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Antioxidant and α-Glucosidase Inhibitory Activities of Cucurbit Fruit Vegetables and Identification of Active and Major Constituents from Phenolic-rich Extracts of *Lagenaria siceraria* and *Sechium edule*

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ABSTRACT: Antioxidant and α-glucosidase activities and total phenolic contents (TPC) in sequential extracts of dried pulps from seven cucurbit fruit vegetables were determined for the first time. The highest TPC and metal chelating activity were obtained from the chloroform extracts of *Luffa acutangula* (28.04 ± 0.37 mg GAE/g extract) and *Benincasa hispida* (EC_{50} = 0.44 ± 0.03 mg/mL), respectively. The ethyl acetate extract of *Sechium edule* showed the highest 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity (951.73 ± 29.14 mM TE/g extract). The highest reducing and anti-α-glucosidase activities were shown by the methanol and ethyl acetate extracts of *Momordica charantia* (692.56 ± 43.38 mM AscAE/g extract; 66.64 ± 2.94%, respectively). The highest correlation (r = 0.99) was observed between the TPC and DPPH values of *S. edule*. Although caffeic acid was quantified as the major constituent in the methanol extract of *Lagenaria siceraria*, isoquercetin was found to be the main contributor to the activities. Gallic acid was identified as both the main and most active antioxidant constituent in the ethyl acetate extract of *S. edule*.

KEYWORDS: *Cucurbitaceae, fruit vegetables, sequential extraction, phenolic content, antioxidant activity*

INTRODUCTION

Seven cucurbit fruit vegetables were selected for this study. These cooking vegetables were selected due to their common availability in markets worldwide. They also share a similar commercial stage of maturity and are usually prepared as soups that are best consumed during hot weather, as they possess a cooling property.[1] Most importantly, the decoctions of their dried pulps (excluding *Luffa acutangula* and *Trichosanthes cucumerina*) are traditionally used to treat diabetes.[2−4] Their antidiabetic activities had been verified by various in vivo pharmacological evaluations.[5,6] However, the screenings for α-glucosidase inhibitory activity of these cucurbit fruits were limited to the extracts of *L. acutangula* and *Momordica charantia*.[7,8]Antidiabetic agents that possess antioxidant activities might provide better healing efficacy as they would not only improve the glucose tolerance of diabetic patients by delaying glucose absorption or by stimulating insulin secretion but also alleviate the oxidative milieu by counteracting hypoglycemia-generated free radicals.

A comprehensive literature review of antioxidant activities and chemical constituents found in the vegetables is shown in Table 1. The data are not comparable because various edible portions, sample matrices, extractions, and quantification procedures were used in the different experiments. The antioxidant activities were mostly investigated on individual bases.[9−18] Moreover, the antioxidant activity of *Sechium edule* was determined only from aerial part extracts.[19,20] Earlier comparative studies mostly used a single polar solvent (such as 80% methanol) for extraction and reported lower antioxidant activity for this group of vegetables compared with other vegetables.[21−28] However, this information is inadequate to categorize them as vegetables with low antioxidant activity. Thus, to optimize the recovery of various hydrophilic and lipophilic antioxidants from these vegetables, sequential extraction using a Soxhlet extractor was used in this study.

This study aimed to (i) successively extract the dried fruit pulps using four different polarities of solvents, (ii) determine antioxidant activities (using three different colorimetric assays), α-glucosidase inhibitory activity, and total phenolic content of the extracts, (iii) analyze the possible correlation between total phenolic contents and activities of the extracts, (iv) identify active compounds from the phenolic-rich extracts, and (v) quantify phenolic compounds in the extracts.

MATERIALS AND METHODS

Plant Materials. The fruits of all vegetables examined were harvested at their commercial maturity stage (i.e., unripe). *Benincasa hispida*, *Cucurbita maxima*, *Lagenaria siceraria*, *Luffa acutangula*, *Momordica charantia*, and *Trichosanthes cucumerina* were freshly obtained from a vegetable farm in Teluk Kumbar, Pulau Pinang, Malaysia, whereas *Sechium edule* was harvested from a farm in Cameron Highlands, Pahang, Malaysia. Their identities were checked by morphological comparison with authentic herbarium specimens. The pulps were cleaned and cut into small pieces prior to drying in a hot air-blowing oven at 35 °C to a constant weight. The dried samples were ground to fine powders (40 mesh) in a mechanical grinder. The powders were kept at room temperature prior to extraction.

Chemicals. The chemicals used in this study were of analytical grade. *n*-Hexane, chloroform, ethyl acetate, methanol, acetonitrile, butanol, and ethanol were purchased from R&M Chemicals (UK). Folin−Ciocalteu phenol reagent, 1,1-diphenyl-2-picrylhydrazyl radical

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Table 1. Extracts from Fruits of the Seven Cucurbit Vegetables Previously Used for Antioxidant Evaluations and Their Active Antioxidant Chemicals

scientific name	extract	phenolic compound and/or content	other compound and/or content
Benincasa hispida	water, 50% methanol, and 75% methanol extracts of freeze-dried pulp and fresh juice extract[9]	three flavonoids: astilbin, catechin, and naringenin[48]	vitamin A: 20 mg/100 g of edible portion[40]
	methanol and water extracts of fresh edible portion[23]	total phenolic content in fresh juice: 169.1 mg GAE/g fresh weight[9]	thiamin (vitamin B1): 0.06 mg/100 g of edible portion[40]
	acetone/water/acetic acid (70:29.5:0.5) extract of freeze-dried sample[26]	total phenolic content in acetone/water/acetic acid (70:29.5:0.5) extract: 0.17 mg GAE/g fresh weight[26]	vitamin C: 1 mg/100 g of edible portion[40]
	aqueous extract of fresh sample[27]		
Cucurbita maxima	water-soluble polysaccharide in fresh pulp[14]	a phenolic acid: syringic acid[53]	total carotenoids: 2120 μg/100 g fresh weight[39]
	methanol extract of fresh edible part[24]	total phenolic content in 60% methanolic extract containing 0.1% hydrochloric acid: 46.43 mg GAE/100 g fresh weight[28]	β-carotene: 1180 μg/100 g fresh weight[39]
	dried pulp was successively extracted with petroleum ether, chloroform, and methanol using a Soxhlet apparatus[15]	total phenolic content in methanolic extract: 12 mg GAE/g extract[15]	vitamin A: 1.84 g/100 g of edible portion[40]
	50% ethanolic extract of fresh pulp[25]	total phenolic content in 50% ethanolic extract: 13.3 mg GAE/g extract[25]	thiamin (vitamin B1): 0.06 mg/100 g of edible portion[40]
	60% methanolic extract containing 0.1% hydrochloric acid of fresh edible portion[28]		vitamin C: 2 mg/100 g of edible portion[40]
			a water-soluble polysaccharide[14]
Lagenaria siceraria	methanol and water extracts of fresh edible portion[23]	four flavone *C*-glycosides: isovitexin, isoorientin, saponarin, and saponarin 4′-*O*-glucoside[37]	vitamin A: 60 mg/100 g of edible portion[40]
	ethyl acetate and *n*-butanol extracts of fresh and dried whole fruit samples[13]	two flavonols: isoquercitrin, and kaempferol[33]	thiamin (vitamin B1): 0.03 mg/100 g of edible portion[40]
	dried fruit was successively extracted with dichloromethane, ethyl acetate, and methanol[18]	four hydroxycinnamic acids: (*E*)-4-(hydroxymethyl)phenyl-6-*O*-caffeoyl-β-D-glucopyranoside, 1-(2-hydroxy-4-hydroxymethyl)-phenyl-6-*O*-caffeoyl-β-D-gluco-pyranoside, caffeic acid, and 3,4-dimethoxycinnamic acid[18]	vitamin C: 6 mg/100 g of edible portion[40]
		two phenolic acids: gallic acid, and protocatechuic acid[18]	sitosterol, campesterol, and oleanolic acid[33]
			a water-soluble polysaccharide[54]
Luffa acutangula	80% methanol extract of fresh pulp[21]	total phenolic content in 60% methanolic extract containing 0.1% hydrochloric acid: 27.04 mg GAE/100 g fresh weight[28]	total carotenoids: 991 μg/100 g fresh weight[39]
	aqueous extract of fresh sample[27]		β-carotene: 324 μg/100 g fresh weight[39]
	60% methanolic extract containing 0.1% hydrochloric acid of fresh edible portion[28]		vitamin A: 56 mg/100 g of edible portion[40]
	sequential extraction using hexane, methanol and water of fresh fruit sample[17]		thiamin (vitamin B1): 0.07 mg/100 g of edible portion[40]
			vitamin C: 5 mg/100 g of edible portion[40]
Momordica charantia	80% methanol extract of fresh pulp[21]	catechin, gallic acid, gentisic acid, chlorogenic acid, and epicatechin[11,16]	total carotenoids: 967 μg/100 g fresh weight[39]
	methanol extract of dried fruit[22]	total phenolic content in acetone/water/acetic acid (70:29.5:0.5) extract: 0.4 mg GAE/g fresh weight[26]	β-carotene: 84 μg/100 g fresh weight[39]
	methanol and water extracts of fresh edible portion[23]	total phenolic content in 60% methanolic extract containing 0.1% hydrochloric acid: 139.67 mg GAE/100 g fresh weight[28]	vitamin A: 210 mg/100 g of edible portion[40]
	freeze-dried fruits (mature and ripe) boiled in distilled water[11]	total phenolic content in aqueous extract: 68.8 mg GAE/g extract[12]	thiamin (vitamin B1): 0.07 mg/100 g of edible portion[40]

