

Effect of different extraction solvents on the yield and enzyme inhibition (α -amylase, α -glucosidase, and lipase) activity of some vegetables

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Abstract. Maser WH, Maiyah N, Nagarajan M, Kingwascharapong P, Senphan T, Ali AMM, Bavisetty SCB. 2023. Effect of different extraction solvents on the yield and enzyme inhibition (α -amylase, α -glucosidase, and lipase) activity of some vegetables. *Biodiversitas* 24: 3320-3331. The present study investigated the *in vitro* inhibition of α -amylase, α -glucosidase, and lipase, and antioxidant activities (DPPH, metal chelating, and FRAP) of *Allium cepa* L., *Apium graveolens* L., *Coriandrum sativum* L., and *Petroselinum crispum* (Mill.) Fuss using solvent 80% ethanol, absolute ethanol, acetone, methanol, *n*-hexane, chloroform, and hot water. The total phenolic content (TPC) and the FTIR and GC-MS characterization spectra were compared with the activities. The TPC of 80% ethanol extract in *A. graveolens* L. was the highest among the extracts, with 23.78 mg GAE/g extract. The 80% ethanol extract exhibited the most potent antioxidant activity on DPPH radical scavenging (*A. graveolens*, 125.57 mg AEAC/g extract), metal chelating (*C. sativum*, 92.85 mg EECC/g extract), and FRAP activities (*C. sativum*, 46.98 mg AEAC/g extract). The 80% ethanol extract showed the highest anti- α -amylase (*P. crispum*, 30.61 mmol ACE/g extract) and anti- α -glucosidase (*A. cepa*, 595.28 mmol ACE/g extract) activities. In comparison, the absolute ethanol extract of *C. sativum* showed the highest anti-lipase activity (42.10% inhibition). According to the FTIR spectra, the 80% ethanol extract of the four green leafy vegetables is predicted to have several active compounds. GC-MS identified the compounds responsible for the activity. Studies on the recovery potential of active compounds as oral agents to treat diabetes mellitus with certain solvents are very useful in producing oral agents and functional foods to prevent diabetes mellitus.

Keywords: Antidiabetic, anti-obesity, FTIR, GC-MS, green leafy vegetables

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia caused by insulin deficiency and resistance (Bashkin et al. 2021), resulting in high blood glucose levels, which can increase oxidative stress (Justino et al. 2018). In addition, excessive consumption of carbohydrates can lead to obesity, resulting in various health issues (Vu et al. 2020). If not properly managed, diabetes mellitus poses significant health risks. Prolonged hyperglycemia can lead to various complications, affecting multiple organ systems in the body (Bashkin et al. 2021). These complications include cardiovascular diseases, retinopathy (eye damage), nephropathy (kidney damage), neuropathy (nerve damage), and increased susceptibility to infections (Bashkin et al. 2021). The presence of antioxidants and plant metabolites during digestion and metabolism is known to suppress the absorption of glucose

and fat absorption, postprandial hyperglycemia, and reduce oxidative stress, finally leading to the improvement of diabetes mellitus (Justino et al. 2018).

The use of plants as a natural preventive and treatment option for diabetes by reducing the complications of type 2 diabetes mellitus (T2DM) with negligible side effects has been recognized worldwide (Bashkin et al. 2021). Various phytochemicals from plants have been widely reported to exhibit antidiabetic, anti-obesity, and antioxidant properties (Suttisansanee et al. 2021). In addition, secondary metabolites from a few green leafy vegetables (GLVs), including polyphenols, steroids, tannins, alkaloids, saponins, terpenes, organosulfides, and other organic acids, have been reported to possess these beneficial effects (Randhawa et al. 2015). For example, leaves of *Petroselinum crispum* (Mill.) Fuss and outer skins of *Allium cepa* L. have been reported to have antioxidant and antidiabetic activities (Vu et al. 2020; Aissani et al. 2021;

Bashkin et al. 2021). In addition, leaves of *Apium graveolens* L. and *Coriandrum sativum* L. were reported to have antioxidant, α -amylase, α -glucosidase inhibitory, and lipase inhibitory properties (Sreelatha and Inbavalli 2012; Neagu et al. 2019; Khongrum et al. 2021; Liu et al. 2020; Suttisansanee et al. 2021).

Identifying and extracting the bioactive compounds are crucial in harnessing their potential benefits. Extraction solvents play a vital role in this process, as they affect the yield and bioactivity of the extracted compounds (Lefebvre et al. 2021). Research on the extraction of various bioactive compounds from plants has been developed for a long period. However, the demand for the extraction method to recover compounds with high bioactivity is still high, especially from plants (Bashkin et al. 2021). Therefore, to maximize the benefits of secondary metabolites with specific bioactivity, it is important to recover the compound in an appropriate solvent system and characterize it. As plant metabolites are complex in structure with multiple mixtures, thus recovery of individual compounds with specific bioactivity is highly challenging (Lefebvre et al. 2021). General extraction techniques cannot recover compounds with diversified chemical structures and properties, as the solvents comprise different polarity and extractability, which can be the crucial deciding factor in achieving optimal extraction efficiency for particular extracts (Lefebvre et al. 2021). As an extraction medium, solvent relies on soluble affinity, which requires various interactions to achieve high extraction yields and different bio-activity (Lefebvre et al. 2021). E.g., Hellal et al. (2020) reported that 80% ethanolic extract of *Anacyclus valentinus* L. and *Marrubium vulgare* L. had higher α -glucosidase inhibitory and antioxidant activities. On the other hand, Deveci et al. (2021) reported that n-hexane extract from *Pleurotus ostreatus* (Jacq.) P.Kumm. had the strongest inhibitory effect on α -glucosidase (IC₅₀ of 0.10 mg/mL) compared to methanolic extract.

Therefore, the present study aimed to investigate the effect of seven different solvents as extraction mediums of metabolites from *A. cepa*, *A. graveolens*, *C. sativum*, and *P. crispum*. The extract was subjected to yield and bioactivity through enzyme inhibition, i.e., α -amylase inhibitory (AAI), α -glucosidase inhibitory (AGI), and lipase inhibitory (LPI) activities. In addition, the characterization of metabolites was done using Fourier-transform infrared (FTIR) spectroscopy and gas chromatography-mass spectrometry (GC-MS).

MATERIALS AND METHODS

Materials

Enzymes such as α -glucosidase, lipase (Type II), and α -amylase (Type IV-B) from porcine pancreas were purchased from Sigma-Aldrich (St. Louis, MO, USA). Chemicals including p-nitrophenyl- α -D-glucopyranoside (p-NPG), acarbose, 4-methylumbelliferyl oleate (4MUO), pyridine, N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane, and

methoxyamineHCl, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethylenediaminetetraacetic acid (EDTA), gallic acid monohydrate, L-ascorbic acid, Folin-Ciocalteu reagent, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p-disulfonic acid monosodium salt hydrate (Ferrozine), and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were purchased from Acros Organics (Fair Lawn, NJ, USA). 2,2-Diphenyl-1-picrylhydrazil (DPPH) was purchased from Thermo Fisher Scientific (Waltham, MA, USA). All chemicals were of analytical grade.

