Saponins, indicated by stable foam after the addition of 2 N HCl, were detected only in purple kale. Purple kale therefore carries a somewhat broader metabolite profile than green kale, and its saponins may act alongside flavonoids and other phenolics in producing the stronger α-glucosidase inhibition described below. This remains an inference from qualitative data, since the individual contribution of saponins was not measured. The overall profile corresponds to that reported for the closely related broccoli (*B. oleracea* var. *italica*), in which alkaloids, flavonoids, tannins, and saponins were the principal metabolites detected (Purba et al., 2023).

3.3. Extraction yield

Sequential reflux extraction was used to fractionate metabolites according to polarity and so improve the selectivity of each step. Reflux maintains continuous contact between solvent and plant material under controlled heating, which improves mass transfer and shortens extraction time; continuous condensation limits solvent loss, and the technique is well suited to thermostable constituents.

Yield increased with solvent polarity in both varieties (Table 4). 96% ethanol was the most productive solvent, giving 12.31% for purple kale and 11.15% for green kale, which indicates that most of the extractable material in kale leaves is polar; ethanol is likewise an effective solvent for polar phytochemicals such as flavonoids and phenolic compounds (Khairunnisa et al., 2022). The low *n*-hexane yields (5.48% and 5.91%) point to a small lipophilic fraction, non-polar solvents recovering mainly lipids and other lipophilic material from leaf tissue (Azzahra et al., 2022). Purple kale yielded more extract than green kale with every solvent — the first of several observations indicating a larger pool of extractable metabolites in the pigmented variety.

Table 3. Phytochemical screening of green and purple kale leaves

Metabolite class	Reagent or test	Green kale leaves	Purple kale leaves
Alkaloids	Mayer's	–	–
	Dragendorff's	+	+
	Wagner's	+	+
Flavonoids	Amyl alcohol	+	+
Tannins	1% $FeCl_3$	+	+
	1% gelatin	–	–
Saponins	Foam test	–	+
Steroids/triterpenoids	Liebermann–Burchard	–	–
Quinones	1 N NaOH	–	–

+, detected; –, not detected.

Table 4. Extraction yields of green and purple kale leaves obtained with solvents of increasing polarity

Solvent	Green kale leaves (%)	Purple kale leaves (%)
n-Hexane	5.48	5.91
Ethyl acetate	8.24	10.49
96% Ethanol	11.15	12.31

3.4. Thin-layer chromatography monitoring

Each extract was monitored by TLC on silica gel 60 F_{254} developed with *n*-hexane–ethyl acetate (7:3, v/v). Spraying with 10% H_2SO_4 produced brown, green, black, and gray zones (Figure 1d), indicating that several compound classes were present. 5% $AlCl_3$ gave green and pale yellow zones (Figure 1e) and 10% $FeCl_3$ gave black zones (Figure 1f), pointing respectively to flavonoids and to phenolic compounds.

$AlCl_3$ detects flavonoids because Al^{3+} chelates the hydroxyl and carbonyl groups of the flavonoid nucleus, producing a bathochromic shift in the absorption maximum and a yellow chromophore (Shraim et al., 2021). The ethanol extracts of green and purple kale each showed one major $AlCl_3$-positive zone, at R_f 0.62 and 0.60 respectively. A difference of 0.02 R_f unit falls within the run-to-run variation expected from plate thickness, chamber saturation, mobile phase composition, and sample application, so the two zones plausibly correspond to flavonoids of comparable polarity. These observations corroborate the screening results but do not identify individual compounds.

3.5. Total flavonoid content

The aluminum chloride method exploits the acid-stable complex formed between Al^{3+} and the C-4 carbonyl together with the C-3 or C-5 hydroxyl group of flavones and flavonols; the resulting yellow complex absorbs in the 400–450 nm region, and its absorbance is proportional to flavonoid concentration (Bag et al., 2015; Shraim et al., 2021).

Both variety and solvent affected the amount of flavonoid recovered (Figure 2A). Two-way ANOVA showed significant effects of solvent ($F(2,12) = 8318$, $p < 0.001$, partial $\eta^2 = 0.999$) and variety ($F(1,12) = 1360$, $p < 0.001$, partial $\eta^2 = 0.991$), together with a significant interaction ($F(2,12) = 102.6$, $p < 0.001$) indicating that the size of the varietal difference depended on the solvent used. Tukey HSD separated all six extracts from one another ($p < 0.05$). Ethyl acetate gave the highest values in both varieties — 32.71 ± 0.18 mg QE/g extract for green kale and 35.97 ± 0.18 mg QE/g extract for purple kale — and *n*-hexane the lowest. That a semi-polar solvent outperformed both *n*-hexane and 96% ethanol is consistent with the solubility behaviour of flavonoids, particularly the aglycones, which partition preferentially into solvents of intermediate polarity (Muhamad et al., 2014). Ethanol produced the largest mass of extract but not the most flavonoid-rich one, so the high ethanol yield in Table 4 evidently reflects co-extraction of highly polar constituents that dilute the flavonoid fraction.