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Table 1. continued

scientific name	extract	phenolic compound and/or content	other compound and/or content
	aqueous and 95% ethanol extracts of dried fruit[12]	total phenolic content in 95% ethanol extract: 51.6 mg GAE/g extract[12]	vitamin C: 88 mg/100 g of edible portion[40]
	aqueous, 20, 40, 60, 80, 95% ethanol extracts of dried immature, mature and ripe pulps[16]	total phenolic contents in aqueous, 20, 40, 60, 80, 95% ethanol extracts of dried immature, mature, and ripe pulps ranged from 4.3 to 15.7 mg GAE/g extract[16]	momordicoside K (a triterpenoid glycoside)[42]
	acetone/water/acetic acid (70:29.5:0.5)		
	extract of freeze-dried sample[26]		
	aqueous extract of fresh sample[27]		
	60% methanolic extract containing 0.1% hydrochloric acid of fresh edible portion[28]		
Sechium edule		trace amount of vicenin 2, vitexin, and luteolin 7-*O*-rutinoside[34]	total carotenoids: 97 μg/100 g fresh weight[39] β-carotene: 2 μg/100 g fresh weight[39]
Trichosanthes cucumerina	methanol and water extracts of fresh edible portion[23]	total phenolic content in aqueous extract: 71.90 mg GAE/100 g fresh weight[10]	α-carotene: 10.7 mg/100 g fresh weight[10]
	20% acetone extract of freeze-dried fully ripe pulp.[10]		β-carotene: 2.80 mg/100 g fresh weight[10]

(DPPH), ferric chloride hexahydrate, ferrous chloride hexahydrate, ferrozine, ascorbic acid, caffeic acid, gallic acid, isoquercitrin, luteolin, vitexin, sodium phosphate, sodium acetate, 4-nitrophenyl-α-D-glucopyranoside (PNPG), α-glucosidase, acetic acid, acarbose, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), and trichloroacetic acid were purchased from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Potassium ferricyanide, aluminum chloride, anhydrous sodium carbonate, potassium acetate, and dimethyl sulfoxide (DMSO) were purchased from Fluka (Switzerland).

Extraction. The extraction process for each sample was carried out in triplicate using fruits from different vines. Each sample (50 g) was successively extracted by four different solvents (*n*-hexane, chloroform, ethyl acetate, and methanol) with ascending order of polarity using a Soxhlet apparatus. The extracts were filtered and concentrated using a rotary evaporator (EYELA, Japan). The dried extracts were stored at 4 °C until further analysis. The extracts were weighed using a microbalance (Sartorius, Germany) and reconstituted with 99.9% (v/v) DMSO to prepare a stock solution at a concentration of 10 mg/mL.

Determination of Total Phenolic Content (TPC). TPC was determined according to the Folin−Ciocalteu method.[29] In brief, 0.5 mL of each extract (1.0 mg/mL in concentration) was mixed with 1 mL of 2 N (10% v/v) Folin−Ciocalteu reagent. After 3 min, 3 mL of 2% (w/v) sodium carbonate was added to the previous mixture. The absorbance of the reaction products was measured at 760 nm using a Genesys 20 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) after 2 h of incubation at room temperature. The same procedure was repeated using different concentrations (0−200 μg/mL) of gallic acid. The total phenolic content of a sample was calculated from the gallic acid calibration curve, and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight of extract (mg GAE/g extract).

Chelating Power Assay. Chelating power was determined according to the method of Ooi et al.[30] Different concentrations of each extract (50 μL) were mixed with 5 μL of 2.0 mM ferrous chloride hexahydrate and 130 μL of methanol in different wells of a 96-well plate. After 5 min of incubation, the reaction was initiated by the addition of 15 μL of ferrozine (5.0 mM). After 10 min of incubation at room temperature, the absorbance was measured at 562 nm using a Multiskan EX microplate reader (Thermo Fisher Scientific, Finland). The chelating power activity (%) was calculated according to the following equation: chelating power activity (%) = [(absorbance of negative control − absorbance of extract)/absorbance of negative control] × 100%. Each EC_{50} value, the effective concentration that could chelate 50% of ferrous [Fe(II)] ions, was derived from a nonlinear sigmoidal dose response curve (GraphPadPrism, San Diego, CA, USA).

DPPH Free Radical Scavenging Assay. Free radical scavenging activities of the extracts were measured using the method described by Sulaiman et al.[31] The reaction mixture in each well of the 96-well plate used consisted of 50 μL of extract (1.0 mg/mL) and 150 μL of 300 μM DPPH ethanolic solution. The plate was wrapped with aluminum foil to avoid exposure to light and was incubated at 37 °C for 30 min. The decrease in absorbance was determined at 515 nm using a Multiskan EX microplate reader (Thermo Fisher Scientific, Finland). Trolox was used as a reference for this assay. A standard curve was obtained using different concentrations of Trolox (from 31.25 to 4000 μM). The absorbance of the reaction sample was compared to that of the Trolox standard curve, and all results were expressed in terms of millimolar Trolox equivalents (TE) per gram dry weight of extract (mM TE/g extract).

Reducing Power Assay. Reducing powers of the extracts were determined according to the method of Zhuang et al.[32] with a slight modification. An aliquot (125 μL) of each extract (1.0 mg/mL) was mixed with 125 μL of sodium phosphate buffer (0.2 M, pH 6.6) and 125 μL of 1% potassium ferricyanide, and the reaction mixture was incubated at 50 °C for 20 min. After the addition of 125 μL of 10% trichloroacetic acid, the mixture was centrifuged at 704*g* for 10 min using an EBA 20 Centrifuge (Hettich, Japan). The supernatant solution (100 μL) was mixed with 100 μL of distilled water and 20 μL

Table 2. Total Phenolic Contents and Antioxidant and α-Glucosidase Inhibitory Activities of Dried Extracts Obtained from the Pulps of the Seven Cucurbit Vegetables[a]

