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Evaluation of Antioxidant and α-Glucosidase Inhibitory Activities of Some Subtropical Plants

[1,2]Amalia Indah Prihantini, [3]Sanro Tachibana and [3]Kazutaka Itoh
[1]United Graduate School of Agricultural Science, Ehime University, 3-5-7 Tarumi Matsuyama, Ehime, 790-8566, Japan
[2]Forest Research and Development Agency (FORDA), Ministry of Forestry-Republic of Indonesia, Manggala Wanabhakti Building, Jl. Jend. Gatot Subroto, Jakarta, Indonesia
[3]Department of Applied Bioscience, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime, 790-8566, Japan

Abstract: The antioxidant and α-glucosidase inhibitory activities of the methanolic leaf extracts of some subtropical plants were evaluated in the present study. Antioxidant activity was evaluated based on 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, reducing power, hydrogen peroxide and β-carotene bleaching assays. α-Glucosidase inhibitory activity and enzyme kinetics as well as the total phenolic content of the extracts were also investigated. *Elaeocarpus sylvestris* extract had the highest activities on all the antioxidant assays performed such as DPPH scavenging activity (IC_{50} 12.7±0.5 μg mL^{-1}), reducing power (491.1±6.3 mg QE g^{-1} dry extract), hydrogen peroxide (IC_{50} 65.6±0.4 μg mL^{-1}) and β-carotene bleaching assays (IC_{50} 5.1±1.9 μg mL^{-1}). The total phenolic content of the *E. sylvestris* extract also had the highest values for gallic acid, quercetin and rutin equivalents (353.8±28.6 mg GAE g^{-1} dry extract; 294.9±24.5 mg QE g^{-1} dry extract; 663.0±52.3 mg RE g^{-1} dry extract, respectively). α-Glucosidase inhibition assay revealed that *Distylium racemosum* had the highest activity with an IC_{50} value of 22.6±1.9 μg mL^{-1}. The results of the present study revealed the potencies of *E. sylvestris*, *D. racemosum*, *Acer mono Maxim* and *Liquidambar styraciflua* as alternative sources for antioxidants and α-glucosidase inhibitors.

Key words: Antioxidant, α-glucosidase inhibitor, alternative sources, leaf extracts, subtropical plants

INTRODUCTION

Free radicals are known to play a critical role in our health. Free radicals generated from oxygen are called Reactive Oxygen Species (ROS). Excessive oxidation caused by ROS is harmful because the resultant DNA damage, protein carbonylation and lipid peroxidation have been shown to lead to several chronic diseases such as cancer, aging, atherosclerosis, hypertension, heart attacks, diabetes and other degenerative diseases (Basma *et al*., 2011; Meziti *et al*., 2012). Natural antioxidant defense systems can protect against free radicals. However, the balance between antioxidant defense system and free radicals may be altered in our body due to the lack of antioxidants or excessive production of free radicals derived from bodily activities or the environment (Chanda and Dave, 2009). The intake of antioxidant supplements is considered to be an important strategy to help reduce oxidative damage (Meziti *et al*., 2012).

ROS also play an important role in complications associated with diabetes. Moreover, the production of ROS has been shown to be increased under diabetic conditions (Noh and Ha, 2011; Ha *et al*., 2008). Previous studies demonstrated that α-glucosidase inhibitors (AGIs) helped control postprandial blood glucose levels in diabetic patients (Sancheti *et al*., 2011; Gurudeeban *et al*., 2012). AGIs were previously slow the digestion and delay the absorption of carbohydrates, which relieved the postprandial increase in blood glucose levels (Cheng and Josse, 2004). This approach may be considered as a strategy for the management of diabetes. Furthermore, an ideal anti-diabetes treatment should possess both hypoglycemic and antioxidant properties (Shibano *et al*., 2008).

The use of currently available synthetic antioxidants, such as Butylated Hydroxyl Anisole (BHA), Butylated Hydroxyl Toluene (BHT) and Tertiary Butylated Hydroquinon (TBH) has been limited because of their

Corresponding Author: Sanro Tachibana, Department of Applied Bioscience, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime, 790-8566, Japan
Tel: +81-89-9469864 Fax: +81-89-9774364

toxicities and negative health effects (Pourmorad *et al*., 2006; Madhavi and Salunkhe, 1995). Well-known commercial AGIs such as acarbose, voglibose and miglitol also lead to gastrointestinal side effects (Bachhawat *et al*., 2011). These conditions have justified the search for alternative natural medicines that are safer, cause fewer side effects and can be derived from natural sources including plants, food and fungi.