Purple kale contained more flavonoids than green kale with every solvent. The difference is most readily attributed to the accumulation of anthocyanins, the flavonoid subclass responsible for the purple pigmentation and a recognized contributor to total phenolic and flavonoid measurements (Lee et al., 2015; Wang et al., 2024). Because anthocyanins themselves respond only weakly in the aluminum chloride assay (Shraim et al., 2021), the higher values recorded for purple kale probably reflect a generally larger flavonol pool accompanying anthocyanin accumulation rather than the anthocyanins alone. The result is in line with Lučić et al. (2023), who described kale as a rich source of flavonoids and polyphenols with marked antioxidant activity, and with Subedi et al. (2024), who raised the polyphenol content of kale by fermentation and recorded a corresponding gain in biological activity. Taken together, solvent polarity and variety both determine how much flavonoid is recovered, and the flavonoid-rich ethyl acetate extracts — purple kale in particular — were also the most active against α-glucosidase.

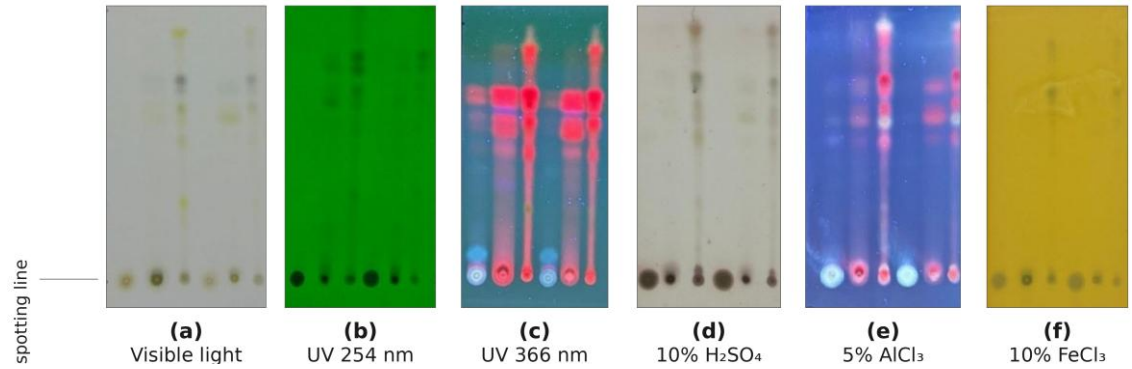


Figure 1. Thin-layer chromatograms of green and purple kale leaf extracts on silica gel 60 F_{254} developed with *n*-hexane–ethyl acetate (7:3, v/v), visualized as indicated beneath each panel. Tracks 1–6, left to right in every panel: (1) green kale ethanol extract, (2) green kale ethyl acetate extract, (3) green kale *n*-hexane extract, (4) purple kale ethanol extract, (5) purple kale ethyl acetate extract, (6) purple kale *n*-hexane extract. Panels are aligned on the spotting line and are shown at a common plate length; they were cropped and scaled for presentation only, with no tonal, contrast, or selective adjustment applied.

3.6. α-Glucosidase inhibitory activity

The assay follows the enzymatic release of yellow p-nitrophenol from pNPG: an inhibitor bound to α-glucosidase slows hydrolysis of the substrate and so lowers the absorbance recorded at 405 nm (Nasution et al., 2013).

All six extracts inhibited α-glucosidase, with potency varying by solvent and variety (Figure 2B). Two-way ANOVA across the six extracts showed significant effects of solvent ($F(2,12) = 254.8$, $p < 0.001$, partial $\eta^2 = 0.977$) and variety ($F(1,12) = 18.9$, $p = 0.001$, partial $\eta^2 = 0.612$), with a weaker interaction ($F(2,12) = 4.59$, $p = 0.033$). The ethyl acetate extract of purple kale was the most potent (IC_{50} 52.25 ± 1.90 µg/mL), followed by the ethyl acetate extract of green kale (59.87 ± 3.53 µg/mL); the *n*-hexane extracts were the least potent (78.35 ± 0.79 µg/mL for purple and 82.70 ± 1.82 µg/mL for green kale). A lower IC_{50} denotes greater potency, less extract being required to halve enzyme activity.

