



Antioxidative and digestive enzymes inhibitory activities of 27 edible plants

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Abstract

The contents of total phenol and flavonoid of 27 edible plants as well as their antioxidative and digestive enzymes inhibitory activities were evaluated. The total phenol content of the 27 plants ranged from 1.28-46.80 mg gallic acid equivalent/g. DPPH, ABTS and reducing power assay explored that *Rubus idaeus* Linn, *Hibiscus sabdariffa* Linn. and *Crataegus pinnatifida* Bge. exhibited stronger antioxidant capacity among the 27 plants. 4 plants showed inhibitory percentage more than 50% on pancreatic lipase. 10 plants showed inhibitory percentage more than 50% on α -glucosidase. 3 plants simultaneously possess significant inhibitory activities on pancreatic lipase and α -glucosidase, which were *Alpinia officinarum* Hance, *Ginkgo biloba* Linn. leaves and *Vitis vinifera* Linn. Seeds. Strong positive correlation between total phenol content and antioxidative capacity were found. However, digestive enzymes inhibitory activity was less correlated with total phenol and total flavonoids content.

Keywords: edible plants; antioxidative activity; digestive enzymes; correlation.

Practical application: Obesity becomes seriously around the world. Plant-based food is an important part of the human diet. In the present study, the total phenol and flavonoid contents in 27 edible plants as well as their antioxidative and digestive enzymes inhibitory activities were compared. The results may have practical application in searching diet for obesity prevention without side-effects.

1 Introduction

Plant-based food is an important part of the human diet. Besides nutrients of protein, carbohydrate, vitamins and minerals, plants also contain many secondary metabolites (e.g. polyphenols, saponins, etc) which exhibit many physiological activities *in vivo* (Crozier et al., 2009). Epidemiological studies confirmed that diets rich in fruit and vegetables can reduce the incidence of many diseases, particularly, cardiovascular diseases and cancer (Mladěnka et al., 2010; Amado et al., 2011). Polyphenols, particularly flavonoids, play important role in these protective effects (Crozier et al., 2009).

Because of excessive intake of calorie and lacking of exercise, obesity and its comorbidities (e.g. cardiovascular diseases, diabetes) become seriously around the world. The energy components in the diet (e.g. fat, starch) are not absorbed directly in human gastrointestinal tract, but need be hydrolyzed by digestive enzymes first. Orlistat and acarbose, the inhibitors of lipase and α -glucosidase, respectively, are approved for long-term use in clinical on obesity and diabetes, while uncomfortable symptoms are always accompanied (Buyukhatipoglu, 2008; Holman & Dinneen, 1999). Edible plants with digestive enzymes inhibitory activity are promising resource for obesity prevention without side-effects (Fu et al., 2016). Reactive oxygen species (ROS) can be produced by oxidative metabolism *in vivo* and induced by exogenous substances, e.g. lipid peroxides. Therefore, obesity usually lead to the imbalance of oxidation-antioxidant system in the body. The accumulation of ROS in body may attack biological macromolecules and contribute to the occurrence

of many diseases (Ou et al., 2002; Gutteridge & Halliwell, 2000). Antioxidants in body defense system play a crucial role in preventing the accumulation of ROS. Therefore, increasing consumption of antioxidants in food or dietary supplements may be an available approach to maintain an adequate antioxidant status (Kaur & Kapoor, 2001; Record et al., 2001). Thus, searching for medicinal and/or edible plants with strong digestive enzymes inhibitory and antioxidative activities has gained increasing attention (Nowicka et al., 2018; Buchholz & Melzig, 2016). The anti-obesity effects of some natural extracts were explored *in vivo* studies, for instance, tea extract (Lu et al., 2019), mulberry extract (Peng et al., 2011), Microalga *Nitzschia laevis* extract (Guo et al., 2019), etc.