The potencies of some subtropical plants as antioxidant and α-glucosidase inhibitor sources were investigated in the present study using several assays. Because phenolic compounds are considered to play roles as antioxidants or α-glucosidase inhibitors, the total phenolic contents of plant extracts were examined. The objective of this study was to evaluate the antioxidant as well as α-glucosidase inhibitory activities of some subtropical plants *in vitro*.

MATERIALS AND METHODS

Chemicals: Quercetin, gallic acid, 1,1-diphenyl -2-picrylhydrazyl (DPPH), β-carotene, p-nitrophenyl-α-D-glucopyranoside and α-glucosidase were obtained from Wako Pure Chemical Industries Ltd., Osaka, Japan. Folin-Ciocalteu reagent was purchased from Sigma Aldrich, Japan. All the solvents used were of analytical grade.

Plant materials: The following subtropical plants: *Buxus microphylla*, *Osmanthus fragrans*, *Cedrus deodara*, *Pinus thunbergii*, *Xylosma congestum*, *Pinus palustris*, *Liquidambar styraciflua*, *Ternstroemia gymnanthera*, *Metasequioia glyptostroboides*, *Magnolia grandiflora*, *Elaeocarpus sylvestris*, *Acer mono Maxim*, *Distylium racemosum* and *Cryptomeria japonica* were collected at Ehime University (Japan) between October and November 2011. The plants are the collection plants of Ehime University. Voucher specimens were deposited in the Laboratory of Plant Chemistry, Faculty of Agriculture, Ehime University. The samples were dried at room temperature, crushed and freshly used for further experiments.

Preparation of extracts: Approximately 20 g of dried leaves were extracted with methanol twice for a week. The extracts were filtered and concentrated under a rotary evaporator and then vacuum dried.

DPPH radical scavenging activity assay: The scavenging activity of DPPH free radicals was assessed using the method described by Yen and Chen (1995). Various concentrations of each sample were mixed with 0.5 mL of the 1 mM DPPH radical solution in methanol. Similar solution with the absence of sample was used as control. Absorbance (A) was measured with a spectrophotometer at 517 nm after incubation at room temperature under dark conditions for 30 min. Quercetin and gallic acid were used as positive standards. The percentage of scavenging activity was determined by the following equation:

$$\text{Scavenging activity (\%)} = \left[\frac{A_{control} - A_{sample}}{A_{control}}\right] \times 100\%$$

Reducing power assay: The reducing power assay was performed according to a previously described method by Yen and Chen (1995) with minor modifications. Approximately 0.5 mL of various concentrations of samples in methanol was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferrycyanide (2.5 mL, 1%). Trichloroacetic acid (2.5 mL, 10%) was added to the mixture after 20 min incubation at 50°C. The mixture was then centrifugated at 3000 rpm for 10 min. The upper layer solution (2.5 mL) was mixed with distilled water (2.5 mL) and ferric chloride solution (0.5 mL, 0.1%). Absorbance was then measured with a spectrophotometer at 700 nm. The reducing power assay was measured as quercetin equivalent.

Hydrogen peroxide assay: The hydrogen peroxide assay was conducted according to a method adapted from Ruch *et al*. (1989) with minor modifications. Various concentrations of each sample were mixed with phosphate buffer saline (pH 7.4) up to 3.4 mL. Approximately 0.6 mL of 40 mM hydrogen peroxide was added to the mixture and was left to stand for 10 min. The absorbance of the mixture was measured with a spectrophotometer at 230 nm.