	total phenolic content (mg GAE/g extract)	EC_{50} values of chelating activity (mg/mL)	DPPH free radical scavenging activity (mM TE/g extract)	reducing power (mM AscAE/g extract)	α-glucosidase inhibition activity (%)
Benincasa hispida					
hexane	9.99 ± 0.15no	8.37 ± 0.42p	327.35 ± 5.84k	8.70 ± 4.99l	37.93 ± 1.31f
chloroform	14.87 ± 0.08h	0.44 ± 0.03a	380.76 ± 3.66j	118.06 ± 6.18g	42.93 ± 0.48e
ethyl acetate	20.29 ± 0.24d	2.52 ± 0.04ghij	161.53 ± 14.78p	279.64 ± 17.98d	48.73 ± 0.98d
methanol	17.73 ± 0.27e	7.47 ± 1.31o	66.30 ± 7.06q	275.83 ± 8.64d	18.94 ± 0.55kl
Cucurbita maxima					
hexane	12.99 ± 0.11i	2.05 ± 0.07fgh	268.39 ± 1.76lm	8.16 ± 4.90l	11.76 ± 1.85m
chloroform	11.59 ± 0.04k	3.65 ± 0.27kl	395.45 ± 5.87ij	78.89 ± 13.88hi	10.63 ± 1.06m
ethyl acetate	6.04 ± 0.27s	1.15 ± 0.05bcd	598.38 ± 22.26d	99.56 ± 12.95gh	22.11 ± 0.90jk
methanol	10.50 ± 0.04l	1.95 ± 0.22efg	252.53 ± 2.10m	207.28 ± 23.02f	9.93 ± 0.93m
Lagenaria siceraria					
hexane	4.03 ± 0.07u	3.05 ± 0.48jk	273.80 ± 3.03lm	18.50 ± 3.40kl	26.44 ± 3.30i
chloroform	11.66 ± 0.11k	0.62 ± 0.09ab	469.30 ± 7.57g	84.87 ± 1.63hi	38.61 ± 1.12f
ethyl acetate	10.33 ± 0.04lm	0.98 ± 0.14abc	556.94 ± 10.62f	218.16 ± 24.93f	56.04 ± 1.72c
methanol	20.85 ± 0.12c	4.39 ± 0.37m	649.32 ± 17.70c	324.25 ± 30.29c	61.25 ± 2.57b
Luffa acutangula					
hexane	8.34 ± 0.15q	9.56 ± 0.19q	201.12 ± 6.11o	43.52 ± 4.11jk	22.81 ± 2.82j
chloroform	28.04 ± 0.37a	2.02 ± 0.09efgh	226.16 ± 0.71n	367.77 ± 9.97b	50.82 ± 3.25d
ethyl acetate	15.55 ± 0.07g	1.72 ± 0.21def	274.66 ± 1.32l	348.19 ± 22.28bc	43.93 ± 1.28e
methanol	13.27 ± 0.04i	4.16 ± 0.67lm	698.14 ± 19.87b	248.63 ± 10.87e	11.59 ± 2.99m
Momordica charantia					
hexane	2.34 ± 0.04v	8.37 ± 0.55p	37.04 ± 1.40r	22.31 ± 0.94kl	19.93 ± 2.31jkl
chloroform	8.92 ± 0.07p	3.51 ± 0.25k	448.23 ± 5.06h	84.87 ± 10.70hi	21.62 ± 0.21jk
ethyl acetate	16.43 ± 0.31f	4.28 ± 0.19lm	393.05 ± 11.15ij	221.97 ± 17.20f	66.64 ± 2.94a
methanol	10.08 ± 0.10mn	1.36 ± 0.34cde	403.87 ± 7.91i	692.56 ± 43.38a	18.04 ± 0.47l
Sechium edule					
hexane	4.02 ± 0.19u	8.11 ± 0.28p	313.53 ± 3.75k	75.62 ± 6.18hi	34.59 ± 2.22g
chloroform	9.08 ± 0.20p	1.16 ± 0.17bcd	443.02 ± 11.29h	126.76 ± 7.36g	27.62 ± 2.82hi
ethyl acetate	22.69 ± 0.15b	2.76 ± 0.07ij	951.73 ± 29.14a	289.97 ± 22.52d	51.49 ± 2.13d
methanol	9.74 ± 0.23o	2.13 ± 0.18fghi	439.23 ± 4.86h	216.53 ± 21.30f	29.96 ± 3.35h
Trichosanthes cucumerina					
hexane	5.29 ± 0.19t	1.59 ± 0.14cdef	38.87 ± 0.89r	68.55 ± 11.42ij	30.87 ± 0.79h
chloroform	12.15 ± 0.23j	5.19 ± 0.23n	256.00 ± 2.46lm	192.59 ± 12.32f	38.08 ± 0.00f
ethyl acetate	17.45 ± 0.27e	2.66 ± 0.07hij	577.04 ± 30.46e	212.72 ± 8.38f	61.91 ± 1.96b
methanol	7.36 ± 0.18r	4.47 ± 0.31m	378.08 ± 11.46j	277.46 ± 4.32d	12.62 ± 1.75m

[a]Values are means ± standard deviations of triplicate analyses. The results from different assays were analyzed separately. Values for each assay followed by different letters are significantly different ($p < 0.05$) as measured by Duncan's test.

of 1% ferric chloride hexahydrate to react for 10 min. The absorbance was then measured at 700 nm using a Multiskan EX microplate reader (Thermo Fisher Scientific, Finland). Ascorbic acid was used as a standard antioxidant compound to produce a standard curve at concentrations between 1.95 and 4000 μM. Values were expressed as millimolar ascorbic acid equivalents (AscAE) per gram dry weight of extract (mM AscAE/g extract).

α-Glucosidase Inhibitory Assay. The α-glucosidase inhibitory activity was determined according to the method of Ooi et al.[30] The extract (25 μL; 10 mg/mL) was mixed with 25 μL of 0.1 M sodium phosphate buffer (pH 7), 25 μL of 15 mM PNPG, and 25 μL of α-glucosidase enzyme solution (5 U/mL). The mixture was then incubated for 40 min at 37 °C. One hundred microliters of 0.2 M sodium carbonate solution was added to terminate the reaction. Acarbose was used as a positive control. The increase in absorbance due to hydrolysis of PNPG by α-glucosidase was measured using a Multiskan EX microplate reader (Thermo Fisher Scientific, Finland). The inhibition percentage was calculated as follows: % α-glucosidase inhibition = [(absorbance of negative control − absorbance of extract)/absorbance of negative control] × 100%. All measurements were done in triplicate.

Bioactivity-Guided Fractionation of the Methanol Extract of *L. siceraria* and the Ethyl Acetate Extract of *S. edule*. The methanol extract of *L. siceraria* and the ethyl acetate extract of *S. edule* were selected for further studies because they showed high TPCs as well as scavenging and reducing values and α-glucosidase inhibitory activity. The extracts were also fractionated by applying each of them separately as a streak on 15−20 sheets of 3 mm Chr (46 × 57 cm) chromatography paper (Whatman, UK) and run in a solvent of *n*-butanol/acetic acid/water (BAW) in 4:1:5 relative proportions (upper layer) for 16 h. The chromatograms were left to air-dry in a fume cupboard. The dried chromatograms were later viewed under long-wave ultraviolet (UV) light, and the R_f values and colors for each band were recorded prior to cutting of the bands. The separated bands were eluted using methanol. The dried fractions (1.0 mg/mL) were tested for their antioxidant activities using the DPPH and ferric reducing assays and α-glucosidase inhibitory activity. Fraction LS3 from the methanol extract of *L. siceraria*, with an R_f value (×100) of 65 in BAW, and fraction SE4 from the ethyl acetate extract of *S. edule*, with an R_f value (×100) of 75 in BAW, were further purified. The fractions were streaked and rerun on chromatography paper in a 15% acetic acid (v/v) solvent. Subsequently, the separated bands were cut out and eluted in methanol. To test its purity, each fraction was spotted onto thin layer chromatography plates (Polygram Cel 400 UV254, Macherey Nagel, Germany) and rerun with BAW, 15% acetic acid, and water. The solvent producing the greatest separation was chosen for further

purification. The fractions were further purified using a Sephadex LH-20 column with methanol. Identification of the compounds was made on the basis of spectroscopic analyses, R_f values, retention times, spiking with authentic markers, and comparison of results with those reported for *L. siceraria*[33] and *S. edule*.[34] The dried compounds at a concentration of 1.0 mg/mL were tested for their antioxidant and α-glucosidase inhibitory activities.

Quantitative Analysis of Phenolic Compounds in *L. siceraria* and *S. edule* Extracts. Quantitative analyses of phenolic compounds in the *L. siceraria* and *S. edule* extracts were performed in triplicate using an Acquity ultraperformance liquid chromatography (UPLC) system (Waters, Milford, MA, USA) equipped with a reverse-phase Acquity UPLC BEH C_{18} column, 1.7 μm (100 mm × 2.1 mm i.d.) and a photodiode array detector. The mobile phase consisted of solvent A (1% acetic acid) and solvent B (methanol/acetic acid/water; 18:1:1). Each extract was separated using a gradient mode that was initially set at an A:B ratio of 85:15 and then linearly increased to 65:35 at 1.5 min, 40:60 at 2 min, and 10:90 at 6.3 min until 7.3 min. The detector was set at 280 nm for quantification of gallic acid and at 350 nm for caffeic acid, isoquercitrin, luteolin, and vitexin, with a flow rate of 0.20 mL/min and an injection volume of 5.0 μL. Phenolic compounds in the extracts were identified and quantified by comparison of their retention times and UV spectra (detected by the photodiode array detector) with those of authentic standards. A standard calibration curve of different concentrations (31.25−1000 μg/mL) of each standard was plotted. The concentration of the phenolic compound in each extract was calculated using the regression equation of its peak area to the peak area of a known concentration of standard from the calibration curve. The results were expressed as milligrams of phenolic compound per 100 g of extract (mg/100 g extract).

Statistical Analysis. All data were expressed as the means ± standard deviations. Data were analyzed by means of a one-way ANOVA using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA), and Duncan's test was used to assess the differences between the means. Pearson's correlation test was used for determination of correlations between the antioxidant activities of the three independent tests (DPPH, reducing power, and metal chelating), α-glucosidase inhibitory activity, and total phenolic contents using Prism 3.02 statistical software (GraphPadPrism). A p value less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSION

Total Phenolic Content. The TPCs of the extracts are shown in Table 2. The polarity and solubility of chemical constituents in an extracting solvent may influence the extraction yield and TPC in an extract. Therefore, in this study, each sample was sequentially extracted using four different polarities of solvents, and the recovery of TPC in each extract was determined.