Accordingly, in this study, the total phenol and flavonoid contents in 27 edible plants were measured. Particularly, their antioxidative and digestive enzymes inhibitory activities were compared. Besides, the correlations among active substances contents, digestive enzymes inhibitory and antioxidant activity were discussed.

2 Materials and methods

2.1 Chemicals and reagents

27 plants were purchased from Yuci Tang store. All plants were smashed and filtered through 40 mesh sieve. α -Glucosidase (EC 3.2.1.20) from *saccharomyces cerevisiae*, α -amylase,

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2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma Co., Ltd. (St. Louis, MO, U.S.A.). Porcine pancreatic lipase was purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Rutin, 4-nitrophenyl- α -D-glucopyranoside (PNPG) and p-nitrophenyl-palmitate (PNPP) was purchased from Shanghai aladdin Biochemical Technology Co., Ltd. (Shanghai, China).

2.2 Preparation of plants extract

0.3 g of plant powers were extracted by 10 mL of 70% ethanol under ultrasound for 30 min. After filtration, the extracts were directly used for total phenol and flavonoid quantification, as well as the antioxidant capacity and digestive enzyme inhibition studies. All extracts were stored at 4 °C before analysis.

2.3 Determination of total phenol and total flavonoid

Total phenol was determined by Folin-Ciocalteu method (Singleton & Rossi, 1965). In brief, 1 mL sample extract was mixed with 0.5 mL of Folin-Ciocalteu reagent in 10 mL volumetric flask. After standing for 5 min, 1.5 mL Na_2CO_3 (10%) was added. Then, the mixture was diluted with water to the volume and incubated at 25 °C for 30 min. The absorbance was measured at 765 nm. The quantification was based on the standard curve generated by gallic acid, which was $Y=152.26X$, where Y is the absorbance, X in the concentration of gallic acid (0-0.01 mg/mL). The total phenol content in the plant was expressed as gallic acid equivalent.

The total flavonoid content was measured as rutin equivalent (Zhishen et al., 1999). Briefly, 1 mL extract was mixed with 0.3 mL NaNO_3 (5%) in 10 mL volumetric flask. After standing for 5 min, 0.3 mL AlCl_3 (10%) was added subsequently. After standing for another 5 min, 4 mL NaOH (4%) was added and then diluted to the final volume with distilled water. The absorbance was measured at 510 nm after standing for 15 min. The calibration curve of rutin was $Y=6.03X$, where Y is the absorbance, X in the concentration of rutin (0-0.20 mg/mL).

2.4 Antioxidative activity of extracts

DPPH radical scavenging assay (Król et al., 2014). Briefly, 50 μL plant extract was mixed with 200 μL DPPH solution (55 $\mu\text{g}/\text{mL}$ in 95% ethanol) in a 96 well microplate. The mixture was placed in the dark at 25 °C for 10 min. Then, the absorbance at 517 nm was read on a Thermo Microplate Spectrophotometer (Multiskan FC, USA). Ascorbic acid was used as the positive control. The scavenging activity was calculated by the following Equation 1:

$$DPPH(\%) = \frac{A_c - A_i}{A_c} \times 100 \quad (1)$$

Where A_i and A_c are the absorbance in the presence and absence of the antioxidants, respectively.

ABTS⁺ radical scavenging activity (Meng et al., 2018). Equal volume of ABTS solution (7 mM) and potassium persulfate

(4.95 mM) were mixed and placed in the dark for 16 h to obtain the ABTS^+ solution. The ABTS^+ solution was properly diluted with water to give an absorbance about 0.7 at 734 nm in a 96 well microplate (200 μL). Then, the ABTS^+ solution (200 μL) was mixed with 30 μL extract. After standing in dark for 10 min, the absorbance at 600 nm was monitored. The scavenging activity was determined according to the following Equation 2:

$$ABTS^+(\%) = \frac{A_c - A_i}{A_c} \times 100 \quad (2)$$

Where A_i and A_c are the absorbance in the presence and absence of the antioxidants, respectively.