β-carotene bleaching assay: A previously reported method by Jayaprakasha *et al*. (2001) with a slight modification was used to conduct the β-carotene bleaching assay. Approximately 0.2 mg of β-carotene, 20 mg of linoleic acid and 200 mg of Tween40 were mixed in 0.5 mL chloroform, which was then removed using a rotary evaporator at 40°C. The resulting mixture was diluted with distilled water and mixed vigorously. The mixtures were made up to 50 mL and aliquots (4.8 mL) were added to test tubes containing various concentrations of each sample and methanol (control). A similar mixture without β-carotene was used for the background of the samples. The tubes were incubated at 50°C for 2 h. The absorbance at 470 nm was measured at 0 min either for the control (A_0) or samples (A_t). The absorbance of both the

control ($Å_0$) and samples ($Å_t$) were measured after 120 min incubation. Furthermore, the Antioxidant Activity (AA) was evaluated with the following equation:

$$\text{Antioxidant activity (\%)} = 100\left[\frac{1-(A_0-A_t)}{Å_0-Å_t}\right]$$

α-Glucosidase inhibitory activity: The α-glucosidase inhibitory activity of the crude extracts was evaluated using a method described by Kim *et al*. (2005) with minor modifications. The reaction mixture containing 5 μL of the sample dissolved in DMSO at various concentrations and 495 μL of 100 mM phosphate buffer (pH 7.0) was added to 250 μL of 3 mM substrate solution p-nitrophenyl-α-D-glucopyranoside (PNPG). The mixtures were pre-incubated for 5 min at 37°C and 250 μL of α-glucosidase (0.065 unit mL^{-1}) was added to start the reaction. The incubation was continued for 15 min and the reaction was terminated by the addition of 1 mL of 0.1 M sodium carbonate. Enzymatic activity was quantified by measuring the released product of p-nitrophenyl at 400 nm. Quercetin was used as a positive standard.

Enzyme kinetics: The inhibition type of the active extracts against α-glucosidase was measured with several concentrations of PNPG as the substrate in the absence or presence of the extracts. The enzyme reaction was assayed with a method similar to the α-glucosidase inhibition assay. The inhibition type was determined by a Lineweaver-Burk plot analysis. The Michaelis-Menten constant (Km) and maximum enzyme reaction rate (Vmax) value were also calculated. Km is expressed in the same units as X-axis whereas Vmax is expressed in the same units of Y-axis on the Lineweaver-Burk plots.

Total phenolic content: The Total Phenolic Content (TPC) of plant extracts was determined using Folin-Ciocalteu reagent (Singleton *et al*., 1999). Approximately 500 μL of the extracts (1.0 mg mL^{-1}) was added with distilled water, made up to 8 mL and then mixed with 500 μL of 2 N Folin-Ciocalteu reagents. The mixture was allowed to stand for 8 min and 1.5 mL of 20% sodium carbonate was then added. The reaction mixture was incubated at room temperature for 2 h. Absorbance was measured at 765 nm and the phenolic content was determined using a calibration curve obtained from various concentrations of gallic acid, quercetin and rutin.

Statistical analysis: All assays were performed in triplicate in independent three experiments. Data were expressed as the Mean±S.D value and analyzed by SPSS version 16.0 for windows followed by Tukey's post hoc test. Values with $p<0.05$ were considered as statistically significant.

RESULTS

Scavenging activity of DPPH radicals: Samples were evaluated for their ability to scavenge DPPH radicals and their activities were compared with quercetin and gallic acid as positive controls (Table 1). The results obtained showed that *E. sylvestris* had the highest activity, with an IC_{50} value of 12.7±0.5 μg mL^{-1}, followed by *A. mono Maxim* (27.1±0.5 μg mL^{-1}), *L. styraciflua* (30.4±1.3 μg mL^{-1}) and *D. racemosum* (33.9±1.6 μg mL^{-1}). However, the IC_{50} values of all extracts were lower than those of quercetin (7.4±0.1 μg mL^{-1}) and gallic acid (3.8±0.1 μg mL^{-1}).