The highest value of TPC was exhibited by the chloroform extract of *L. acutangula* (28.04 ± 0.37 mg GAE/g extract). This suggests that the initial extraction using hexane may have facilitated greater recovery of lipophilic phenolics from the chloroform extract of this dried fruit. According to a comparative assessment of TPCs from extracts of 60% methanol containing 0.1% hydrochloric acid of fresh vegetables that are commonly consumed in India, the lowest value was obtained from the fresh edible portion of *L. acutangula* (Table 1).[28] Meanwhile, another study found that the amount of antioxidant components in the 80% methanol extract of *L. acutangula* fresh pulp prepared by boiling was higher than that obtained by cold maceration.[21] In this study, the heat treatment applied during the Soxhlet extraction may optimize the extraction of phenolic compounds from the samples by accelerating their solubility and diffusion rates.

The ethyl acetate extracts of most of the vegetables (excluding *C. maxima* and *L. siceraria*) were found to have more than 15 mg GAE/g extract of TPC. Several sequential solvent partitioning studies of dry-ground pulps also revealed the highest recovery of TPC from the ethyl acetate fraction.[35,36] Among the extracts of *C. maxima*, the highest TPC was measured from the hexane extract. The TPC in the hexane extract of *C. maxima* (12.99 ± 0.11 mg GAE/g extract) was found to be comparable to those reported by Attarde et al.[15] and Gacche et al.[25] from methanol and 50% ethanol extracts, respectively (Table 1). The methanol extract of *L. siceraria* was found to have the highest TPC (20.85 ± 0.12 mg GAE/g extract) compared with the other methanol extracts and other extracts of *L. siceraria*. As indicated in Table 1, four flavone *C*-glycosides, two flavonols, two phenolic acids, and four hydroxycinnamic acids were isolated from the fruit of *L. siceraria*.[18,33,37]

Antioxidant Assessments. Many antioxidant studies on these vegetables had been carried out using various in vitro and in vivo assays. Table 1 shows the extracts used in the earlier antioxidant evaluations of the vegetables. With the exception of the studies done by Chanwitheesuk et al.[22] and Isabelle et al.,[26] previous comparative studies were found to have utilized fresh samples. The fruit ripening stages were not highlighted in most papers. The use of different assays and sample matrices can lead to different results. In addition, on the basis of our observations, discrepancies in comparative analysis of literature data could be due to the lack of descriptions (of methodologies), the samples used,[26,27] and the definitions of fresh edible portions not being clearly specified.[23,24,28] An edible portion may exclude the peel and the seed or can refer to the entire fruit. For instance, studies by Kubola and Siriamornpun,[11] Wu and Ng,[12] and Chanwitheesuk et al.[22] on *M. charantia* were found to employ the entire dried fruit instead of dried pulp. Only studies by Attarde et al.[15] on *C. maxima* and by Horax et al.[16] on *M. charantia* utilized dried pulp and the DPPH method. However, it is still inappropriate to compare our results with theirs due to the dissimilar quantitative units (i.e., percentage of free radical scavenging activity and EC_{50}, respectively) used.

The metal chelating assay assesses the indirect involvement of an extract as a secondary antioxidant by binding the ferrous [Fe(II)] ion that catalyzes oxidation and subsequently prevents the formation of the Fe(II)−ferrozine complex (intense red-purple in color). The extract with the lowest EC_{50} value is considered to have the highest activity. The chloroform extract of *B. hispida* exhibited the highest activity, with an EC_{50} value of 0.44 ± 0.03 mg/mL (Table 2). This was followed by the chloroform and ethyl acetate extracts of *L. siceraria*, the ethyl acetate extract of *C. maxima*, and the chloroform extract of *S. edule*, with no significant differences ($p < 0.05$).

All of the extracts with promising activities were found to have low TPC (Table 2). Therefore, the results revealed the insignificant contribution of TPC to the chelating power. In addition, the metal chelating ability of polyphenols has been suggested to be related to the presence of an *o*-dihydroxy moiety in their chemical structures or to catechol or galloyl groups.[38] Several compounds that were previously isolated from these fruit vegetables (Table 1), such as astilbin and catechin from *B. hispida*; caffeic acid, isoorientin, and isoquercitrin from *L. siceraria*; catechin, epicatechin, and chlorogenic acid from *M. charantia*; and luteolin 7-*O*-rutinoside from *S. edule*, have *o*-dihydroxy or catechol groups in their chemical structures, whereas gallic acid (a 3,4,5-trihydroxybenzoic acid), which was found at high concentration in *M. charantia*, bears a galloyl moiety.

DPPH free radical scavenging and ferric reducing assays were used to measure the direct involvement of an extract as a primary antioxidant. An extract with antioxidant activity may react with DPPH radicals (purple in color) and convert them to stable diamagnetic molecules (diphenylpicrylhydrazine, which is yellow in color). As shown in Table 2, ethyl acetate is the best solvent for extracting DPPH free radical scavenging constituents from *C. maxima*, *S. edule*, and *T. cucumerina*. The highest activity indicated by the ethyl acetate extract of *S. edule* (951.73 ± 29.14 mM TE/g extract) can be associated with its high TPC (22.69 ± 0.15 mg GAE/g extract; Table 2). This was followed by the methanol extracts of *L. acutangula* (698.14 ± 19.87 mM TE/g extract) and *L. siceraria* (649.32 ± 17.70 mM TE/g extract). This is the first antioxidant evaluation using *S. edule* and *L. acutangula* dried pulp samples because previous antioxidant studies of these two fruits were focused on vegetative parts and fresh fruit samples (Table 1). The scavenging activity of *L. acutangula* and *L. siceraria* was also influenced by the increasing polarity of extracting solvents as the highest activity was observed in the most polar extract (i.e., methanol). The higher activity of the methanol extract of *L. siceraria* was also in line with its TPC (20.85 ± 0.12 mg GAE/g extract; Table 2). Polyphenolics and other chemical constituents that were identified from the fruit (Table 1) might be responsible for the activity.

Due to the low TPC in the methanol extract of *L. acutangula*, it is suggested that its higher scavenging activity might be elicited by a strong antioxidant compound in the extract that could potentiate the activity at a very low concentration. However, such a compound has yet to be isolated from this fruit. Nevertheless, the activity might also be correlated with its moderate total carotenoid, β-carotene,[39] vitamin A, thiamin (vitamin B1), and vitamin C contents (Table 1).[40] Furthermore, higher activities of the chloroform extracts of *B. hispida* and *M. charantia* were also not correlated with their TPC values and might be associated with synergistic effects of the chloroform-soluble phytochemicals. Several sequential extraction studies of dried fruits also revealed the highest recovery of radical scavenging compounds from the chloroform extracts.[31,41] As shown in Table 1, momordicoside K (a cucurbitane-type triterpenoid glycoside) is an antioxidant constituent that was isolated from the dichloromethane extract of fresh fruit of *M. charantia*.[42]

In the ferric reducing assay, the presence of an antioxidant in an extract may lead to the reduction of the Fe(III)/ferric cyanide complex to form ferrous iron [Fe(II)] by donating an electron. As indicated in Table 2, higher activities were mostly shared by the methanol and ethyl acetate extracts. This might be due to the efficiency of these solvents in extracting polar reducing power compounds compared to the other two extracting solvents. The methanol extract of *M. charantia* was found to demonstrate the highest activity (692.56 ± 43.38 mM AscAE/g extract). Catechin, gallic acid, gentisic acid, chlorogenic acid, and epicatechin are major phenolic constituents that have been quantified from the fruit extracts (Table 1). The methanol extract was followed by the chloroform (367.77 ± 9.97 mM AscAE/g extract) and ethyl acetate extracts (348.19 ± 22.28 mM AscAE/g extract) of *L. acutangula*, with no significant difference ($p < 0.05$). Moreover, in accordance with having TPCs of >20 mg GAE/g extract (Table 2), the chloroform extract of *L. acutangula*, the ethyl acetate extracts of *B. hispida* and *S. edule*, and the methanol extract of *L. siceraria* also exhibited among the greatest reducing capacities compared to their other extracts. Furthermore, for the fruits with activities best extracted by methanol, their activities were successively increased due to the increase in polarity of the extracting solvents.

α-Glucosidase Inhibitory Activity. Table 2 also shows the α-glucosidase inhibitory activity of the extracts. The extracts were tested at an initial concentration of 10 mg/mL (a final concentration of 1.25 mg/mL). Only six extracts were found having above 50% of activity. The ethyl acetate extract of *M. charantia* showed the highest percentage of α-glucosidase inhibition activity (66.64 ± 2.94%). The antidiabetic activity of this fruit had been scientifically proven by various pharmacological evaluations.[6] Among the sequential extracts, the ethyl acetate extract of *M. charantia* was found to have the highest recovery of α-glucosidase inhibitors. Thus, sequential extraction using hexane followed by chloroform and ethyl acetate could be considered as the most appropriate procedure for extracting α-glucosidase inhibitors from *M. charantia*.