Reducing power (Yıldırım et al., 2001). Briefly, 200 μL plant extract was mixed with 200 μL phosphate buffer (PB, 0.2 M with pH 6.6) and 200 μL potassium ferricyanide (0.3%). After incubation at 5

2.5 Digestive enzymes inhibition

The inhibitory activities of the extracts on pancreatic lipase, α -glucosidase and α -amylase were determined in accordance with our previous work (Su et al., 2020).

2.6 Statistical analysis

Data were expressed as mean \pm standard deviation of triplicates. Data analysis and plotting were performed with software of Origin 9.0 (Origin Lab Co., Northampton, MA, USA).

3 Results and discussion

3.1 Total phenol and flavonoid content in 27 edible plants

The levels of total phenol and flavonoid in 27 edible plants were presented in Table 1. The flavonoid content of the extracts ranged 2.32-187.26 mg/g. Remarkable differences were found between plants. For instance, the content of total flavonoid in *Forsythia suspensa* (Thunb.) Vahl leaves was 135.91 mg rutin equivalent/g, while in *Nymphaea tetragona* Georgi seeds, the value was only 3.84 mg/g. Similar trend was found in total phenol result. As shown in Table 1, the highest total phenol content was found in *Crataegus pinnatifida* Bge. leaves with value of 46.80 mg gallic acid equivalent/g. In general, plants with high total phenol content usually have high level of total flavonoid. Usually, the biological activities of plant are positively correlated with their content of total phenol and flavonoid (Crozier et al., 2009). The leaves of *Crataegus pinnatifida* Bge. is used as tea material and spice in China. Because of high content of total phenol, it has potential effects on inflammation and cardiovascular disease prevention (Zorniak et al., 2019). The leaves of *Forsythia suspensa* (Thunb.) Vahl exhibited effects on regulating blood lipid (Kang & Wang, 2010).

3.2 Antioxidative activity of 27 edible plants

The antioxidative capacity of 27 edible plants were assayed through DPPH, ABTS and reducing power in present study. DPPH⁺ and ABTS⁺ are coloured radical. After accepting an

Table 1. Total phenolic content, total flavonoid content, antioxidant capacity and digestive enzymes inhibition in 27 edible plants.