Fresh green leafy vegetables (GLVs), such as *A. cepa*, *A. graveolens*, *C. sativum*, and *P. crispum*, were procured from the Hua Takhe market at Latkrabang, Bangkok, Thailand. Each GLV (10 kg) was packed in vegetable crates and sent to the School of Food-Industry, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. Upon arrival, all the GLVs were thoroughly cleansed with tap water to eliminate dirt and drained on the screen to remove any leftover water.

Sample preparation

The cleaned GLVs were dried using a dehydration drier (WRH-100, IKE, Guangdong, China) at 45°C until a constant weight was achieved. The dried GLVs were ground to powder using a blender (Philips HR 2222, Philips Healthcare, Best, Netherlands). The powder sample was used for extraction experiments.

Preparation of extracts

Powdered samples of all four GLVs (100 g) were mixed with different solvents, including 80% ethanol, absolute ethanol, acetone, methanol, n-hexane, chloroform, or distilled water at the ratio of 1:10 (w/v), and homogenized using a hand homogenizer (T25 ULTRA-TURRAX, IKA, Staufen, Germany) at 13,000 rpm for 2 min at room temperature (25 \pm 2°C). Those extracted samples in distilled water were heated at 80°C for 30 minutes using a water bath (WNB 29, Memmert, Schwabach, Germany). All the mixtures were then macerated for 24 h at 4°C. Then the samples were centrifuged (5910 R, Eppendorf, Hamburg, Germany) at 4000 rpm for 15 min at 25°C and filtered using Whatman No. 1 filter paper (Maidstone, Kent, UK). The filtrates were concentrated using a vacuum rotary evaporator (Rotavapor R-300, Buchi, Flawil, Switzerland) at 40°C and then freeze-dried (Model LD0.5, Kinetic, Bangkok, Thailand) for 72 h at -50°C. The obtained extract in the form of powder was used for analysis and characterization.

Extraction yield

The extraction yield was determined on the dry weight basis, and the final values were expressed in percentage (%). The extraction yield was calculated using the formula:

$$\text{Extraction yield (\%)} = w_1/w_2 \times 100\% \quad (1)$$

Where:

w₁: the extract powder after freeze-drying

w₂: the grounded powder of leaves after the dehydration drier.

Determination of total phenolic content (TPC)

TPC was measured as described by Bavisetty and Venkatachalam (2021) with minor changes. A 100 μ L of the sample (1 mg/mL in 50% methanol) was thoroughly mixed with 200 μ L of Folin-Ciocalteu reagent (10%) and 800 μ L of Na_2CO_3 (700 mM) and incubated at room temperature for 2 h. After incubation, the absorbance was measured at 765 nm, gallic acid was used to plot the standard curve, and TPC was expressed as gallic acid equivalents (mg GAE/g extract).

Antioxidant

Samples were prepared by dissolving the crude extracts powder in absolute methanol at 1 mg/mL (w/v). The absorbance was measured using a microplate reader. DPPH radical scavenging and ferric reducing antioxidant power were expressed as L-ascorbic acid equivalent antioxidant capacity in mg AEAC/g extract. The metal chelating activity was expressed as EDTA equivalent chelating capacity in mg EECC/g extract.

DPPH radical scavenging assay

With some modifications, the DPPH radical scavenging assay was measured as described by Ali et al. (2021). The reaction was initiated by mixing 100 μ L of prepared samples with 100 μ L 0.2 mM DPPH solution and incubating in the dark for 30 min at room temperature. The absorbance was recorded at 517 nm.

Metal chelating assay

The metal chelating activity was determined as described by Masood et al. (2021) with slight changes. The reaction was initiated by mixing 100 μ L prepared samples with that 25 μ L of 0.6 mM FeCl_2 solution and 25 μ L of 5 mM Ferrozine solution. The mixture was vortexed and then incubated for 10 min at room temperature, and absorbance was recorded at 562 nm.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed as described by Ali et al. (2021). To the 25 μ L of prepared samples, 175 μ L of FRAP reagent (ten volumes of 300 mM acetate buffer pH 3.6, one volume of TPTZ solution (10 mM TPTZ in 40 mM in HCl), and one volume of 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution) was added. The reaction was incubated in the dark for 30 min, and absorbance was measured at 593 nm.

α -Amylase inhibitory (AAI) assay

A 20 μ L of samples (1 mg/mL in 0.2 M phosphate buffer saline, pH 6.9 at the concentration) was mixed with 20 μ L of 0.2 M phosphate buffer saline (pH 6.9) containing 20 U/mL of α -amylase enzyme and incubated at $37 \pm 2^\circ\text{C}$ for 10 min. Then, 30 μ L of 0.5% starch solution was added and incubated for 8 min at $37 \pm 2^\circ\text{C}$. The reaction was terminated by adding 20 μ L of HCl (1 M) and 100 μ L of iodine solution (0.25 mM). Then the absorbance was measured at 565 nm. Acarbose was used to build the standard curve, and the activity was expressed as acarbose equivalent in mmol ACE/g extract.

α -Glucosidase inhibitory (AGI) assay

A 10 μ L of samples (10 mg/mL in DMSO:) mixed with 50 μ L of 0.1 M phosphate buffer (pH 6.9) and 25 μ L of α -glucosidase enzyme solution (0.1 U/mL). Then 25 μ L of p-NPG was added and incubated at $37 \pm 2^\circ\text{C}$ for 30 min. The enzyme reaction was stopped by adding 100 μ L of 0.2 M sodium carbonate, and the absorbance was measured at 410 nm. Acarbose was used to build the standard curve, and the activity was expressed as acarbose equivalent in mmol ACE/g extract.

Lipase inhibitory (LPI) assay

LPI assay was performed as described by Chatsumpun et al. (2017). The samples 25 μ L [1 mg/mL in a buffer containing 1.3 mM CaCl_2 , 13 mM Tris-HCl, and 150 mM NaCl (pH 8.0)] were mixed with substrate 50 μ L of 0.5 mM 4-methylumbelliferyl oleate (4MUO). Then, 25 μ L of lipase enzyme solution (50 U/mL) was added and incubated for 30 min at $37 \pm 2^\circ\text{C}$. Next, 100 μ L of 0.1 M sodium citrate (pH 4.2) to stop the reaction. Fluorescence intensity was recorded at 355 nm (excitation) and 460 nm (emission). The percentage inhibition of enzyme activity was calculated using the formula:

$$\text{Percentage inhibition (\%)} = (A_0 - A_1) / A_0 \times 100\% \quad (2)$$

Where:

A_0 : the fluorescence intensity without extract

A_1 : the fluorescence intensity with the extract

Fourier-transform infrared (FTIR) spectroscopy

FTIR analysis was carried out as described by Ali et al. (2019). The 5 mg of powder of the crude extracts were subjected to a FTIR spectrophotometer (Invenio-S 100183, Bruker, Ettlingen, Germany). The spectra ranging from $4000\text{--}400\text{ cm}^{-1}$, 32 scans/min in absorbance mode at 4 cm^{-1} resolution were recorded and analyzed using OPUS software (OPUS ver. 4.2, Bruker, Ettlingen, Germany).