Table 1: Antioxidant activities of the extracts

Leaf extracts	IC_{50} on scavenging DPPH (μg mL^{-1})	Reducing power (mg QE g^{-1} dry extract)	IC_{50} on scavenging H_2O_2 (μg mL IC_{50} on scavenging)	IC_{50} on inhibition β-carotene bleaching (μg mL^{-1})
Gallic acid	3.8±0.1^{a}	-	52.2±0.2^{a}	65.9±3.9^{e}
Quercetin	7.4±0.1^{a,b}	-	n.a	n.a
E. sylvestris	12.7±0.5^{a,b,c}	491.1±6.3^{a}	65.6±0.4^{b}	5.1±1.9^{a}
A. mono Maxim	27.1±0.5^{b,c}	266.4±28.7^{b}	103.3±0.2^{c}	31.4±8.3^{b,c}
D. racemosum	33.9±1.6^{c}	221.4±31.0^{c}	137.6±1.2^{f}	22.4±1.1^{b}
L. styraciflua	30.4±1.3^{c}	47.4±2.2^{e}	148.1±0.7^{g}	41.9±2.9^{c,d}
X. congestum	59.6±3.3^{d}	206.5±33.2^{c}	127.3±1.3^{e}	121.5±10.0^{g,h}
B. microphylla	128.4±2.8^{e}	66.7±3.9^{d,e}	105.9±0.5^{c,d}	108.5±10.6^{f,g}
T. gymnanthera	135.7±6.9^{e}	50.8±13.8^{d,e}	471.9±17.8^{i}	175.0±13.3^{i}
C. deodara	148.9±1.1^{e,f}	76.5±8.1^{d,e}	145.3±1.8^{f,g}	165.9±5.4^{i}
O. fragrans	166.8±0.9^{f}	73.5±8.1^{d,e}	162.3±2.6^{h}	100.9±12.9^{f}
M. glyptostroboides	215.0±14.6^{g}	58.6±7.5^{d,e}	114.7±0.5^{d}	130.2±5.3^{h}
M. grandiflora	235.0±12.2^{g}	86.7±14.1^{d}	209.4±0.8^{i}	213.9±11.2^{j}
P. palustris	349.0±3.0^{h}	52.4±17.9^{d,e}	218.9±3.6^{i}	113.2±1.1^{f,g}
P. thunbergii	794.8±24.0^{i}	50.7±17.9^{d,e}	151.5±1.8^{g}	206.6±8.2^{j}
C. japonica	448.6±27.8^{i}	58.8±16.7^{d,e}	145.3±2.6^{f,g}	52.7±1.2^{d,e}

Data is expressed as Mean±S.D values, different letters in the same column indicate significant differences ($p<0.05$), QE: Quercetin equivalent, -: Not tested, n.a: Not available

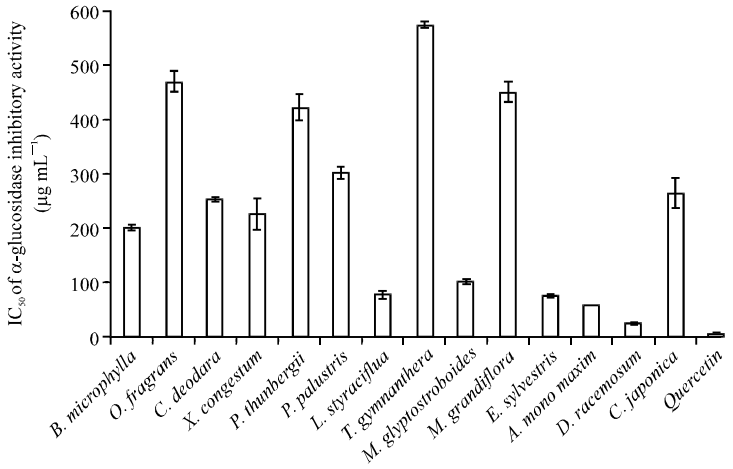

Fig. 1: Inhibitory activities of the extracts against α-glucosidase

Reducing power: Reducing power was shown as a quercetin equivalent (Table 1). The *E. sylvestris* extract had higher activity of approximately 491.1±6.3 mg QE g^{-1} dry extract than the other extracts. *A. mono Maxim* (266.4±28.7 mg QE g^{-1} dry extract); *D. racemosum* (221.4±31.0 mg QE g^{-1} dry extract) and *X. congestum* (206.5±33.2 mg QE g^{-1} dry extract) also had high reducing power activity.

Scavenging activity of hydrogen peroxide radicals: As shown in Table 1, the *E. sylvestris* extract had the strongest hydrogen peroxide scavenging activity with an IC_{50} value of 65.6±0.4 μg mL^{-1}. The other extracts that also had high activities were *A. mono Maxim* (103.3±0.2 μg mL^{-1}), *B. microphylla* (105.9±0.5 μg mL^{-1}) and *M. glyptostroboides* (114.7±0.5 μg mL^{-1}). Gallic acid, the positive standard, had an IC_{50} value of 52.2±0.2 μg mL^{-1}, which was slightly higher than that of the *E. sylvestris* extract.