Scientific name	Use part	Total flavonoid (mg/g)	Total phenolic (mg/g)	Antioxidant capacity			Digestive enzyme inhibition		
				DPPH (%)	ABTS (%)	Reducing power (Abs)	pancreatic lipase	α -glucosidase	α -amylase
<i>Alpinia officinarum</i> Hance	Rhizome	68.67 ± 1.30	24.16 ± 0.10	71.45 ± 2.67	78.45 ± 2.04	2.30 ± 0.07	66.76 ± 0.83	99.33 ± 0.29	12.89 ± 2.04
<i>Ginkgo biloba</i> Linn.	Leaves	42.07 ± 1.09	7.61 ± 1.24	54.28 ± 2.05	34.33 ± 2.88	2.08 ± 0.06	64.56 ± 0.31	99.20 ± 0.28	7.96 ± 4.07
<i>Vitis vinifera</i> Linn.	Seeds	70.94 ± 2.38	28.09 ± 2.43	65.88 ± 0.99	90.71 ± 1.17	2.44 ± 0.04	58.89 ± 4.13	99.84 ± 0.10	17.49 ± 4.20
<i>Glycyrrhiza uralensis</i> Fisch.	Rhizome	81.22 ± 1.42	8.86 ± 0.49	26.91 ± 2.24	27.48 ± 0.84	0.94 ± 0.07	55.61 ± 2.01	78.02 ± 2.44	9.97 ± 1.94
<i>Crataegus pinnatifida</i> Bge.	Leaves	187.26 ± 2.04	46.80 ± 1.83	74.43 ± 0.59	89.53 ± 1.35	2.45 ± 0.045	46.04 ± 3.22	99.34 ± 0.15	21.64 ± 1.92
<i>Forsythia suspensa</i> (Thunb.) Vahl	Leaves	135.91 ± 1.89	33.65 ± 0.89	65.22 ± 0.27	87.12 ± 0.35	2.48 ± 0.11	42.10 ± 2.46	7.22 ± 0.80	12.70 ± 0.48
<i>Lonicera japonica</i> Thunb.	Leaves	83.64 ± 7.51	20.71 ± 0.33	76.38 ± 2.00	75.70 ± 1.58	2.45 ± 0.09	29.12 ± 3.85	17.97 ± 4.62	9.10 ± 2.98
<i>Hippophae rhamnoides</i> Linn.	Leaves	82.42 ± 0.55	34.83 ± 1.59	71.62 ± 0.90	93.16 ± 0.74	2.15 ± 0.02	23.27 ± 2.93	99.37 ± 0.38	8.04 ± 0.82
<i>Nymphaea tetragona</i> Georgi	Leaves	58.25 ± 4.92	21.93 ± 0.44	72.81 ± 3.08	11.62 ± 2.22	2.38 ± 0.04	36.28 ± 2.33	60.72 ± 2.66	6.65 ± 1.25
<i>Moringa oleifera</i> Lam.	Leaves	56.64 ± 2.58	10.97 ± 1.32	42.93 ± 0.05	15.71 ± 0.21	1.65 ± 0.14	16.27 ± 3.65	6.86 ± 1.81	15.78 ± 2.42
<i>Cassia tora</i> Linn.	Seeds	47.49 ± 7.19	9.76 ± 1.74	26.97 ± 3.59	35.06 ± 2.77	0.89 ± 0.10	24.03 ± 2.85	17.67 ± 1.71	9.41 ± 2.03
<i>Eclipta prostrata</i> L.	Acrial part	42.18 ± 1.53	19.80 ± 0.64	32.04 ± 2.70	22.49 ± 2.96	1.54 ± 0.21	29.38 ± 1.92	49.25 ± 2.90	8.55 ± 1.46
<i>Lycium chinense</i> Miller	Seeds	41.98 ± 4.42	9.99 ± 0.32	75.56 ± 4.61	54.33 ± 1.83	2.39 ± 0.01	10.69 ± 2.76	23.52 ± 2.68	10.61 ± 3.27
<i>Hibiscus sabdariffa</i> Linn.	Flower	40.95 ± 2.12	18.18 ± 0.68	86.12 ± 4.38	79.07 ± 0.53	2.37 ± 0.02	25.56 ± 3.49	25.63 ± 1.32	7.30 ± 0.37
<i>Rubus idaeus</i> Linn.	Seeds	39.81 ± 2.98	19.79 ± 1.24	74.10 ± 3.87	89.36 ± 1.79	2.52 ± 0.01	26.63 ± 3.76	99.52 ± 0.25	7.29 ± 1.63
<i>Pueraria lobata</i> (Willd.)Ohwi	Rhizome	39.09 ± 1.41	14.24 ± 0.18	34.01 ± 3.49	30.21 ± 1.94	2.00 ± 0.15	27.96 ± 5.31	35.62 ± 3.31	7.87 ± 1.77
<i>Citrus reticulata</i> Blanco	Husk	37.41 ± 1.66	9.74 ± 0.49	19.93 ± 1.19	17.62 ± 0.83	1.23 ± 0.13	12.81 ± 3.32	7.01 ± 1.08	6.99 ± 0.73
<i>Taraxacum mongolicum</i> Hand.-Mazz.	Acrial part	34.55 ± 4.27	7.40 ± 1.74	27.46 ± 0.72	10.82 ± 2.47	1.29 ± 0.22	29.29 ± 2.44	28.64 ± 2.72	9.13 ± 1.75
<i>Eucommia ulmoides</i> Oliver	Leaves	29.45 ± 0.16	9.17 ± 0.48	48.29 ± 0.74	15.18 ± 1.69	2.31 ± 0.05	28.36 ± 1.49	10.29 ± 1.04	1.01 ± 0.76
<i>Morus alba</i> Linn.	Leaves	28.42 ± 1.71	7.47 ± 1.31	32.66 ± 1.35	23.67 ± 0.69	1.46 ± 0.26	28.73 ± 1.69	52.10 ± 3.91	10.81 ± 0.96
<i>Portulaca oleracea</i> Linn.	Acrial part	17.02 ± 0.42	6.27 ± 0.16	35.75 ± 2.73	10.71 ± 0.59	1.80 ± 0.05	20.61 ± 2.51	7.98 ± 2.76	6.56 ± 1.25
<i>Prunella vulgaris</i> Linn.	Flower	16.54 ± 0.83	5.14 ± 0.12	28.46 ± 3.30	4.00 ± 1.11	1.11 ± 0.07	32.13 ± 3.42	37.21 ± 3.26	5.63 ± 1.33
<i>Punica granatum</i> Linn.	Seeds	14.09 ± 3.66	5.17 ± 0.70	25.09 ± 3.29	77.93 ± 2.93	1.09 ± 0.08	22.20 ± 3.07	64.76 ± 3.26	4.63 ± 1.35
<i>Hordeum vulgare</i> Linn.	Seeds	4.63 ± 0.51	1.97 ± 0.15	12.15 ± 1.67	0.67 ± 1.66	0.45 ± 0.02	16.67 ± 1.69	6.26 ± 1.71	8.23 ± 0.57
<i>Nymphaea tetragona</i> Georgi	Seeds	3.84 ± 0.97	1.90 ± 0.12	7.17 ± 0.72	0.48 ± 0.20	0.29 ± 0.04	16.18 ± 1.23	10.39 ± 2.66	0.46 ± 1.50
<i>Platycodon grandiflorus</i> (Jacq.) A. DC.	Stem	3.06 ± 0.34	1.28 ± 0.23	11.36 ± 1.85	0.41 ± 0.49	0.43 ± 0.04	15.47 ± 1.59	28.79 ± 0.19	17.63 ± 2.15
<i>Euryale ferox</i> Salisb. ex DC	Seeds	2.32 ± 0.28	1.28 ± 0.02	10.94 ± 0.23	1.59 ± 0.98	0.31 ± 0.02	14.27 ± 2.82	22.89 ± 0.08	1.98 ± 1.40