Gas chromatography-mass spectrometry (GC-MS)

Derivatization of extracts was performed as described by Murugesu et al. (2018) with minor modifications. The extract (25 mg) was dissolved with 500 μ L pyridine in a 2 mL glass vial, vortexed, and sonicated for 10 min using a bath sonicator CP200T (Crest Ultrasonics, Cortland, NY, USA). A 100 μ L methoxyamineHCl (20 mg/mL pyridine) was mixed and incubated for 2 h at 60°C , followed by mixing with 300 μ L of MSTFA (N-Methyl-N-(trimethylsilyl)trifluoroacetamide) containing 1% trimethylchlorosilane and incubating for 30 min at 60°C . The derivatized samples were filtered into an autosampler GC-vial using a PTFE syringe filter (0.2 μ m, 13 mm) (Agilent Technologies, Santa Clara, CA, USA) and left overnight at room temperature. The derivatized sample (1 μ L) was injected into the Agilent GC-MS system consisting of a 6890-chromatography gas and an HP-5973 mass selective detector. The GC column and carrier gas used was a 5% DB-5MS phenyl methyl siloxane column (inside diameter of 250 μ m, thickness of 0.25 μ m), and helium was used as a carrier gas (rate of 1 mL/min). The initial temperature of oven was set at 50°C with a holding

time of 3 min, the temperature was increased to 315°C at the rate of 10°C/min and was held for 10 min. The injector and ion source temperatures were set at 330°C and 250°C, respectively. After a solvent delay of 7 min, mass spectra were acquired in full scan and monitoring mode over a mass scan range of 50 to 550 m/z. Each chromatogram peak and retention time of the metabolites were compared with those of Wiley7n.l database. Data were expressed in peak abundance, and the compound similarity with the library data was presented in percentage (%).

Statistical analysis

All the experiments were carried out in triplicates. The results were expressed as means \pm SD. Significance levels of one-way ANOVA were tested (Duncan Multiple Comparison tests) at the confidence level of $p < 0.05$. For normally distributed data, Pearson's correlation coefficient r was expressed in terms of the correlation between parameters (significant at $p < 0.05$ and highly significant at $p < 0.01$). SPSS software was used to analyze the data (SPSS for Windows 28.0, SPSS Inc., IL, USA).

RESULTS AND DISCUSSION

Extraction yield

The results for extraction yield are presented in Table 1. The extracts from hot water had the highest yields from all GLVs (30.21%, 21.26%, 18.71%, and 19.18%; dry wt. basis). That could be due to the polar nature of water, making it a universal solvent. High temperatures can enhance extraction by increasing diffusivity and solubility (Vardanega et al. 2017). In addition, using high temperatures during extraction can facilitate breaking the linkages between compounds and plant tissue, reduce the viscosity, improve the mass transfer, and increase the solubility (Vardanega et al. 2017). Moreover, Lefebvre et al. (2021) documented that many compounds extracted through an aqueous medium were mostly carbohydrates and non-bioactive compounds. Similarly, the present study noted lower bioactivity of water extracts from all the

GLVs. The extracts from 80% ethanol, absolute ethanol, and methanol exhibited moderate extraction yields ranging from 5.49% to 18.95%, with significantly high bioactivity. These results were in agreement with Lee et al. (2014), who reported that hot water extract (8.31%) from onion (*A. cepa*) peel had a higher yield than ethanol extract (4.46%). The other samples extracted using *n*-hexane, chloroform, and acetone had the lowest extraction yield (1.14%–3.20%, dry wt. basis). Moreover, El-Ghorab et al. (2010) reported that *n*-hexane and methanol extract had been reported to have a low extraction yield in ginger and cumin. The present study noted that the higher extraction of plant compounds was more likely in the solvents with high polarity than in non-polar solvent systems.

TPC

Table 1 shows the TPC of GLVs extracts. The two solvents, 80% ethanol and acetone, showed the higher TPC among all four GLVs. For 80% ethanol, *A. cepa*, *A. graveolens*, and *P. crispum*, the TPC were 11.76, 23.78, and 19.94 mg GAE/g extract, respectively. While all four GLVs extracts from acetone showed high TPC, i.e., *A. cepa*, *A. graveolens*, *C. sativum*, and *P. crispum* with values of 11.66, 12.10, 12.58, and 18.41 mg GAE/g extract, respectively. Similarly, Do et al. (2014) documented that ethanol and acetone extracts could recover the TPC with the maximum TPC. The extracts from hot water extraction showed the lowest TPC among all the GLVs, except for *A. graveolens*. Besides, Juan and Chou (2010) discovered that the acetone extract had the highest TPC (26.60 mg GAE/g extract), while the water extract had the lowest (6.04 mg GAE/g extract) of black soybeans. The results for TPC agreed with these extracts' functional properties. It was also noted that sample *A. graveolens* showed the highest TPC when extracted with 80% ethanol and yielded low TPC when extracted with hexane with a difference of 5.6 times. In another report by Anwar and Przybylski (2012), the TPC of flaxseeds was highest in 80% ethanol (32.60 mg GAE/g DM) than in absolute ethanol (13.60 mg GAE/g DM) extracts. The results showed that the phenolic compounds were highly extractable in 80% ethanol.

Table 1. Extraction yield and total phenolic content of various solvent extracts from green leafy vegetables

Antioxidant assay	Solvent	Scientific name			
		<i>Allium cepa</i>	<i>Apium graveolens</i>	<i>Coriandrum sativum</i>	<i>Petroselinum crispum</i>
Yield of extraction ¹	80% ethanol	17.15 \pm 0.15 ^b	16.90 \pm 1.56 ^b	10.95 \pm 0.96 ^b	16.87 \pm 1.62 ^b
	Absolute ethanol	13.14 \pm 0.92 ^c	12.03 \pm 0.81 ^c	11.04 \pm 0.85 ^b	7.50 \pm 0.47 ^c
	Acetone	2.96 \pm 0.22 ^d	3.03 \pm 0.25 ^d	3.20 \pm 0.19 ^d	2.97 \pm 0.18 ^d
	Methanol	18.95 \pm 1.48 ^b	11.43 \pm 1.00 ^c	5.49 \pm 0.40 ^c	8.38 \pm 0.83 ^c
	<i>n</i> -hexane	1.29 \pm 0.12 ^d	1.27 \pm 0.11 ^e	3.08 \pm 0.23 ^d	2.32 \pm 0.20 ^d
	Chloroform	1.68 \pm 0.10 ^d	1.14 \pm 0.09 ^e	2.78 \pm 0.14 ^d	2.16 \pm 0.15 ^d
	Hot water	30.21 \pm 3.00 ^a	21.26 \pm 1.40 ^a	18.71 \pm 0.92 ^a	19.18 \pm 1.84 ^a
Total phenolic content ²	80% ethanol	11.76 \pm 0.16 ^a	23.78 \pm 1.46 ^a	10.34 \pm 0.44 ^b	19.94 \pm 1.98 ^a
	Absolute ethanol	10.71 \pm 0.36 ^b	7.69 \pm 0.18 ^c	7.58 \pm 0.27 ^d	11.19 \pm 0.07 ^b
	Acetone	11.66 \pm 0.27 ^a	12.10 \pm 0.79 ^b	12.58 \pm 0.79 ^a	18.41 \pm 0.90 ^a
	Methanol	6.90 \pm 0.14 ^d	7.81 \pm 0.14 ^c	5.55 \pm 0.07 ^f	8.85 \pm 0.12 ^c
	<i>n</i> -hexane	9.99 \pm 0.27 ^c	4.20 \pm 0.24 ^d	6.38 \pm 0.14 ^e	11.23 \pm 0.94 ^b
	Chloroform	10.31 \pm 0.24 ^{bc}	7.69 \pm 0.18 ^c	8.45 \pm 0.18 ^c	9.40 \pm 0.07 ^c
	Hot water	4.04 \pm 0.04 ^e	7.28 \pm 0.18 ^c	3.99 \pm 0.07 ^g	4.30 \pm 0.17 ^d