Inhibition activity of β-carotene bleaching: The *E. sylvestris* extract also performed the best in this assay with an IC_{50} of 5.1±1.9 μg mL^{-1}. *D. racemosum*, *A. mono Maxim* and *L. styraciflua* also had high activities with IC_{50} values of 22.4±1.1, 31.4±8.3 and 41.9±2.9 μg mL^{-1}, respectively (Table 1). The activities of these extracts were also stronger than those of gallic acid (65.9±3.9 μg mL^{-1}).

α-Glucosidase inhibitory activity: The results obtained showed that *D. racemosum* had the highest activity against α-glucosidase with an IC_{50} value of 22.6±1.9 μg mL^{-1} (Fig. 1). The following extracts also had high inhibitory activities against α-glucosidase: *A. mono Maxim* (56.5±0.7 μg mL^{-1}), *E. sylvestris* (74.4±0.9 μg mL^{-1}) and *L. styraciflua* (76.8±6.4 μg mL^{-1}). However, all extracts had lower activities than that of quercetin (4.3±0.2 μg mL^{-1}).

Enzyme kinetics: Since, the extracts of *D. racemosum*, *A. mono Maxim*, *E. sylvestris* and *L. styraciflua* had higher activities than those of the other extracts, the inhibitory mechanisms in these extracts were analyzed further. A Lineweaver-Burk plot analysis revealed that the inhibition type of these extracts was mixed inhibition (Fig. 2). The inhibitors effect the reduction of Vmax and the increase of Km. *D. racemosum* extract contains inhibitor(s) of α-glucosidase that reduced Vmax value from 3.4 mM min^{-1} to 2.6 mM min^{-1} and increased Km value from 1.5 mM to 2.5 mM at concentration 0.1 and 1 μg mL^{-1}, respectively. Similar effects, *A. mono Maxim* extract contains inhibitor(s) of α-glucosidase that reduced Vmax value from 0.8 mM min^{-1} to 0.1 mM min^{-1} and increased Km value from 2.3 mM to 3.1 mM at concentration 5 and 25 μg mL^{-1}, respectively. Another extract, *E. sylvestris* extract contains inhibitor(s) of α-glucosidase that reduced Vmax value from 1.3 mM min^{-1} to 0.3 mM min^{-1} and increased Km value from 5.5 mM to 15.5 mM at concentration 1 and 5 μg mL^{-1}, respectively. Likewise, *L. styraciflua* extract contains inhibitor(s) of α-glucosidase that reduced Vmax value from 3.4 mM min^{-1} to 3.3 mM min^{-1} and increased Km value from 1.8 mM to 7.4 mM at concentration 5 and 25 μg mL^{-1}, respectively.

Total phenolic content: Total phenolic content was shown as gallic acid, quercetin and rutin equivalents (Table 2). For gallic acid equivalent, *E. sylvestris* had the highest value (353.8±28.6 mg GAE g^{-1} dry extract) followed by *A. mono Maxim* (179.5±11.5 mg GAE g^{-1} dry