electron from the antioxidant, they became colourless. The degree of color change is correlated with the reduction capacity of sample (Huang et al., 2005). As shown in Table 1, in term of DPPH radical scavenging, *Hibiscus sabdariffa* Linn. exhibited strongest ability with value of 86.1%, followed by *Lonicera*

japonica Thunb. and *Lycium chinense* Miller with values of 76.4% and 75.6%, respectively. In contrast, some plants showed very weak scavenging ability. For instance, *Nymphaea tetragona* Georgi (7.1%), *Euryale ferox* Salisb. ex DC (10.9%), which was attributed to their low content of total phenol. The ABTS⁺ scavenging

results between plants varied from 0.4% to 93.2%. For many plants, the ABTS⁺ scavenging ability was in accordance with its DPPH results. The positive correlation coefficient (R) between the two indexes was 0.766 (Table 2).

In reducing powder study, the reduction of $[\text{Fe}(\text{CN})_6]^{3-}$ to $[\text{Fe}(\text{CN})_6]^{4-}$ can reflect the electron-donating activity of sample. The higher the absorbance, the more $[\text{Fe}(\text{CN})_6]^{4-}$ is formed in the test, indicating the stronger antioxidant capacity of the sample (Huang et al., 2005). As shown in Table 1, the reducing powder results was in accordance with DPPH radical scavenging. The two results exhibited strong correlation with R of 0.934 (Table 2).