Notes: ¹Results are expressed as a yield percentage (%) of extract; ²Results are expressed as mg of gallic acid per g of extract. Mean \pm SD from triplicate determinations. Different superscript alphabetic letters in the same column indicate significant differences ($p < 0.05$)

DPPH

The results of the DPPH assay are presented in Table 2. All the extracts from 80% ethanol extract showed the highest DPPH radical scavenging activity, i.e., *A. cepa*, *A. graveolens*, *C. sativum*, and *P. crispum* with values of 12.67, 125.57, 119.42, and 21.04 mg AEAC/g extract, respectively ($p < 0.05$). These values were 7 to 49 times higher when compared with that of all four-sample extracted from hot water, which exhibited the lowest DPPH radical scavenging activity. Similarly, Sepahpour et al. (2018) reported that the DPPH activity of 80% ethanolic extract (41.74%) was highest in curry leaves, and water extract had the least activity. In this study, it was noted that the DPPH activities of all extracts in different solvent extractions showed a highly significant correlation with TPC ($p < 0.01$) (Table 4). The results suggest organic solvents were much better at extracting functional compounds, especially polyphenols, when compared to that hot water extraction concerning DPPH antioxidant activity.

Metal chelating

Table 2 presents the metal chelating activity of all samples. Similar to DPPH activity, all the samples extracted in 80% ethanol showed high metal chelating activity, E.g., *A. graveolens*, *C. sativum*, and *P. crispum* had values of 70.33, 92.85, and 77.96 mg EECC/g extract, respectively. Except for the sample, *A. cepa* extracted in acetone had relatively high metal chelating activity, i.e., 11.91 mg EECC/g extract ($p \geq 0.05$). Likely, Saravanan and Parimelazhagan (2014) reported that acetone extract (134.53 mg EDTA/g extract) had the highest metal chelating activity compared to chloroform and methanol

extracts in *P. ligularis* Juss fruit pulp. These results for metal chelating activity directly correlated with the TPC. Based on the correlation studies shown in Table 4, the metal chelating activity of all GLVs was closely correlated with TPC ($p < 0.01$). These findings suggest that phenolic compounds, such as flavonoids, phenolic acids, and condensed tannins, mainly contribute to the metal chelating property (Cai et al. 2004).

FRAP

The highest FRAP activity was recorded for 80% ethanolic extracts of *A. cepa*, *A. graveolens* L., and *C. sativum*, which was 21.61, 34.78, and 46.98 mg AEAC/g extract, respectively (Table 2). These values were 7 to 20 times higher than the hot water extracts, resulting in the lowest FRAP activity ($p < 0.05$). Moreover, the *n*-hexane extract of *P. crispum* had comparably high FRAP activity with 34.50 mg AEAC/g extract. Likely, Akhir et al. (2017) reported that the FRAP activity of *n*-hexane extract in Bee bread (2.41 mmol FE/g) and propolis (6.64 mmol FE/g) was higher than that of ethanol extract (0.85 and 0.87 mmol FE/g). We noticed that FRAP activity was in a linear correlation with TPC ($p < 0.01$) (Table 4). Likely, Gan et al. (2017) reported that FRAP was significantly correlated with TPC ($r = 0.941$, $p < 0.01$) in *L. meyenii*, indicating that polyphenols were the primary antioxidants in natural products, capable of eliminating radicals by donating hydrogen. In addition, FRAP activity from all the GLVs was highly correlated with DPPH and metal chelating activities, except *P. crispum*. This difference could be attributed to various antioxidant groups in the resulting extracts, contributing to antioxidant activity.

Table 2. Antioxidant activities of various solvent extracts from green leafy vegetables

Antioxidant assay	Solvent	Scientific name			
		<i>Allium cepa</i>	<i>Apium graveolens</i>	<i>Coriandrum sativum</i>	<i>Petroselinum crispum</i>
DPPH radical scavenging activity ¹	80% ethanol	12.67 ± 1.03 ^a	125.57 ± 3.09 ^a	119.42 ± 0.02 ^a	21.04 ± 1.46 ^a
	Absolute ethanol	7.95 ± 0.28 ^c	72.43 ± 7.24 ^c	58.80 ± 1.61 ^c	14.45 ± 0.98 ^c
	Acetone	10.23 ± 0.33 ^b	86.01 ± 2.29 ^b	83.02 ± 1.70 ^b	17.16 ± 0.78 ^b
	Methanol	5.92 ± 0.43 ^d	63.63 ± 1.95 ^d	48.71 ± 3.92 ^d	9.27 ± 0.80 ^d
	<i>n</i> -hexane	10.20 ± 0.46 ^b	68.83 ± 1.01 ^{cd}	43.11 ± 2.46 ^e	16.91 ± 1.16 ^b
	Chloroform	10.45 ± 0.25 ^b	68.78 ± 1.28 ^{cd}	56.64 ± 1.60 ^e	17.61 ± 0.90 ^b
	Hot water	1.97 ± 0.04 ^e	2.87 ± 0.02 ^e	2.43 ± 0.03 ^f	2.73 ± 0.04 ^e
Metal chelating activity ²	80% ethanol	11.07 ± 1.02 ^a	70.33 ± 5.65 ^a	92.85 ± 5.23 ^a	77.96 ± 5.53 ^a
	Absolute ethanol	9.34 ± 0.81 ^b	33.61 ± 1.83 ^c	15.75 ± 1.52 ^e	9.21 ± 0.62 ^e
	Acetone	11.91 ± 1.33 ^a	61.74 ± 5.16 ^b	64.47 ± 2.27 ^b	39.40 ± 1.97 ^b
	Methanol	5.03 ± 0.40 ^d	31.88 ± 2.63 ^{cd}	44.37 ± 1.74 ^c	34.19 ± 1.51 ^c
	<i>n</i> -hexane	4.38 ± 0.34 ^d	26.42 ± 1.45 ^{de}	23.44 ± 1.93 ^d	8.47 ± 0.72 ^e
	Chloroform	7.98 ± 0.68 ^c	23.61 ± 1.22 ^e	26.00 ± 1.38 ^d	14.01 ± 1.37 ^d
	Hot water	0.03 ± 0.00 ^e	0.02 ± 0.00 ^f	0.04 ± 0.00 ^f	0.05 ± 0.00 ^f
Ferric reducing antioxidant power ³	80% ethanol	21.61 ± 1.58 ^a	34.78 ± 3.42 ^a	46.98 ± 4.64 ^a	19.25 ± 0.97 ^c
	Absolute ethanol	6.16 ± 0.19 ^e	13.17 ± 0.43 ^d	10.63 ± 0.73 ^c	11.86 ± 0.19 ^e
	Acetone	9.10 ± 0.84 ^c	22.53 ± 0.81 ^b	15.03 ± 0.96 ^b	25.84 ± 2.37 ^b
	Methanol	7.51 ± 0.52 ^d	13.83 ± 0.66 ^d	11.32 ± 0.50 ^c	12.15 ± 0.22 ^e
	<i>n</i> -hexane	8.42 ± 0.35 ^{cd}	13.10 ± 0.38 ^d	8.39 ± 0.25 ^c	34.50 ± 0.53 ^a
	Chloroform	11.32 ± 0.54 ^b	16.86 ± 0.54 ^c	10.93 ± 0.14 ^c	16.61 ± 0.25 ^d
	Hot water	2.84 ± 0.10 ^f	4.51 ± 0.23 ^e	2.28 ± 0.05 ^d	2.46 ± 0.07 ^f