This comparative evaluation confirmed the contribution of α-glucosidase inhibitors in enhancing the antidiabetic potential of the extract. Several antidiabetic compounds with low ability to inhibit α-glucosidase had been isolated from the fruit.[43,44] According to a study by Kwon et al.,[45] phenolic compounds such as protocatechuic acid, vanillic acid, caffeic acid, and catechin, which were identified from *M. charantia* fruit (Table 1), have high α-glucosidase inhibitory activity. Therefore, these results suggest that phenolic compounds in *M. charantia* may play an important role in the inhibition of α-glucosidase. The ethyl acetate extract of *T. cucumerina* (61.91 ± 1.96%) and the methanol extract of *L. siceraria* (61.25 ± 2.57%) were ranked top two, when analyzed by the assay. Their activities are in line with their high TPCs (17.45 ± 0.27 μg GAE/mg extract and 20.85 ± 0.12 μg GAE/mg extract).

This is the first study highlighting the antidiabetic activity of *T. cucumerina* fruit. Earlier studies had reported the hypoglycemic activity of the seed and leaf extracts.[46,47] Meanwhile, the presence of various classes of phenolic compounds in *L. siceraria* fruit (Table 1) may be responsible for the activity of the methanol and ethyl acetate extracts. The results also revealed lower α-glucosidase inhibitory activity of the sequential extracts of *L. acutangula* in comparison with the results obtained by Pimple et al.[7] using the crude water and methanol extracts.

The results obtained from this study may provide more data to the compilation of α-glucosidase inhibitors from plants by Benalla et al.[8] *L. acutangula* and *M. charantia* are no longer the representatives of the cucurbit fruits that are reputed for having α-glucosidase inhibitory activity, but the fruit extracts of *L. siceraria*, *S. edule*, and *T. cucumerina* also demonstrated positive results.

Correlation between Total Phenolic Content and Bioactivities. As indicated in Table 3, the highest positive linear correlation ($r = 0.99$) was observed between the TPCs and DPPH free radical scavenging activities of the extracts of *S. edule*. Similarly, the TPCs in the extracts of *B. hispida* were notably correlated with their reducing power values ($r = 0.97$). These findings strongly suggested that the antioxidant activities of these two fruits were related to their TPCs. Several flavones have been identified from different plant parts of *S. edule*, with trace amounts of vicenin 2, vitexin, and luteolin 7-*O*-rutinoside in the methanol extract of the fruit (Table 1).[34] Meanwhile, three flavonoids isolated from *B. hispida* fruit pulp were astilbin, catechin, and naringenin (Table 1).[48] These compounds might

Table 3. Pearson's Correlation Coefficients (r) between Total Phenolic Contents and Antioxidant Activities (Obtained from Three Independent Tests) within the Extracts Obtained from Each Vegetable[a]

	metal chelating activity	DPPH	reducing power	α-glucosidase inhibition
Benincasa hispida	0.43	−0.69	0.97*	0.04
Cucurbita maxima	−0.62	−0.86	−0.34	−0.89
Lagenaria siceraria	0.41	0.91	0.88	0.82
Luffa acutangula	0.73	−0.22	0.79	0.76
Momordica charantia	0.60	0.76	0.34	0.80
Sechium edule	0.47	0.99*	0.91	0.83
Trichosanthes cucumerina	−0.14	0.81	0.37	0.86

[a]An asterisk (*) indicates significance at $p < 0.05$.

act synergistically or individually to enhance the activities of the extracts. The scavenging, reducing, and α-glucosidase inhibitory activities of *L. siceraria* and *S. edule* extracts were found to be strongly correlated with their TPCs (with r values >0.80).

In contrast, the highest correlation between TPC and chelating capacity, with a lower r value of 0.73, was obtained among the extracts of *L. acutangula*. The low and inverse correlations of TPC with this antioxidant activity proved that the mechanism of action of this assay was not influenced by the TPC, but may possibly rely on the structure−activity relationships of the active compounds.[38] For the extracts of *C. maxima*, inverse correlations were observed between their TPCs and bioactivities. This means that there were compounds other than phenolics that possibly contributed to the activities. As indicated in Table 1, the major contents of total carotenoids, β-carotene,[39] and vitamin A[40] and the presence of a water-soluble polysaccharide[14] in *C. maxima* fruit might also account for the increased antioxidant activities. Our findings also assisted in verifying the weak to moderate correlations between the TPCs and antioxidant activities of *M. charantia*[16] and *T. cucumeria* as previously reported.[10] However, their TPCs were well correlated with the α-glucosidase inhibitory activities (with r values >0.80). A study by Wongsa et al.[49] found a higher contribution of TPC than individual phenolic (caffeic acid and *p*-coumaric acid) in increasing the potential inhibition against α-glucosidase of 30 culinary herbs commonly consumed in Thailand.

Different reports were found in the literature whereby some authors suggested correlations between TPCs and antioxidant activities, but others found no such relationship.[16,31,32] These variations in correlations could be due to the possible interaction (either synergistic or antagonistic) of diverse types and relative amounts of phytochemicals in the extracts that might have led to different responses in different antioxidant assays.[28]

Fractionation of the Methanol Extract of *L. siceraria* and the Ethyl Acetate Extract of *S. edule*. The results in Table 3 reveal a strong correlation between TPC and primary antioxidant activity (measured by the DPPH and ferric reducing assays) and the α-glucosidase inhibitory activity of extracts obtained from *L. siceraria* and *S. edule* pulps. Among their extracts, the methanol extract of *L. siceraria* and the ethyl acetate extract of *S. edule* with the highest TPCs and activities (Table 2) were further analyzed. Four fractions were separated from each extract and were subjected to DPPH, ferric reducing, and α-glucosidase inhibitory tests. The results are shown in Table 4. Fraction LS3 from the methanol extract of *L. siceraria* and fraction SE4 from the ethyl acetate extract of *S. edule* with the highest activities in all assays were further purified.

Isoquercitrin (quercetin-3-*O*-glucoside), which was previously reported by Gangwal et al.[33] in *L. siceraria*, was purified and identified from fraction LS3, whereas gallic acid was identified from fraction SE4. The identities of the compounds were confirmed by cochromatographic comparisons with authentic markers (using various mobile phases on paper chromatography and UPLC). The R_f values in paper chromatographies, UPLC retention times, and UV spectra of the compounds were found to be similar to those of the markers or standards. After spiking with the standard (1:1), the peak area on the UPLC chromatogram and the absorbance value of the UV spectrum (detected by photodiode array

Table 4. R_f Values and Colors of Bands Developed in Paper Chromatograms of Fractions and Compounds Isolated from the Methanol Extract of *Lagenaria siceraria* and the Ethyl Acetate Extract of *Sechium edule* and Their Antioxidant Activities[a]

	R_f value (×100) in BAW	color (in long-wave UV light)	DPPH (mM TE/g sample)	reducing power (mM AscAE/g sample)	α-glucosidase inhibition (%)
methanol extract of *Lagenaria siceraria*			625.24 ± 19.88f	337.33 ± 12.09f	61.25 ± 2.57b
fraction LS1	30	yellow	171.24 ± 3.28h	226.70 ± 5.66g	na
fraction LS2	55	blue	606.12 ± 11.02f	636.12 ± 14.29e	na
fraction LS3	65	dark	801.76 ± 10.24e	1722.92 ± 12.86d	63.90 ± 6.00b
fraction LS4	90	blue	267.64 ± 8.58gh	349.12 ± 3.93f	na
isoquercitrin (isolated from fraction LS3)	65	dark	3128.40 ± 22.71b	2536.32 ± 16.45b	83.03 ± 0.68a
ethyl acetate extract of *Sechium edule*			946.88 ± 22.24d	312.39 ± 7.49f	51.49 ± 2.13c
fraction SE1	30	yellow	286.08 ± 15.12gh	28.11 ± 2.08h	na
fraction SE2	40	dark	302.36 ± 11.92g	181.36 ± 5.50g	na
fraction SE3	60	dark	349.32 ± 10.92g	401.71 ± 4.16f	na
fraction SE4	75	blue	1404.04 ± 14.68c	2132.80 ± 17.79c	58.76 ± 3.45b
gallic acid (isolated from fraction SE4)	75	blue	5371.20 ± 17.07a	4285.54 ± 11.76a	35.07 ± 0.98d

[a]Values are means ± standard deviations of triplicate analyses. Values followed by different letters are significantly different ($p < 0.05$) as measured by Duncan's test. na, no activity.