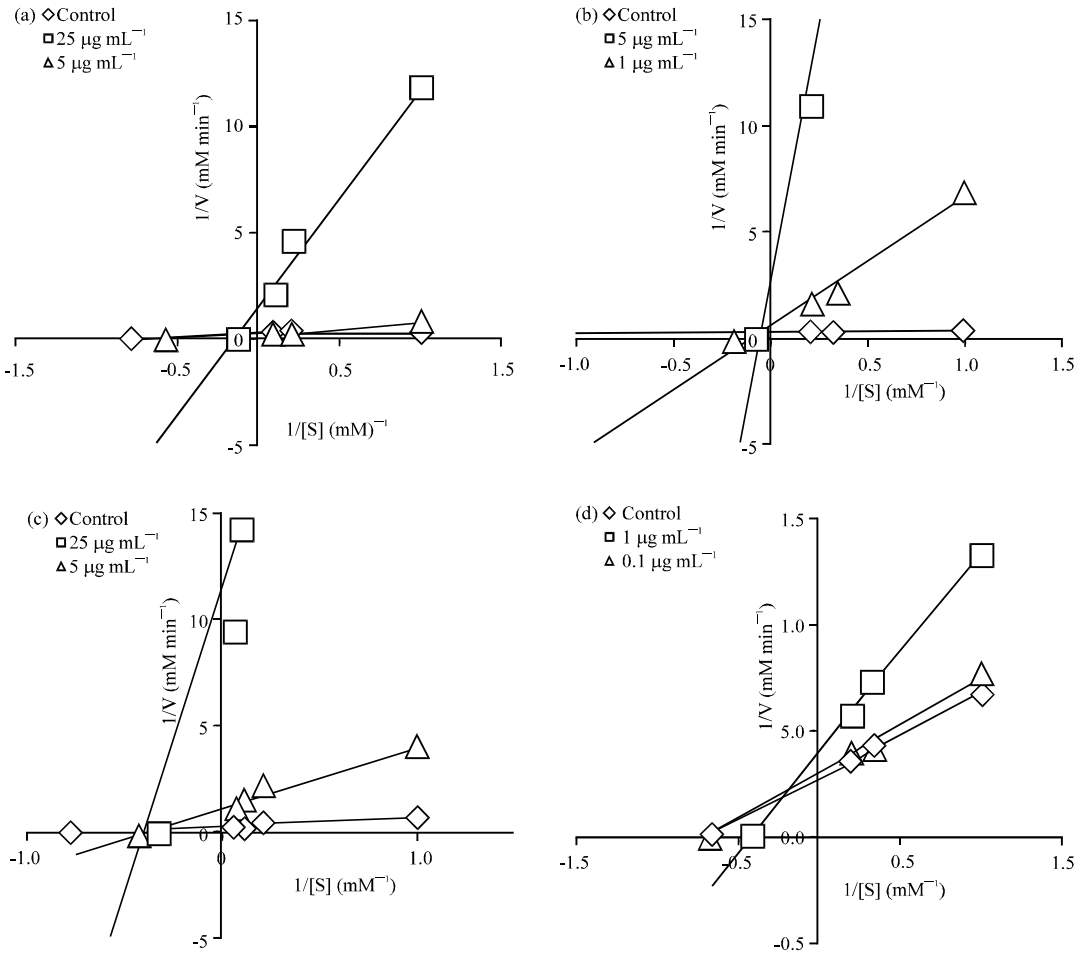

Fig. 2(a-d): Lineweaver-Burk plots of yeast α-glucosidase and PNPG in the presence of the following extracts: (a) *L. styraciflua*, (b) *E. sylvestris*, (c) *A. mono Maxim* and (d) *D. racemosum*

Table 2: Total phenolic content of the extracts

	Total phenolic content		
Leaf extracts	mg GAE g^{-1} dry extract	mg QE g^{-1} dry extract	mg RE g^{-1} dry extract
E. sylvestris	353.8±28.6^{a}	294.9±24.5^{a}	663.0±52.3^{a}
A. mono Maxim	179.5±11.5^{b}	145.9±9.8^{b}	345.6±20.9^{b}
D. racemosum	127.7±9.3^{d}	101.5±7.9^{d}	251.1±16.9^{d}
L. styraciflua	99.9±11.2^{e}	76.4±8.8^{e}	186.0±21.1^{e}
X. congestum	174.7±13.5^{b}	135.3±10.6^{b,c}	327.3±25.5^{b,c}
B. microphylla	152.0±9.9^{c}	122.4±8.5^{c}	295.4±18.1^{c}
T. gymnanthera	19.2±0.8^{g,h}	13.1±0.3^{g}	33.6±1.8^{h,i}
C. deodara	36.5±5.6^{f}	26.5±4.7^{f}	135.5±0.4^{e,f}
O. fragrans	84.5±0.0^{e,f}	64.3±0.0^{e,f}	156.9±0.0^{e,f}
M. glyptostroboides	74.5±2.4^{f}	55.9±2.0^{f}	153.9±4.3^{e,f}
M. grandiflora	63.2±8.6^{f}	46.2±7.4^{f}	133.3±15.7^{f}
P. palustris	27.9±0.5^{g,h}	19.7±0.4^{g}	50.5±0.9^{h,i}
P. thunbergii	13.3±1.8^{h}	8.2±1.4^{g}	22.4±3.5^{i}
C. japonica	40.6±0.2^{g}	26.8±0.1^{g}	92.0±0.3^{g}