Notes: ¹Results are expressed as mg of L-ascorbic acid equivalent radical scavenging capacity per g of extract; ²Results are expressed as mg of EDTA equivalent chelating capacity per g of extract; ³Results are expressed as mg of L-ascorbic acid equivalent reducing capacity per g of extract. Mean ± SD from triplicate determinations. Different superscript alphabetic letters in the same column indicate significant differences ($p < 0.05$)

α -Amylase inhibitory (AAI) activity

AAI activity of GLVs extracts is presented in Table 3. The results indicated that 80% ethanolic extract showed high activity from *P. crispum* 30.61 mg AEAC/g extract), followed by *C. sativum* (26.06 mg AEAC/g extract) ($p < 0.05$). Similarly, Mumtaz et al. (2018) stated that AAI activity in 80% ethanol extract (IC_{50} 13.08 μ g/mL) of *Ficus benjamina* L. was the highest compared to other solvents. The chloroform extract of *A. graveolens* (10.93 mmol ACE/g extract), and acetone extract of *A. cepa* (11.05 mmol ACE/g extract), showed moderate activity compared with that of acarbose. Furthermore, for the sample *A. cepa*, 80% ethanol and acetone showed comparable AAI activity ($p \geq 0.05$). Moreover, the extract from hot water showed low AAI activity ($p < 0.05$). Similarly, Zaharudin et al. (2018) reported that methanol and water extract of *Undaria pinnatifida* (Harv.) Suringar showed the lowest activity. The varying activities of extracts might be due to differences in their compounds acting as AAI, which are reliant on the corresponding solvent polarity (Aristatile and Alshammari 2017). Table 4 shows that AAI activity was positively correlated with TPC and antioxidant activity from *A. cepa*, *P. crispum*, and *C. sativum* ($p < 0.01$). Phenolic acids are important in the inhibitory activity of α -amylase on carbohydrate metabolism because phenolic compounds can covalently bind to this enzyme and cause activity alteration (Iftikhar et al. 2020). Miras-Moreno et al.

(2016) found that *A. graveolens* had the highest levels of phytosterols (2,650 g/g). Akbar (2020) identified α -tocopherol as a significant molecule in *A. graveolens*.

 α -Glucosidase inhibitory (AGI) activity

Table 3 shows the AGI activity of extracts from GLVs extracted in different solvents. *N*-hexane extracts from *A. graveolens*, *C. sativum*, and *P. crispum* demonstrated elevated AGI activity (287.98, 430.46, and 415.80 mmol ACE/g extract, respectively). Deveci et al. (2021) reported that *n*-hexane extracts from *P. ostreatus*, *Macrolepiota procera* (Scop.) Singer, *Phaeolus schweinitzii* (Fr.) Pat., *Leucopaxillus gentianeus* (Quél.) Kotl., and *Phellinus pini* (Brot.) Pilát had the strongest inhibitory effect on α -glucosidase. In addition, 80% ethanolic extract showed the highest activity from *A. cepa* (595.28 mmol ACE/g extract). However, the 80% ethanol extracts from *C. sativum* (131.55 mmol ACE/g extract) and *P. crispum* (111.57 mmol ACE/g extract) had lower activity. Easmin et al. (2017) found that 80% ethanolic extract was less active than absolute ethanolic extract in *P. macrocarpa*. In addition, hot water extracts from all four samples resulted in lower AGI activity (6.35 to 19.51 mmol ACE/g extract). The correlation results revealed that AGI activity from *A. cepa* had correlated with AAI ($r = 0.844$) activities at the significance level of $p < 0.01$.

Table 3. Enzyme inhibitory activities of various solvent extracts from green leafy vegetables.

In-vitro assay	Solvent	Scientific name			
		<i>Allium cepa</i>	<i>Apium graveolens</i> L.	<i>Coriandrum sativum</i>	<i>Petroselinum crispum</i>
α -Amylase inhibitory activity ¹	80% ethanol	10.16 \pm 0.95 ^{ab}	7.27 \pm 0.21 ^c	26.06 \pm 1.56 ^a	30.61 \pm 2.38 ^a
	Absolute ethanol	6.42 \pm 0.53 ^c	9.59 \pm 0.35 ^d	8.80 \pm 0.26 ^d	7.14 \pm 0.54 ^c
	Acetone	11.05 \pm 0.76 ^a	10.22 \pm 0.33 ^{bc}	13.39 \pm 0.89 ^b	4.11 \pm 0.13 ^d
	Methanol	6.23 \pm 0.36 ^c	10.61 \pm 0.30 ^{ab}	8.38 \pm 0.65 ^d	10.78 \pm 0.20 ^b
	<i>n</i> -hexane	9.77 \pm 0.16 ^b	9.98 \pm 0.39 ^{cd}	11.71 \pm 0.67 ^c	3.18 \pm 0.18 ^d
	Chloroform	7.09 \pm 0.37 ^c	10.93 \pm 0.35 ^a	12.13 \pm 0.87 ^{bc}	4.25 \pm 0.25 ^d
	Hot water	4.24 \pm 0.32 ^d	2.66 \pm 0.24 ^f	4.65 \pm 0.32 ^e	3.41 \pm 0.12 ^d
α -Glucosidase inhibitory activity ²	80% ethanol	595.28 \pm 48.97 ^a	130.50 \pm 7.49 ^b	131.55 \pm 10.93 ^f	111.57 \pm 7.94 ^f
	Absolute ethanol	145.68 \pm 12.75 ^c	32.34 \pm 2.54 ^c	266.57 \pm 5.36 ^c	201.93 \pm 14.18 ^d
	Acetone	340.03 \pm 5.71 ^b	31.92 \pm 2.74 ^c	234.25 \pm 7.16 ^d	130.78 \pm 10.13 ^e
	Methanol	198.78 \pm 11.76 ^d	125.11 \pm 10.48 ^b	197.31 \pm 15.45 ^e	320.51 \pm 8.36 ^b
	<i>n</i> -hexane	358.29 \pm 7.62 ^b	287.98 \pm 8.85 ^a	430.46 \pm 2.74 ^a	415.80 \pm 15.23 ^a
	Chloroform	280.21 \pm 26.66 ^c	129.73 \pm 5.36 ^b	320.51 \pm 14.55 ^b	288.82 \pm 9.69 ^c
	Hot water	14.73 \pm 1.18 ^f	6.35 \pm 0.60 ^d	19.51 \pm 1.86 ^g	14.17 \pm 1.18 ^g
Lipase inhibitory activity ³	80% ethanol	17.74 \pm 1.20 ^a	27.45 \pm 1.17 ^a	13.33 \pm 1.21 ^{de}	31.07 \pm 0.89 ^a
	Absolute ethanol	10.74 \pm 0.98 ^b	21.45 \pm 0.54 ^b	42.10 \pm 3.09 ^a	27.31 \pm 0.44 ^b
	Acetone	12.07 \pm 1.09 ^b	20.10 \pm 1.24 ^c	11.58 \pm 0.62 ^e	12.98 \pm 1.27 ^e
	Methanol	7.42 \pm 0.45 ^c	12.27 \pm 0.17 ^d	32.19 \pm 1.99 ^b	24.20 \pm 0.58 ^c
	<i>n</i> -hexane	11.28 \pm 0.19 ^b	3.02 \pm 0.26 ^f	15.14 \pm 1.45 ^d	19.48 \pm 1.46 ^d
	Chloroform	11.14 \pm 0.64 ^b	4.42 \pm 0.25 ^e	18.35 \pm 1.26 ^c	5.91 \pm 0.37 ^f
	Hot water	6.78 \pm 0.28 ^c	3.68 \pm 0.25 ^{ef}	5.89 \pm 0.06 ^f	2.97 \pm 0.17 ^g