The ranking of solvents mirrors that obtained for flavonoid content, which suggests that the principal α-glucosidase inhibitors in kale leaves are semi-polar. The weaker activity of the *n*-hexane extracts is consistent with preferential recovery of lipophilic material that contributes little to enzyme inhibition. Purple kale gave a lower IC_{50} than green kale with every solvent, in keeping with its higher flavonoid and, presumably, anthocyanin content, although Tukey HSD did not resolve the two varieties within any single solvent (Figure 2B); the varietal effect emerges as a main effect across solvents rather than as a pairwise difference.

Across the three extracts of each variety, total flavonoid content and IC_{50} were strongly and negatively correlated ($r = -0.961$ for green kale and $r = -0.928$ for purple kale; Table 5): higher flavonoid content accompanied stronger inhibition. Two qualifications apply. Each coefficient rests on only three solvent extracts, so the estimate is imprecise and no significance test is meaningful at this sample size; and a correlation of this kind cannot establish that flavonoids are themselves the active constituents. Kale extracts also contain phenolic acids, tannins, saponins, glucosinolates, and anthocyanins, whose contributions may be additive, synergistic, or mutually opposing.

A one-way ANOVA including the positive control was also significant ($F(6,14) = 40.2$, $p < 0.001$), and Tukey HSD placed the two ethyl acetate extracts in a single group distinct from the four remaining extracts and from acarbose. Under the conditions used here, therefore, the ethyl acetate extracts of both varieties were significantly more potent than acarbose (74.63 ± 6.37 µg/mL; $p < 0.001$ in both comparisons), whereas the *n*-hexane and ethanol extracts did not differ significantly from the positive control. This should not be read as evidence that kale extracts are clinically more effective than acarbose. IC_{50} values from this assay depend on the enzyme source, substrate concentration, and protocol; the yeast enzyme used here is not identical to the mammalian intestinal α-glucosidase that acarbose targets clinically; and acarbose is a single purified molecule, whereas the extracts are complex mixtures whose active constituents remain unidentified. The direction of the result is nevertheless consistent with earlier work on the genus. Purba et al. (2023) reported antidiabetic activity of an ethanolic broccoli extract in alloxan-induced rats, and Kusumawardianingrum and Lindawati (2022) recorded glucose-lowering activity of an ethanolic kale extract with an EC_{50} of 11.13 ppm. Das et al. (2022) obtained a much lower IC_{50} (2.29 µg/mL) using silver nanoparticles synthesized with kale extract, which illustrates how strongly the physical form of a preparation can alter apparent potency.