Data is expressed as Mean±S.D values, different letters in the same column indicate significant differences ($p<0.05$), GAE: Gallic acid equivalent, QE: Quercetin, RE: Rutin equivalent

extract), *X. congestum* (174.7±13.5 mg GAE g^{-1} dry extract), *B. microphylla* (152.0±9.9 mg GAE g^{-1} dry extract) and *D. racemosum* (127.7±9.3 mg GAE g^{-1} dry extract). Moreover, for quercetin equivalent, *E. sylvestris*

had the highest value (294.9±24.5 mg QE g^{-1} dry extract) followed by *A. mono Maxim* (145.9±9.8 mg QE g^{-1} dry extract), *X. congestum* (135.3±10.6 mg QE g^{-1} dry extract), *B. microphylla* (122.4±8.5 mg QE g^{-1} dry extract) and *D. racemosum* (101.5±7.9 mg QE g^{-1} dry extract). Likewise, for rutin equivalent, *E. sylvestris* had the highest value (663.0±52.3 mg RE g^{-1} dry extract) followed by *A. mono Maxim* (345.6±20.9 mg RE g^{-1} dry extract), *X. congestum* (327.3±25.5 mg RE g^{-1} dry extract), *B. microphylla* (295.4±18.1 mg RE g^{-1} dry extract) and *D. racemosum* (251.1±16.9 mg RE g^{-1} dry extract).

DISCUSSION

Since, the complexity of the nature, several methods are necessary to evaluate antioxidant. This study evaluated antioxidant activity by several assays including DPPH radicals scavenging activity, reducing power, hydrogen peroxide and β-carotene bleaching assays. DPPH is a stable radical that is widely used to evaluate free radical scavenging activity. The antioxidant effect was qualified by the discoloration of purple as the color of DPPH radical. Antioxidant molecules reduce radicals by donating hydrogen (El-Haci *et al*., 2013), which result in discoloration to the colorless stable molecule 2,2-diphenyl-1-hydrazine. The DPPH radicals scavenging activities are in dose dependent manner and the activities were shown as an IC_{50} value, which indicated the concentration required to scavenge 50% of the free radicals. *E. sylvestris* extract had the highest activity on scavenging DPPH radicals whereas *A. mono Maxim, L. styraciflua* and *D. racemosum* extracts also had high activity. The statistical analysis resulted that there were no significant different activities ($p<0.05$) among these active extracts. Furthermore, it was only *E. sylvestris* that had no significancy of DPPH radicals scavenging activity with the standards, gallic acid and quercetin.

The other evaluation of antioxidant activity was reducing power. The reducing power assay may significantly reflect antioxidant activity in which extracts with high reducing power have an enhanced capability to donate electrons. The presence of antioxidants results in the reduction of Fe^{3+} to Fe^{2+} due to the donation of an electron (Ebrahimzadeh *et al*., 2010). In this assay, *E. sylvestris* extract also had the highest activity from other extracts. The statistical data resulted that its activity was significantly different ($p<0.05$) from other extracts. However, *A. mono Maxim, D. racemosum* and *X. congestum* extracts also had high reducing power activity.

To evaluate antioxidant activity, it was also considered important to investigate the ability of extracts to scavenge hydrogen peroxide. Although, hydrogen peroxide itself is not very reactive, it can increase hydroxyl radical levels in cells and cause cytotoxicity (El-Haci *et al*., 2013). Even though all extracts exhibited significant lower activities ($p<0.05$) than gallic acid, *E. sylvestris* extract was the highest active extract and it was significantly different ($p<0.05$) from other extracts on scavenging hydrogen peroxide. However, *A. mono Maxim, B. microphylla* and *M. glyptostroboides* extracts also had high activities. The scavenging activity of H_2O_2 reflects the ability of the extracts to donate electrons to hydrogen peroxide and neutralize it to water (Ebrahimzadeh *et al*., 2010).

Another evaluation of antioxidant activity, β-carotene bleaching assay, also revealed *E. sylvestris* extract that had the highest activity and it was also significant different ($p<0.05$) from other extracts. β-carotene is typically used to determine the capability of antioxidants to reduce the discoloration of β-carotene. The presence of antioxidants reduces this discoloration by stabilizing the linoleat-free radical and other radicals formed in the system, which may attack the double bonds of β-carotene and cause discoloration (Jayaprakasha *et al*., 2001). Other extracts, *D. racemosum*, *A. mono Maxim* and *L. styraciflua* extracts also had high activities.