Notes: ¹Results are expressed as mmol of acarbose equivalent inhibition capacity per g of extract; ²Results are expressed as mmol of acarbose equivalent inhibition capacity per g of extract; ³Results are expressed as percentage (%) inhibition of lipase enzyme activity. Mean \pm SD from triplicate determinations. Different superscript alphabetic letters in the same column indicate significant differences ($p < 0.05$)

Table 4. Correlation coefficients (r) of the activities.

Green leafy vegetables	Parameters	TPC	DPPH	MC	FRAP	AAI	AGI	LPI
<i>Allium cepa</i>	TPC	1.000	0.927**	0.896**	0.639**	0.800**	0.749**	0.804**
	DPPH		1.000	0.787**	0.783**	0.836**	0.896**	0.851**
	MC			1.000	0.691**	0.683**	0.652**	0.737**
	FRAP				1.000	0.602**	0.913**	0.905**
	AAI					1.000	0.844**	0.737**
	AGI						1.000	0.898**
	LPI							1.000
<i>Apium graveolens</i>	TPC	1.000	0.703**	0.768**	0.880**	-0.162	-0.139	0.768**
	DPPH		1.000	0.913**	0.924**	0.526*	-0.300	0.734**
	MC			1.000	0.916**	0.370	0.040	0.843**
	FRAP				1.000	0.255	0.140	0.741**
	AAI					1.000	0.416	0.117
	AGI						1.000	-0.331
	LPI							1.000
<i>Coriandrum sativum</i>	TPC	1.000	0.830**	0.733**	0.566**	0.657**	0.181	-0.131
	DPPH		1.000	0.911**	0.886**	0.901**	0.105	0.049
	MC			1.000	0.871**	0.875**	-0.094	-0.152
	FRAP				1.000	0.946**	-0.187	-0.123
	AAI					1.000	0.019	-0.219
	AGI						1.000	0.281
	LPI							1.000
<i>Petroselinum crispum</i>	TPC	1.000	0.803**	0.806**	0.556**	0.585**	-0.107	0.529*
	DPPH		1.000	0.547*	0.710**	0.416	0.309	0.449*
	MC			1.000	0.208	0.881**	-0.205	0.580**
	FRAP				1.000	-0.022	0.575**	0.235
	AAI					1.000	-0.242	0.675**
	AGI						1.000	0.225
	LPI							1.000

Notes: AAI: α -amylase inhibitory activity; AGI: α -glucosidase inhibitory activity; DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; FRAP: ferric reducing antioxidant power; LPI: lipase inhibitory activity; MC: metal chelating activity; TPC: total phenolic content. *Significant at $p < 0.05$; ** Highly significant at $p < 0.01$

Lipase inhibitory (LPI) activity

In the crude extracts from all solvent types, except for hot water, high to moderate LPI activities was seen, as indicated in Table 3. Among the GLVs, *A. cepa*, *A. graveolens*, and *P. crispum* strongly inhibited lipase enzymes (17.74, 27.45, and 31.07%, respectively), mainly extracted in 80% ethanol, which were noted to be 2 to 10 folds higher than extracted in hot water. Zhang et al. (2018) also made similar observations, where 80% ethanol extract has higher activity than other solvent systems (methanol and acetone) from *Rhus chinensis* Mill. The highest activity among all the GLVs and solvents was observed from *C. sativum* (42.10%) extracted in absolute ethanol. Generally, the hot water extract from *A. cepa*, *C. sativum*, and *P. crispum* resulted in the lowest LPI activity of 6.78, 5.98, and 2.97%, respectively. Sample *A. graveolens* showed the lowest activity (3.02%) when extracted in *n*-hexane. These findings agree with Wunjuntuk et al. (2022), who reported that the IC₅₀ values of *Lentinus edodes* (Berk.) Singer extract in 80% ethanol had the highest activity, and *n*-hexane extract had the weakest.

LPI activity in *A. cepa*, *A. graveolens*, and *P. crispum* was significantly correlated with TPC and antioxidant activity, as shown in Table 4. LPI activity is very significant with TPC ($r = 0.804$, $p < 0.01$) and AGI ($r = 0.898$, $p < 0.01$) activities, as shown in Table 4. Although the LPI ability of the various GLVs varied significantly, the phenolic compounds in the extracts were discovered to be responsible for the LPI activity in some GLVs. The difference in extract performance to inhibit lipase activity could be attributed to phenolic composition differences (Zhang et al. 2018).

FTIR

The FTIR spectra of GLVs are shown in Figure 1. The graph at 3202 to 3022 cm⁻¹ had the highest peak from 80% ethanolic extract of *A. graveolens* (Table 2). Khoshnamvand et al. (2020) reported that the peak at 3243 cm⁻¹ might correspond to the presence of phenols or alcohols because of O-H stretching vibration in the *A. graveolens* stem. The phenolic acid, tannin, and flavonoid content had been reported to be highly significant in *A. graveolens* leaf extract (Sameh et al. 2011).