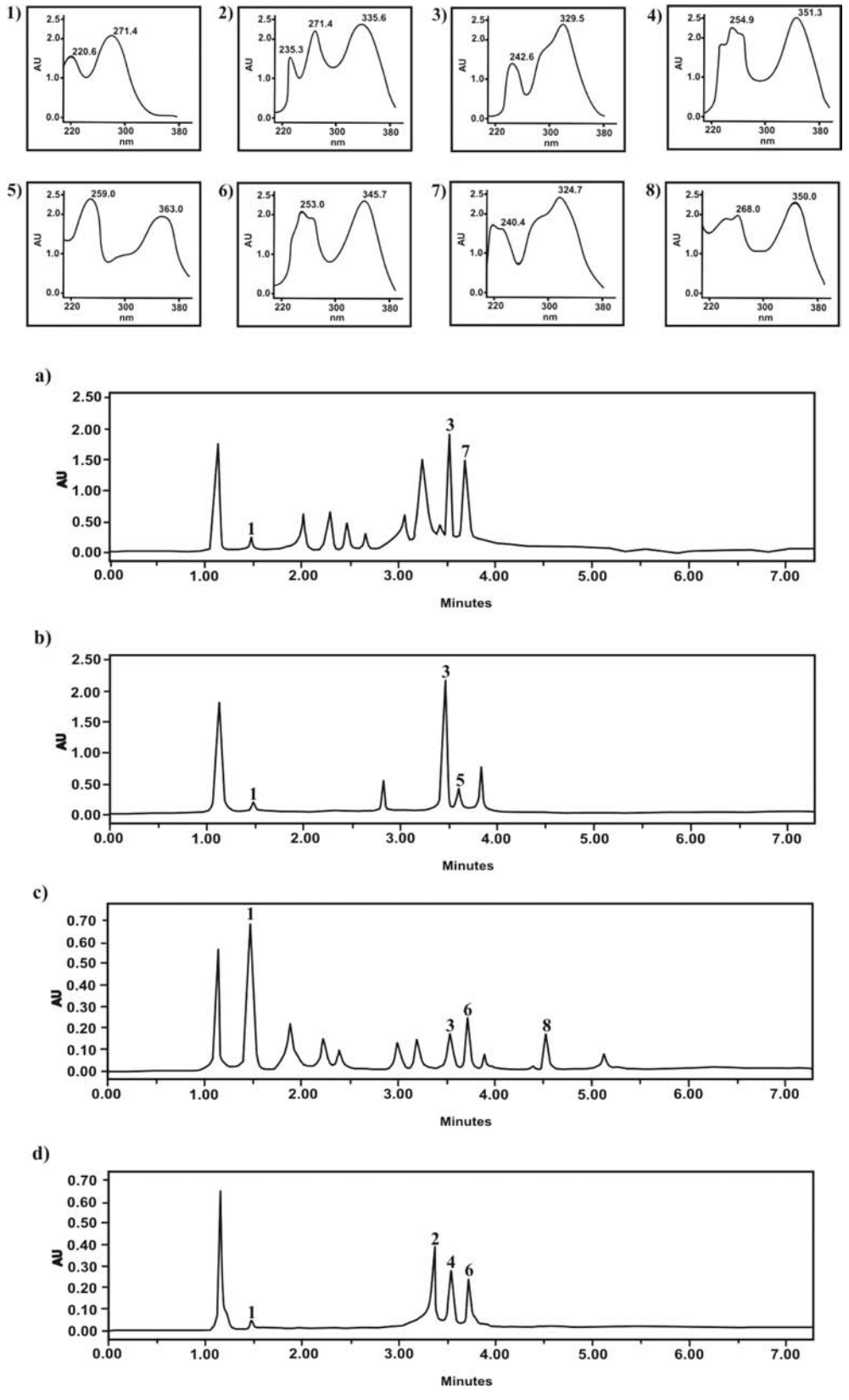

Figure 1. UPLC chromatograms of (a) ethyl acetate extract of *Lagenaria siceraria* (at 280 nm), (b) methanol extract of *Lagenaria siceraria* (at 350 nm), (c) ethyl acetate extract of *Sechium edule* (at 280 nm), and (d) methanol extract of *Sechium edule* (at 350 nm). The UV spectra of peaks 1−8 assessed by a photodiode array detector are also indicated.

detector) of each isolated compound were found to be increased. No effect on the maximum wavelength (λ_{max}, nm) of each UV spectrum was observed. We believe that this is the first report on the occurrence of gallic acid in an *S. edule* fruit extract. A study had found only a trace amount of three glycosidic flavones in the methanol extract of *S. edule* fruit.[33] In

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Table 5. Contents of Phenolic Constituents in Extracts of *Lagenaria siceraria* and *Sechium edule*[a]

	1 (gallic acid)	2 (vitexin)	3 (caffeic acid)	4 (luteolin derivative 1)[b]	5 (isoquercitrin)	6 (luteolin derivative 2)[b]	7 (caffeic acid derivative)[c]	8 (luteolin)
retention time (min)	1.45	3.38	3.49	3.53	3.62	3.71	3.76	4.50
Lagenaria siceraria								
hexane								
chloroform							52.39 ± 3.92	
ethyl acetate	8.99 ± 0.71		97.69 ± 5.35				63.25 ± 3.28	
methanol	5.70 ± 0.59		95.37 ± 3.83		13.70 ± 1.23			
Sechium edule								
hexane								
chloroform								
ethyl acetate	93.41 ± 10.86		7.22 ± 0.48			31.48 ± 3.57		24.58 ± 2.01
methanol	4.82 ± 0.32	5.35 ± 0.14		10.49 ± 0.81		10.03 ± 0.83		

[a]Values are means ± standard deviations (mg/100 g extract, except as noted) of triplicate analyses. [b]Expressed as luteolin equivalents. [c]Expressed as caffeic acid equivalents.

this study, the flavones in the ethyl acetate extract appeared as dark bands (in long-wave UV light) and were separated as SE2 and SE3. However, the antioxidant activities of these flavone fractions were significantly lower than that of fraction SE4.

The antioxidant activities of the isolated compounds were found to be higher than those of their original extracts and fractions (Table 4). Thus, they could be considered as major contributors to the antioxidant activities of the extracts. The highest activity of gallic acid in both assays could be related to its chemical structure containing galloyl groups. In comparison, isoquercitrin is a glycosidic quercetin with glycosylation of the glucoside at the 3-position of the C-ring, which has been found to decrease its efficiency as an antioxidant compared with that of quercetin.[50] Its quercetin structure with antioxidant functional groups such as *o*-dihydroxy groups at the B-ring and a 2,3-double bond in conjugation with a 4-oxo function are essential structural elements that strengthen its activities.[51]

The isolated compounds were also tested for their α-glucosidase inhibitory activity, and the results revealed the highest inhibitory activity of isoquercitrin from the methanol extract of *L. siceraria*. According to Shibano et al.,[52] the α-glucosidase inhibiting potential of isoquercitrin is comparable with that of 1-deoxynojirimycin (a commercial α-glucosidase inhibitor). They also found higher activity of this compound compared with other glycosidic flavonols and flavones (such as isorhamnetin 3-*O*-rutinoside and vitexin). Thus, isoquercitrin is identified as the major contributor to the α-glucosidase inhibitory activity of the methanol extract of *L. siceraria*. Gallic acid (with higher antioxidant activities) showed lower ability as an α-glucosidase inhibitor than the ethyl acetate extract of *S. edule*. Thus, the higher activity of the extract might be contributed by the synergistic effect of the compounds in the extract. Gallic acid was previously reported to have low α-glucosidase activity in comparison with other phenolic compounds.[45]

Quantification of Phenolic Constituents in *L. siceraria* and *S. edule* Extracts. As indicated in Table 1, many polyphenolics have been identified from extracts of *L. siceraria*[18,33,37] and *S. edule* fruits.[34] Thus, some of the polyphenolics were used as standards. Figure 1 shows the UPLC chromatograms of the ethyl acetate extracts of *L. siceraria* and *S. edule* (at 280 nm), the methanol extracts of *L. siceraria* and *S. edule* (at 350 nm), and the UV spectra of eight labeled peaks determined by the photodiode array detector. Only five peaks (1, 2, 3, 5, and 8) were positively identified by comparisons with standards. The UV spectra of peaks 4 and 6 are very similar to those derived from luteolin and were quantified as luteolin equivalents. The UV spectrum of peak 7 is most likely a derivative of caffeic acid and was quantified as caffeic acid equivalents. The contents of these compounds in the extracts are summarized in Table 5.

No phenolic compounds were detected in the hexane extracts of both samples and the chloroform extract of *S. edule*. Only peak 7 (caffeic acid derivative; UV λ_{max} 240.4 and 324.7 nm) was detected in the chloroform extract of *L. siceraria*. Caffeic acid (peak 3; UV λ_{max} 242.6 and 329.5 nm) and the caffeic acid derivative (peak 7) were measured at the highest level from the ethyl acetate extract of *L. siceraria* (Figure 1a; Table 5). Mohan et al.[18] had isolated caffeic acid and three caffeic acid derivatives from the ethyl acetate extract of *L. siceraria* (Table 1). One of the derivatives was 3,4-dimethoxycinnamic acid, which is derived from caffeic acid (a 3,4-dihydroxycinnamic acid) by the substitution of the *o*-dihydroxy at the 3- and 4-positions with methoxy groups. The other two derivatives originate from the coupling of caffeic acid with the glucosidic phenolic acids *p*-hydroxybenzoic acid [to form (*E*)-4-(hydroxymethyl)phenyl-6-*O*-caffeoyl-β-D-glucopyranoside] and protocatechuic acid [to form 1-(2-hydroxy-4-hydroxymethyl)phenyl-6-*O*-caffeoyl-β-D-glucopyranoside]. The caffeic acid derivative that was detected in this study is most likely one of these. The UV spectrum and retention time of peak 5 in the UPLC chromatogram of the methanol extract of *L. siceraria* (Figure 1b) corresponded to that of the isoquercitrin standard (UV λ_{max} 259 and 363 nm). However, the low content of this compound was measured from the extract and caffeic acid (peak 3) was also identified as the major compound. The catechol moiety of isoquercitrin and caffeic acid might be the key contributor to the antioxidant activity of this extract.