All the three *in vitro* testes confirmed that *Rubus idaeus* Linn., *Hibiscus sabdariffa* Linn. and *Crataegus pinnatifida* Bge. leaves exhibited stronger antioxidative capacity among the 27 plants. Although the data can't directly be applied to biological system, it is a guide that these plant extracts deserves further *in vivo* antioxidative study. Generally, the positive effect of polyphenols on various cardiovascular diseases were mainly attributed to their antioxidant activity (Mladěnka et al., 2010).

3.3 Inhibitory activity of 27 edible plants on digestive enzyme

Pancreatic lipase is responsible for the hydrolysis of 50-70% of total dietary fats, while α -glucosidase and α -amylase are related to the hydrolysis of carbohydrate. Therefore, the inhibition of these digestive enzymes can reduce fat and glucose absorption. The inhibition of 27 edible plants on pancreatic lipase, α -glucosidase and α -amylase were listed in Table 1. As shown, most plants exhibited lower inhibitory activity on pancreatic lipase. Only 4 plants showed inhibitory percentage more than 50%, which were *Alpinia officinarum* Hance, *Ginkgo biloba* Linn. leaves, *Vitis vinifera* Linn. seeds and *Glycyrrhiza uralensis* Fisch. The inhibitory performance of some plants on lipase were shown in Figure 1. After hydrolysis by lipase, substance PNPP released coloured PNP, which exhibited maximum absorption around 405 nm. Hence, in control group, its absorbance linearly increased with the hydrolysis time with biggest slope. However, with the addition of *Vitis vinifera* Linn. seeds extract, the slope notably decreased, which revealed the inhibition on enzyme activity. In contrast, the addition of *Lycium chinense* Miller showed very weak effect.

α -glucosidase and α -amylase are essential for starch hydrolysis *in vivo*. As shown in Table 1, remarkable different inhibitory

results of plants were found between α -glucosidase and α -amylase. None of the tested plants exhibited significant inhibition on α -amylase with maximum of only 21.6%. In contrast, 10 plants showed inhibitory percentage more than 50% on α -glucosidase, while 6 plants even exhibited 99% inhibition, e.g. *Crataegus pinnatifida* Bge., *Hippophae rhamnoides* Linn., etc. The results implied that the inhibitors of these two enzymes are specific and/or the enzyme inhibition is acted in different ways. The inhibitory performance of some typical plants on the hydrolysis of PNPG by α -glucosidase were shown in Figure 2. The experimental phenomenon was similar to that of pancreatic lipase.

Usually, high contents of secondary metabolites in plants results significant biological activities. However, the phytochemical types, and even the chemical structure of single component always play critical roles (Chen et al., 2018). Hence, the plants with strong α -glucosidase inhibition do not necessarily have corresponding pancreatic lipase inhibition. Luckily, 3 plants simultaneously possess significant inhibitory activities on pancreatic lipase and α -glucosidase, which were *Alpinia officinarum* Hance, *Ginkgo biloba* Linn. leaves and *Vitis vinifera* Linn. leaves. Hence, these plants deserved further anti-obesity investigation *in vivo* in future study.

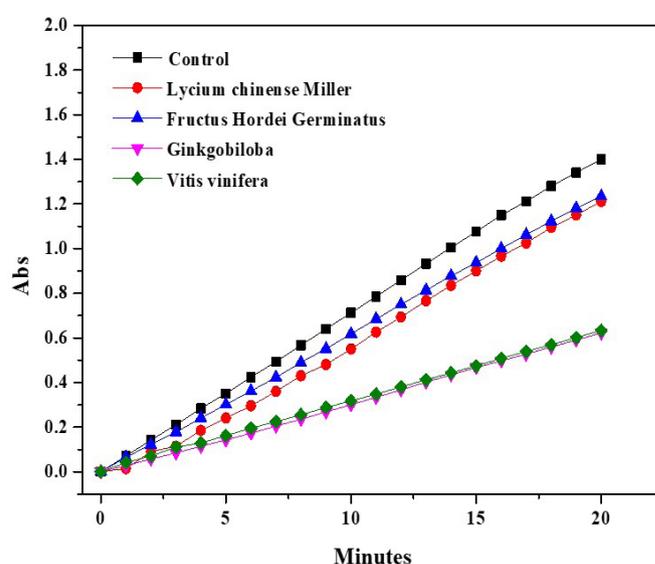


Figure 1. The inhibitory performance of some representative plants on lipase.