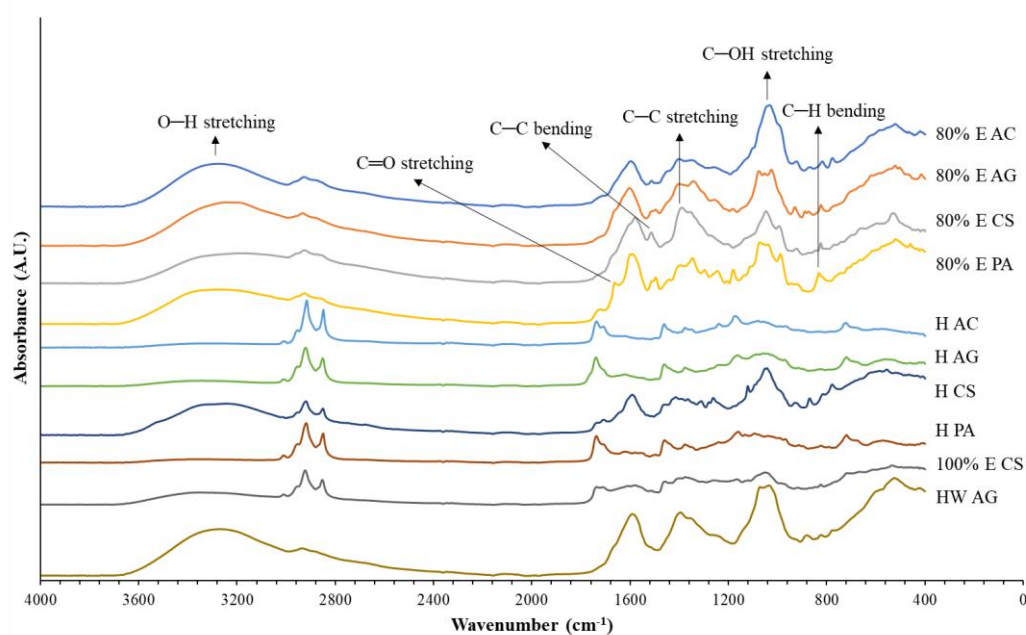


Figure 1. Fourier transforms infrared spectroscopy spectra of green leafy vegetables. Code of samples: 80%E: 80% ethanol extract, 100%E: absolute ethanol extract, H: *n*-hexane extract, HW: hot water extract, AC: *Allium cepa*, AG: *Apium graveolens*, CS: *Coriandrum sativum*, PS: *Petroselinum crispum*.

The spectra ranging from 1678 to 1652 cm^{-1} and 846 to 813 cm^{-1} show the highest peak of 80% ethanol in *P. crispum*, which is related to its highest AAI and LPI activity (Table 3). Like Poureini et al. (2020), the FTIR spectra in *P. crispum* leaves revealed C=O stretching vibrations in the bands 1600 to 1400 cm^{-1} and 800 to 520 cm^{-1} could be assigned to C-H bending. The presence of apigenin is indicated by this FTIR spectrum (Poureini et al. 2020). Apigenin has been reported to be therapeutic for modulating postprandial hyperglycemia and antifungal therapy (Sahnoun et al. 2018). In addition, C-H bending vibrations could be attributed to mannitol, which was reported to be abundant in *P. crispum* (Pharr et al. 1999). C=O stretching and C-H bending vibrations could be indicated to succinic acid, which is significantly higher in *P. crispum* (Saleh et al. 2018). Both compounds had high antidiabetic and anti-obesity activities (Ives et al. 2020; Wu et al. 2021).

The peaks at 1582 to 1500 cm^{-1} and 1420 to 1290 cm^{-1} showed the highest peak in the 80% ethanol extract of *C. sativum*, corresponding to the high metal chelating and FRAP activities (Table 2). This result is in line with Jiao et al. (2021), who reported that C-C bending on the aromatic ring bond in the range of 1600 to 1500 cm^{-1} indicated the presence of an aromatic group. While the aromatic stretch of the C-C bond was identified in the range of 1500 to 1400 cm^{-1} , which was significant for the aromatic backbone associated with the main content in the leaf extract of *C. sativum*. This band has been reported to be present in coriander leaf, which was recognized as flavonoids and xanthone linked to significant strong antioxidant and analgesic activities in coriander leaf (Jiao et al. 2021).

The spectra in wavenumber 1062 to 894 cm^{-1} show the highest peak in the 80% ethanol extract of *A. cepa*, indicating the presence of dialkyl-disulfides compounds, as stated by Krähmer et al. (2021), which appeared around 1100 to 1000 cm^{-1} . The AGI activity results revealed that the 80% ethanol extract in *A. cepa* had the highest inhibition (Table 2), which correlated with dialkyl-disulfides. Dialkyl disulfide is the principal bioactive compound in *Allium* species and may be an active compound in the treatment of diabetes in both animals and humans (Padiya and Banerjee 2013). Moreover, Shanmugam et al. (2022) reported that 1057 cm^{-1} corresponded to the C-OH stretching, which indicated propanoic acid, succinic acid, and myoinositol. Propanoic acid, succinic acid, and myoinositol reported to be high in *A. cepa* (Rozpadek et al. 2016; Medina-Melchor et al. 2022). These compounds have been reported to have antidiabetic activity (Heimann et al. 2015; Chhetri 2019; Ives et al. 2020).

GC-MS

The chromatogram of 80% ethanol extract of *A. cepa* and *P. crispum* are shown in Figure 2. All identified metabolites were confirmed by scrutinizing the spectral pattern and comparison with Wiley7n.l database library. The metabolites of 80% ethanol from *A. cepa* identified were primarily fructose, glucose, propanoic acid, 2,3,4-trihydroxybutyric acid, succinic acid, myoinositol, and glycerol (Figure 2.A). The main compound of 80% ethanol from *P. crispum* was glucose, mannitol, succinic acid, valine, butanoic acid, and glycerol (Figure 2.B). All metabolites analyzed in both extracts are shown in Table 5.

The high antidiabetic activity of 80% ethanol extract from *A. cepa* correlated with the high abundance of several compounds that have been reported to have antidiabetic activity, such as propanoic acid (Heimann et al. 2015), succinic acid (Ives et al. 2020), and myoinositol (Chhetri 2019). Besides, isoleucine, phenylalanine, proline, threonine, valine, and hexadecanoic acid with low abundance could correlate with antidiabetic activity, as reported by Gori et al. (2020); Srinivasan et al. (2019) that these compounds have antidiabetic activity.

In the 80% ethanol extract of *P. crispum*, the high antioxidant activity was supported by the high abundance of mannitol, succinic acid, and valine, which have been reported to have antioxidant activity (Xu et al. 2017; Ives et al. 2020; Wu et al. 2021). Myoinositol, alanine, asparagine, aspartic acid, glutamine, isoleucine, phenylalanine, proline, threonine, hexadecanoic acid, linolenic acid, 9,12-octadecadienoic, citric acid, gluconic acid, phosphoric acid, and succinic acid with low abundance were also reported to have antioxidant activity (Ramis-Ramos 2003; Canete-

Rodriguez et al. 2016; Xu et al. 2017; Chhetri 2019; Ryan et al. 2019; Gori et al. 2020; Ives et al. 2020; Saffaryazdi et al. 2020). On the other hand, several compounds, which have been analyzed, correlated with the high activity of AMI and LPI on 80% ethanol extract of *P. crispum*. Mannitol, succinic acid, valine, butanoic acid, myoinositol, alanine, asparagine, aspartic acid, glutamine, isoleucine, phenylalanine, proline, threonine, hexadecanoic acid, linolenic acid, 9,12-octadecadienoic, propanoic acid, citric acid, and succinic acid had been reported to have the antidiabetic activity (Heimann et al. 2015; Jovanovski et al. 2017; Mustafa et al. 2018; Chhetri 2019; Srinivasan et al. 2019; Gori et al. 2020; Ives et al. 2020; Wu et al. 2021; Yoon et al. 2021). Meanwhile, metabolites reported to have LPI activity were mannitol (Wu et al. 2021), valine (Srinivasan et al. 2019), butanoic acid (Heimann et al. 2015), myoinositol (Chhetri 2019), alanine, asparagine, aspartic acid, glutamine, isoleucine, phenylalanine, proline, threonine, (Srinivasan et al. 2019), propanoic acid (Heimann et al. 2015), and citric acid (Mustafa et al. 2018).