The ethyl acetate extract of *S. edule* (Figure 1c) was found to have the highest content of gallic acid (peak 1; UV λ_{max} 220.6 and 271.4 nm). Low content of caffeic acid (peak 3) and moderate contents of luteolin (peak 8; UV λ_{max} 268 and 350 nm) and luteolin derivative 2 (peak 6; UV λ_{max} 253 and 345.7

nm) were also quantified from the extract. Andarwulan et al.[20] also quantified a low content of caffeic acid in a hydrolyzed leaf extract of this plant. As indicated in Table 5, low contents of gallic acid (peak 1), vitexin (peak 2; UV λ_{max} 271.4 and 335.6 nm), and two luteolin derivatives (peak 4; UV λ_{max} 254.9 and 351.3 nm and peak 6) were also detected from the methanol extract of *S. edule* (Figure 1d). Luteolin derivative 1 (peak 4) is most likely luteolin 7-*O*-rutinoside (an *O*-glycosidic flavone), which was detected in a methanol extract of *S. edule* fruit.[34] The results in Table 5 also revealed 100% recovery of this compound in the methanol extract, which was similar to that of another *C*-glycosidic flavone (vitexin; peak 2) and an *O*-glycosidic flavonol (isoquercitrin; peak 5). Moreover, 100% recovery of luteolin (peak 8) was observed in the ethyl acetate extract of *S. edule*. Luteolin derivative 2 (peak 6), which was 76% recovered by ethyl acetate, might be a methylated luteolin. Two methylated luteolins that were previously detected in the leaves and stems of *S. edule* are chrysoeriol (3′-methoxyluteolin) and diosmetin (4′-methoxyluteolin).[34] Furthermore, the recoveries of gallic acid (peak 1), caffeic acid (peak 3), and the caffeic acid derivative (peak 7) were also found to be higher in ethyl acetate extracts than in extracts acquired using methanol.

The results obtained from this study revealed the effect of sequential extraction using ascending polarity of extracting solvents on the TPCs and antioxidant and α-glucosidase inhibitory activities of the fruit vegetables. Although the pulps of these vegetables were obtained from the same family (Cucurbitaceae) at similar commercial maturity stages (unripe), different solvent systems were required to optimize the recovery of their TPCs and antioxidant compounds. The correlation analysis suggested a strong relationship between TPC and primary antioxidant and α-glucosidase inhibitory activities of extracts obtained from *L. siceraria* and *S. edule* fruits. On the basis of the results obtained from the bioactivity-guided fractionation of two phenolic-rich extracts (the methanol extract of *L. siceraria* and the ethyl acetate extract of *S. edule*), two phenolic compounds (isoquercitrin and gallic acid), isolated from the respective extracts, were found to be responsible for enhancing the antioxidant activities of the extracts. Isoquercitrin was also identified as an α-glucosidase inhibitor. Gallic acid was measured as the most abundant phenolic in the ethyl acetate extract of *S. edule*, whereas only a low content of isoquercitrin was detected in the methanol extract of *L. siceraria*. As these vegetables are widely available, the antioxidant information obtained by this study would be useful for promoting their consumption and for further epidemiology research.

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Notes

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REFERENCES

(1) Ong, H. C. *Sayuran: Khasiat Makanan & Ubatan*; Utusan Publication and Distributors: Kuala Lumpur, Malaysia, 2004; pp 2−9.

(2) Him-Che, Y. *Handbook of Chinese Herbs and Formulas*; Institute of Chinese Medicine: Los Angeles, CA, USA, 1985; pp 120−201.

(3) Covington, M. B. Traditional Chinese medicine in the treatment of diabetes. *Diabetes Spectr.* **2001**, *14*, 154−159.

(4) Andrade-Cetto, A.; Heinrich, M. Mexican plants with hypoglycaemic effect used in the treatment of diabetes. *J. Ethnopharmacol.* **2005**, *99*, 325−348.

(5) Zhang, Y.; Yao, H. Study on the effect of hypoglycemia of different type pumpkin. *J. Chin. Food Sci.* **2002**, *23*, 118−120.

(6) Dhiman, K.; Gupta, A.; Sharma, D. K.; Gill, N. S.; Goyal, A. A review on the medicinally important plants of the family Cucurbitaceae. *Asian J. Clin. Nutr.* **2012**, *4*, 16−26.

(7) Pimple, B. P.; Kadam, P. V.; Patil, M. J. Antidiabetic and antihyperlipidemic activity of *Luffa acutangula* fruit extracts in *Streptozotocin* induced NIDDM rats. *Asian J. Pharm. Clin. Res.* **2011**, *4*, 156−163.

(8) Benalla, W.; Bellahcen, S.; Bnouham, M. Antidiabetic medicinal plants as a source of α-glucosidase inhibitors. *Curr. Diabetes Rev.* **2010**, *6*, 247−254.

(9) Huang, H. Y.; Huang, J. J.; Tso, T. K.; Tsai, Y. C.; Chang, C. K. Antioxidant and angiotension-converting enzyme inhibition capacities of various parts of *Benincasa hispida* (wax gourd). *Nahrung* **2004**, *48*, 230−233.

(10) Adebooye, O. C. Phyto-constituents and antioxidant activity of the pulp of snake tomato (*Trichosanthes cucumerina* L.). *Afr. J. Tradit. Complem.* **2008**, *5*, 173−179.

(11) Kubola, J.; Siriamornpun, S. Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia* L.) leaf, stem and fruit fraction extracts *in vitro*. *Food Chem.* **2008**, *110*, 881−890.

(12) Wu, S.-J.; Ng, L.-T. Antioxidant and free radical scavenging activities of wild bitter melon (*Momordica charantia* Linn var. *abbreviata* Ser.) in Taiwan. *LWT−Food Sci. Technol.* **2008**, *41*, 323−330.

(13) Erasto, P.; Mbwambo, Z. H. Antioxidant activity and HPTLC profile of *Lagenaria siceraria* fruits. *Tanzan. J. Health Res.* **2009**, *11*, 79−83.

(14) Nara, K.; Yamaguchi, A.; Maeda, N.; Koga, H. Antioxidative activity of water soluble polysaccharide in pumpkin fruits (*Cucurbita maxima* Duchesne). *Biosci., Biotechnol., Biochem.* **2009**, *73*, 1416−1418.

(15) Attarde, D. L.; Kadu, S. S.; Chaudhari, B. J.; Kale, S. S.; Bhamber, R. S. *In vitro* antioxidant activity of pericarp of *Cucurbita maxima* Duch. ex Lam. *Int. J. PharmTech Res.* **2010**, *2*, 1533−1538.

(16) Horax, R.; Hettiarachchy, N.; Chen, P. Extraction, quantification, and antioxidant activities of phenolics from pericarp and seeds of bitter melons (*Momordica charantia*) harvested at three maturity stages (immature, mature, and ripe). *J. Agric. Food Chem.* **2010**, *58*, 4428−4433.

(17) Reddy, B. P.; Mohan, S. V.; Sarma, P. N. The protective role of *Luffa acutangula* fruit methanolic fraction against t-BHP induced oxidative damage in human erythrocytes. *Curr. Trends Biotechnol. Pharm.* **2011**, *5*, 1073−1082.

(18) Mohan, R.; Birari, R.; Karmase, A.; Jagtap, S.; Bhutani, K. K. Antioxidant activity of a new phenolic glycoside from *Lagenaria siceraria* Stand. fruits. *Food Chem.* **2012**, *132*, 244−251.

(19) Ordonez, A. A. L.; Gomez, J. D.; Vattuone, M. A.; Isla, M. I. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food Chem.* **2006**, *97*, 452−458.

(20) Andarwulan, N.; Kurniasih, D.; Apriady, R. A.; Rahmat, H.; Roto, A. V.; Bolling, B. W. Polyphenols, carotenoids, and ascorbic acid in underutilized medicinal vegetables. *J. Funct. Foods* **2012**, *4*, 339−347.