Table 2. Linear correlation coefficients, R, for relationships among active substances contents, digestive enzymes inhibitory and antioxidant activity for plants.

	Total flavonoid	Total phenol	DPPH	ABTS	Reducing power	Pancreatic lipase	α -glucosidase
Total flavonoid	1.000						
Total phenol	0.898	1.000					
DPPH	0.631	0.812	1.000				
ABTS	0.645	0.799	0.767	1.000			
Reducing power	0.615	0.782	0.934	0.702	1.000		
Pancreatic lipase	0.511	0.422	0.400	0.393	0.416	1.000	
α -Glucosidase	0.389	0.502	0.442	0.560	0.404	0.688	1.000

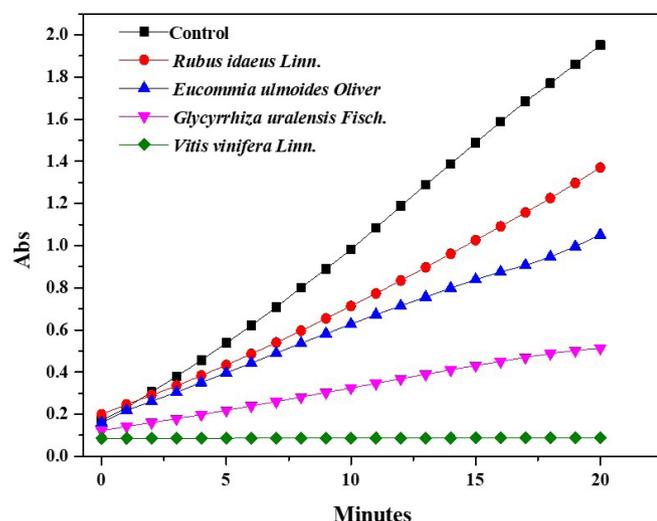


Figure 2. The inhibitory performance of some representative plants on α -glucosidase.

3.4 Correlation between the assays

The correlations between total phenol, total flavonoid, antioxidative activity and digestive enzymes inhibitory activity was shown in Table 2. As shown, the total flavonoid and phenol content of plants was positively correlated with R of 0.897. The R values between DPPH, ABTS and reducing power were all > 0.7, indicating the consistency between the three antioxidative testes. Many studies confirmed that the antioxidative activity of plants was mainly derived from their phenolic constituents (Scalbert et al., 2005). In the present study, high R values (> 0.78) between total phenol content and antioxidative activity results were found. Besides, the R values of total flavonoid with antioxidative results were > 0.61, indicating flavonoid in the total phenol contributed main antioxidant capacity. Comparing with antioxidant capacity, digestive enzymes inhibitory activity was less correlated with total phenol and total flavonoid content. The results implied that antioxidative and enzyme inhibitory activity of plants second metabolites were acted in different way. Total phenol content was not the decisive factor to determine the enzymes inhibitory activity, which was also affected by phytochemical types and chemical structures of the component.

4 Conclusions

The antioxidative activity of plants was mainly derived from their phenolic constituents. The two indexes had strong positive correlation. However, total phenol content of plant exhibited weak correlation with its digestive enzymes inhibitory activity. The phytochemical types and chemical structure of single secondary metabolite in plant may play critical role in such bioactivities. Among the 27 edible plants in present study, *Alpinia officinarum* Hance, *Ginkgo biloba* Linn. leaves and *Vitis vinifera* Linn. seeds simultaneously possess significant inhibitory activities on both pancreatic lipase and α -glucosidase, as well as moderate antioxidant capacity. Hence, these plants deserve further constituent identification and bioactivities screening *in vivo*.

Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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