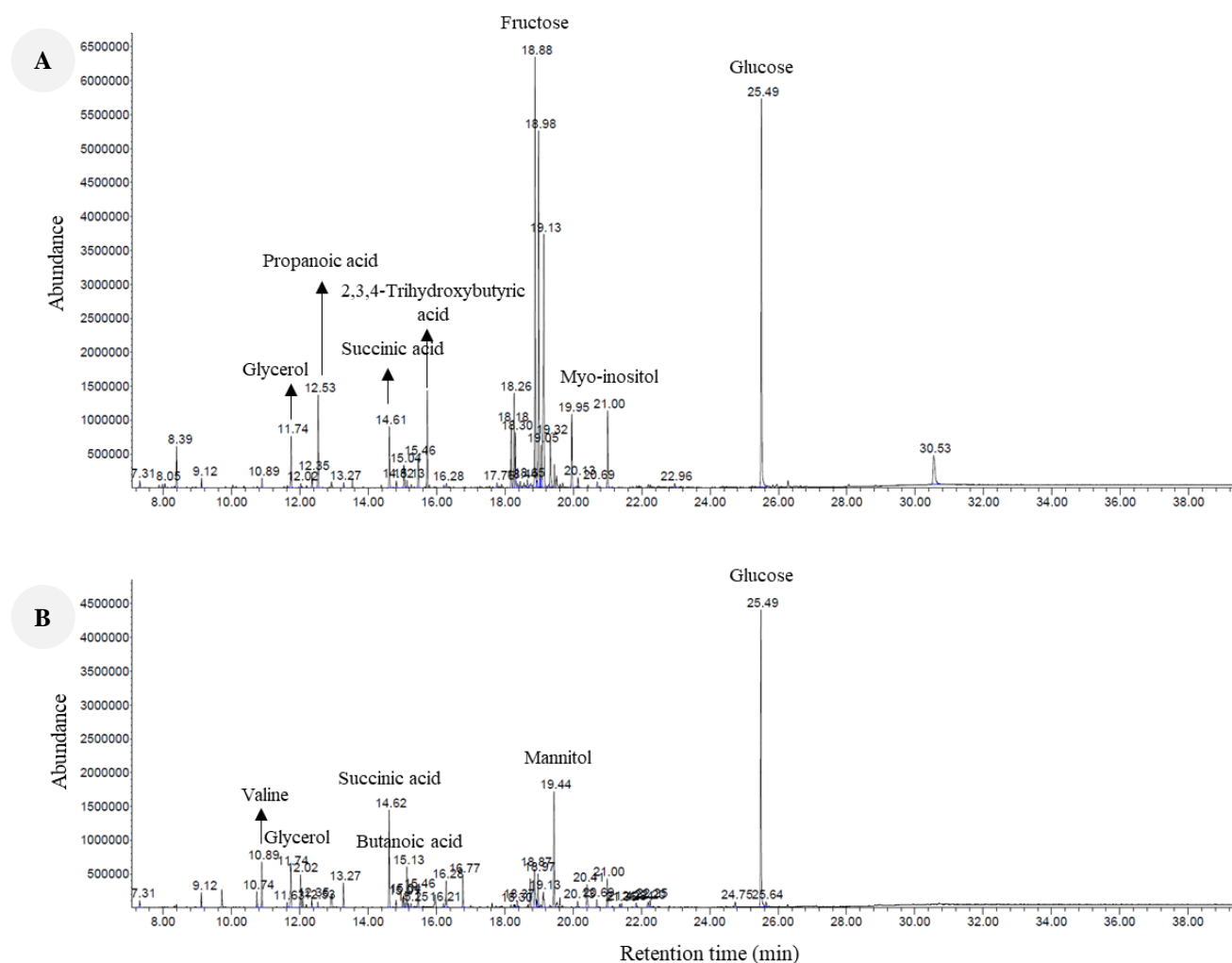


Figure 2. Gas chromatography-mass spectrometry (GC-MS) chromatograms of 80% ethanol extracts of A. *Allium cepa* and B. *Petroselinum crispum*

Table 5. The metabolites of 80% ethanol extracts of *Allium cepa* and *Petroselinum crispum* by GC-MS

Compounds	<i>Allium cepa</i>		<i>Petroselinum crispum</i>	
	Abund. ¹	Sim. (%)	Abund. ¹	Sim. (%)
Sugars				
Arabinofuranose	13.93	52	-	-
Fructose	63.34	91	5.68	94
Fructose oxime	52.47	90	4.80	91
Galactose	0.61	80	-	-
Galactose oxime	7.49	90	-	-
Glucose	57.26	95	44.01	90
Glucose oxime	37.16	90	-	-
Maltose	-	-	0.78	58
Myoinositol	11.32	86	4.26	86
Ribofuranose	0.75	87	-	-
Sorbopyranose	8.10	87	0.44	86
Sugar alcohols				
Mannitol	-	-	17.15	91
Amino acids				
Alanine	-	-	2.19	90
Asparagine	-	-	4.71	98
Aspartic acid	-	-	1.43	86
Glutamine	-	-	0.58	87
Isoleucine	0.62	86	4.84	90
Phenylalanine	0.64	68	3.89	90
Proline	3.32	90	1.74	90
Threonine	0.78	91	3.66	90
Valine	1.43	87	6.72	87
Fatty acids				
Butanoic acid	-	-	6.01	72
Hexadecanoic acid	0.87	99	1.17	99
Linolenic acid	-	-	1.04	99
9,12-Octadecadienoic acid	-	-	0.81	95
Propanedioic acid	-	-	2.35	92
Propanoic acid	13.70	99	0.83	91
Organic acids				
Citric acid	-	-	0.95	91
Gluconic acid	1.45	90	0.92	93
Phosphoric acid	-	-	0.69	87
Succinic acid	9.00	98	14.41	99
2,3,4-Trihydroxy-butyric acid	4.40	86	2.45	62
Other compounds				
Glycerol	7.61	91	5.90	91

Notes: ¹Values are expressed as abundance (x 10⁵). -, not detected

Overall, using various solvents effectively selects extracts to obtain compounds with high activities. In the α -glucosidase and α -amylase inhibitory tests, the 80% ethanol extracts of *A. cepa* and *P. crispum* demonstrated the highest activity. The absolute ethanol extract of *C. sativum* had the greatest lipase inhibitory activity, while the 80% ethanol extracts of *A. graveolens* L. and *C. sativum* had the strongest antioxidant activity. Interestingly, the *n*-hexane extracts of *A. graveolens*, *C. sativum*, and *P. crispum* demonstrated high activity in the α -glucosidase inhibitory activity test, indicating the presence of non-polar active compounds in the extract. Thus, ethanol and *n*-hexane solvents can produce extracts widely used in food and health applications for diabetes mellitus treatment.

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