(21) Ansari, N. M.; Houlihan, L.; Hussain, B.; Pieroni, A. Antioxidant activity of five vegetables traditionally consumed by South-Asian migrants in Bradford, Yorkshire, UK. *Phytother. Res.* **2005**, *19*, 907−911.

(22) Chanwitheesuk, A.; Teerawutgulrag, A.; Rakariyatham, N. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. *Food Chem.* **2005**, *92*, 491−497.

(23) Yang, R.-Y.; Tsou, S. C. S.; Lee, T.-C.; Wu, W.-J.; Hanson, P. M.; Kuo, G.; Engle, L. M.; Lai, P.-Y. Distribution of 127 edible plant

species for antioxidant activities by two assays. *J. Sci. Food Agric.* **2006**, *86*, 2395−2403.
(24) Stangeland, T.; Remberg, S. F.; Lye, K. A. Total antioxidant activity in 35 Ugandan fruits and vegetables. *Food Chem.* **2009**, *113*, 85−91.
(25) Gacche, R. N.; Kabaliye, V. N.; Dhole, N. A.; Jadhav, A. D. Antioxidant potential of selected vegetables commonly used in diet in Asian subcontinent. *Indian J. Nat. Prod. Resour.* **2010**, *1*, 306−313.
(26) Isabelle, M.; Lee, B. L.; Lim, M. T.; Koh, W.-P.; Huang, D.; Ong, C. N. Antioxidant activity and profiles of common vegetables in Singapore. *Food Chem.* **2010**, *120*, 993−1003.
(27) Raghu, K. L.; Ramesh, C. K.; Srinivasa, T. R.; Jamuna, K. S. DPPH scavenging and reducing power properties in common vegetables. *Res. J. Pharm. Biol. Chem. Sci.* **2010**, *1*, 399−406.
(28) Sreeramulu, D.; Raghunath, M. Antioxidant and phenolic content of roots, tuber and vegetables commonly consumed in India. *Food Res. Int.* **2010**, *43*, 1017−1020.
(29) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. *Method Enzymol.* **1999**, *299*, 152−178.
(30) Ooi, K. L.; Muhammad, T. S. T.; Tan, M. L.; Sulaiman, S. F. Cytotoxic, apoptotic and anti-α-glucosidase activities of 3,4-di-*O*-caffeoyl quinic acid, an antioxidant isolated from the polyphenolic-rich extract of *Elephantopus mollis* Kunth. *J. Ethnopharmacol.* **2011**, *135*, 685−695.
(31) Sulaiman, S. F.; Yusoff, N. A. M.; Eldeen, I. M.; Seow, E. M.; Sajak, A. A. A.; Supriatno; Ooi, K. L. Correlation between total phenolic and mineral contents with antioxidant activity of eight Malaysian bananas (*Musa* sp.). *J. Food Compos. Anal.* **2011**, *24*, 1−10.
(32) Zhuang, Y.; Chen, L.; Sun, L.; Cao, J. Bioactive characteristics and antioxidant activities of nine peppers. *J. Funct. Foods* **2012**, *4*, 331−338.
(33) Gangwal, A.; Parmar, S. K.; Sheth, N. R. Triterpenoid, flavonoids and sterols from *Lagenaria siceraria* fruits. *Pharm. Lett.* **2010**, *2*, 307−317.
(34) Siciliano, T.; De Tommasi, N.; Morelli, I.; Braca, A. Study of flavonoids of *Sechium edule* (Jacq) Swartz (Cucurbitaceae) different edible organs by liquid chromatography photodiode array mass spectrometry. *J. Agric. Food Chem.* **2004**, *52*, 6510−6515.
(35) Liu, X.; Cui, C.; Zhao, M.; Wang, J.; Luo, W.; Yang, B.; Jiang, Y. Identification of phenolics in the fruit of emblica (*Phyllanthus emblica* L.) and their antioxidant activities. *Food Chem.* **2008**, *109*, 909−915.
(36) Prasad, K. N.; Chew, L. Y.; Khoo, H. E.; Kong, K. W.; Azlan, A.; Ismail, A. Antioxidant capacities of peel, pulp, and seed fractions of *Canarium odontophyllum* Miq. Fruit. *J. Biomed. Biotechnol.* **2010**, 871379.
(37) Krauze-Baranowska, M.; Cisowski, W. Isolation and identification of *C*-glycosides flavone from *Lagenaria siceraria* L. *Acta Pol. Pharm.* **1995**, *52*, 137−139.
(38) Andjelković, M.; Camp, J. V.; Meulenaer, D. B.; Depaemelaere, G.; Socaciu, C.; Verloo, M.; Verhe, R. Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. *Food Chem.* **2006**, *98*, 23−32.
(39) Kandlakunta, B.; Rajendran, A.; Thingnganing, L. Carotene content of some common (cereals, pulses, vegetables, spices and condiments) and unconventional sources of plant origin. *Food Chem.* **2008**, *106*, 85−89.
(40) Rahman, A. H. M. M.; Anisuzzaman, M.; Ferdous, A.; Rafiul Islam, A. K. M.; Naderuzzaman, A. T. M. Study of nutritive value and medicinal uses of cultivated cucurbits. *J. Appl. Sci. Res.* **2008**, *4*, 555−558.
(41) Sahreen, S.; Khan, M. R.; Khan, R. A. Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. *Food Chem.* **2010**, *122*, 1205−1211.
(42) Lin, K.-W.; Yang, S.-C.; Lin, C.-N. Antioxidant constituents from stems and fruits of *Momordica charantia*. *Food Chem.* **2011**, *127*, 609−614.
(43) Nhiem, N. X.; Kiem, P. V.; Minh, C. V.; Ban, N. K.; Cuong, N. X.; Tung, N. H.; Ha, L. M.; Kim, Y. H. α-Glucosidase inhibition properties of cucurbitane-type triterpene glycosides from the fruits of *Momordica charantia*. *Chem. Pharm. Bull.* **2010**, *58*, 720−724.
(44) Mata, R.; Cristians, S.; Escandón-Rivera, S.; Juárez-Reyes, K.; Rivero-Cruz, I. Mexican antidiabetic herbs: valuable sources of inhibitors of α-glucosidases. *J. Nat. Prod.* **2013**, *76*, 468−483.
(45) Kwon, Y.-I.; Apostolidis, E.; Shetty, K. Inhibitory potential of wine and tea against α-amylase and α-glucosidase for management of hyperglycemia linked to type 2 diabetes. *J. Food Biochem.* **2008**, *32*, 15−31.
(46) Kar, A.; Choudhary, B. K.; Bandyopadhyay, N. G. Comparative evaluation of hypoglycemic activity of some Indian medicinal plants in alloxan diabetic rats. *J. Ethnopharmacol.* **2003**, *84*, 105−108.
(47) Kirana, H.; Srinivasan, B. P. *Trichosanthes cucumerina* Linn. improves glucose tolerance and tissue glycogen in non insulin dependent diabetes mellitus induced rats. *Indian J. Pharmacol.* **2008**, *40*, 103−106.
(48) Du, Q.; Zhang, Q.; Ito, Y. Isolation and identification of phenolic compounds in fruit of *Benincasa hispida* by HSCCC. *J. Liq. Chromatogr. Relat. Technol.* **2005**, *28*, 137−144.
(49) Wongsa, P.; Chaiwarit, J.; Zamaludien, A. *In vitro* screening of phenolic compounds, potential inhibition against α-amylase and α-glucosidase of culinary herbs in Thailand. *Food Chem.* **2012**, *131*, 964−971.
(50) Firuzi, O.; Lacanna, A.; Petrucci, R.; Marrosu, G.; Saso, L. Evaluation of the antioxidant activity of flavonoids by "ferric reducing antioxidant power" assay and cyclic voltammetry. *Biochim. Biophys. Acta* **2005**, *1721*, 174−184.
(51) Soobrattee, M. A.; Neergheen, V. S.; Luximon-Ramma, A.; Aruoma, O. I.; Bahorun, T. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutat. Res.−Fundam. Mol. Mech. Mutagen.* **2005**, *579*, 200−213.
(52) Shibano, M.; Kakutani, K.; Taniguchi, M.; Yasuda, M.; Baba, K. Antioxidant constituents in dayflower (*Commelina communis* L.) and their α-glucosidase inhibitory activity. *J. Nat. Med.* **2008**, *62*, 349−353.
(53) Dragovic-Uzelac, V.; Delonga, K.; Levaj, B.; Djakovic, S.; Pospisil, J. Phenolic profiles of raw apricots, pumpkins and their purees in the evaluation of apricot nectar and jam authenticity. *J. Agric. Food Chem.* **2005**, *53*, 4836−4842.
(54) Ghosh, K.; Chandra, K.; Ojha, A. K.; Sarkar, S.; Islam, S. S. Structural identification and cytotoxic activity of a polysaccharide from the fruits of *Lagenaria siceraria* (Lau). *Carbohydr. Res.* **2009**, *344*, 693−698.