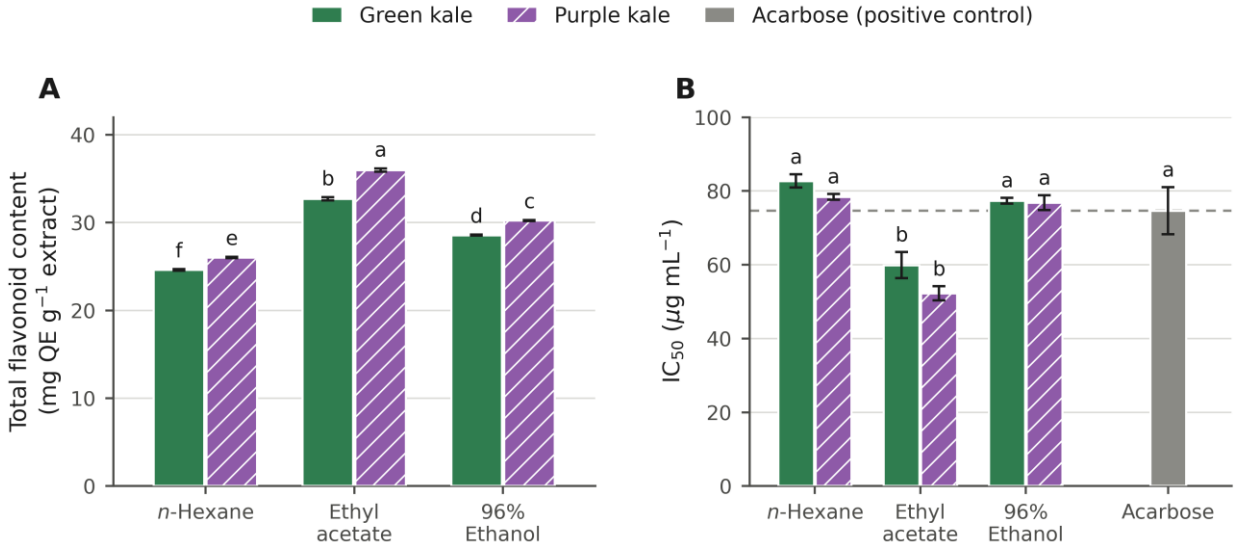


Figure 2. Total flavonoid content (**A**) and α-glucosidase inhibitory activity (**B**) of green and purple kale leaf extracts obtained with solvents of increasing polarity. Bars are mean ± SD of three independent extractions; the dashed line in panel **B** marks the IC_{50} of acarbose. Within each panel, bars that do not share a letter differ significantly (Tukey HSD, $p < 0.05$). Panel **A**: green kale 24.61 ± 0.10, 32.71 ± 0.18, and 28.56 ± 0.06 mg QE/g extract; purple kale 26.02 ± 0.08, 35.97 ± 0.18, and 30.22 ± 0.06 mg QE/g extract, for *n*-hexane, ethyl acetate, and 96% ethanol respectively. Panel **B**: green kale 82.70 ± 1.82, 59.87 ± 3.53, and 77.32 ± 0.77 µg/mL; purple kale 78.35 ± 0.79, 52.25 ± 1.90, and 76.80 ± 2.04 µg/mL; acarbose 74.63 ± 6.37 µg/mL.

Table 5. Pearson correlation between total flavonoid content and IC_{50} value

Kale variety	Correlation coefficient (r)	Number of extracts
Green kale	−0.961	3
Purple kale	−0.928	3

Correlations were calculated across the *n*-hexane, ethyl acetate, and 96% ethanol extracts of each variety. With three data points per variety the coefficients describe the observed trend rather than test it.

3.7. Limitations

Three limitations bound the interpretation offered above. First, the active constituents were not identified: screening and TLC establish that flavonoids are present but not which compounds account for the inhibition, so HPLC or LC–MS/MS profiling of the ethyl acetate extracts is the necessary next step. Second, the assay used yeast α-glucosidase in a cell-free system, so the findings describe enzyme inhibition rather than any glycemic effect in a living organism. Third, with three extracts per variety the relationship between flavonoid content and IC_{50} can be described but not tested statistically, and the dispersion of the flavonoid measurements is small enough that the pairwise separations in Figure 2A should be treated as provisional. Enzyme kinetic analysis would establish the mode of inhibition, and in vivo studies would be required before efficacy could be claimed.

4. CONCLUSION

Green and purple kale (*Brassica oleracea* L.) leaf extracts inhibited α-glucosidase in vitro, and both the extraction solvent and the variety governed the degree of inhibition. Ethyl acetate produced the most active extract of each variety, with IC_{50} values of 59.87 µg/mL for green kale and 52.25 µg/mL for purple kale, and these extracts also carried the highest total flavonoid contents, 32.71 and 35.97 mg QE/g extract respectively. Purple kale exceeded green kale on both measures with every solvent tested, consistent with pigmentation marking a difference in secondary metabolite composition. These results add comparative evidence that solvent polarity governs the recovery of α-glucosidase inhibitors from kale leaves and that varietal pigmentation is associated with their abundance, and they identify semi-polar extraction of purple kale as the most promising starting point for isolating such inhibitors. Identification of the active constituents, kinetic characterization of the inhibition, and in vivo evaluation remain necessary before any antidiabetic application can be claimed.

AUTHOR CONTRIBUTIONS

Conceptualization, W.B. and D.K.; methodology, W.B.; validation, W.B. and D.K.; formal analysis, W.B. and D.K.; investigation, I.R.; resources, W.B. and I.R.; data curation, I.R.; writing—original draft preparation, I.R. and W.B.; writing—review and editing, W.B., D.K., and I.R.; visualization, W.B. and D.K.; supervision, W.B. and D.K.; project administration, W.B.; funding acquisition, W.B. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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The funders had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript the authors used Claude (Anthropic) for English-language editing and to improve the clarity and organization of the text, for formatting the reference list, and for preparing Figures 1 and 2 from data and images the authors had already generated. All experimental data, analyses, and interpretations are the authors' own. The authors reviewed and edited all output and take full responsibility for the content of the publication.

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