Based on the results of evaluation of the antioxidant assays, the *E. sylvestris* extract had higher antioxidant activities in all the tested assays than those of the other extracts. This result was consistent with a previous study in which the antioxidant properties of gallotannin were isolated from *E. sylvestris* (Piao *et al*., 2008, 2009). However, *A. mono Maxim*, *D. racemosum*, *L. styraciflua*, *X. congestum* and *B. microphylla* also exhibited high antioxidant activities, which indicated their potencies as antioxidant sources. The activity of *E. sylvestris* can be attributed to the highest phenolic content in this extract because phenolics have a chemical structure that stabilizes unpaired electrons (Chanda and Dave, 2009; Seal, 2012). Therefore, phenolics can donate their electron without become reactive radicals, which is one of the requirements to be considered a good antioxidant.

α-Glucosidase is an important enzyme that catalyzes the final digestion process of carbohydrates. It breaks down oligosaccharides and disaccharides into monosaccharides. A delay in the absorption of carbohydrates is considered to improve glycemic control (Cheng and Josse, 2004). Therefore, inhibiting this enzyme activity retards the absorption of glucose, which can be used in the management of diabetes. α-Glucosidase inhibitory activity was shown as an IC_{50} value, which reflected the required concentration for 50% inhibition; higher inhibitory activity was represented by a lower IC_{50}

value. Quercetin was used as positive control in this study because it has been known as a powerful inhibitor for α-glucosidase. Moreover, quercetin has stronger inhibition against α-glucosidase from yeast *S. cereviceae* rather than acarbose (Tadera *et al*., 2006; Li *et al*., 2009b). *D. racemosum* extract had the highest activity against α-glucosidase followed by *A. mono Maxim*, *E. sylvestris* and *L. styraciflua*.

The enzyme kinetic analysis showed that the type inhibition of those extracts was mixed inhibition. This result indicates that the existing inhibitor(s) may bind the free enzyme or enzyme-substrate complex and affect the affinity of substrate binding. The effect of inhibitor(s) that contained in the extracts increased the Km values and reduced the Vmax values. Calculation of Km and Vmax value are necessary to analyze the effects of inhibitor(s) on the reaction. Km is the Michaelis-Menten constant indicating the substrate concentration at which the initial reaction rate is half of maximum, whereas, Vmax is the maximum initial rate of an enzyme catalyzed reaction. The Km value increased because the inhibitor allows the substrate to bind, but reduces its affinity. Meanwhile, the Vmax value decreased as mixed inhibitors inhibit at high substrate concentrations.

It has been known that wide range of phenolic compounds contains in the plant extracts. Furthermore, the phenolics are commonly considered to play a role in antioxidant and α-glucosidase inhibitory activities. Therefore, it is necessary to measure the content of total phenolic compounds using different equivalent chemicals. Three different equivalents that are known as good antioxidant and/or α-glucosidase inhibitor (Sajeeth *et al*., 2010; Tadera *et al*., 2006; Li *et al*., 2009b), gallic acid, quercetin and rutin, are used in this study. Gallic acid and quercetin are commonly used to evaluate total phenolic content from plants extracts (Chanda and Dave, 2009). Gallic acid is a type of phenolic acid that has been known as a well-known antioxidant, whereas quercetin is a type of flavonoid that is known as a strong α-glucosidase inhibitor. It should be also considered that phenolic glycosides contain in the extracts. Hence, rutin as a glycoside of quercetin was also used as equivalent chemical for total phenolic compounds in this study. The evaluation of total phenolic content revealed that *E. sylvestris* extracts had the highest phenolic contents which significantly different from other extracts. On the other hand, *X. congestum*, *A. mono Maxim* and *B. microphylla* were also had high total phenolic contents.

Since, the total phenolic content in plant extracts was considered responsible for antioxidant and α-glucosidase inhibitor activities, correlations between them were analyzed further. A good correlation has been reported between phenolic content and antioxidant activity in several studies (Basma *et al*., 2011; Cai *et al*., 2004). Calculations of correlations used $1/IC_{50}$ values because they paralleled with antioxidant activity (Li *et al*., 2009a). However, the calculation of correlation coefficients (R^2) is affected by the number of samples and wide range of raw materials (Cai *et al*., 2004).

The results obtained showed that the correlation between total phenolic content with DPPH radical scavenging activity, as a representative of a general antioxidant assay, was moderate with $R^2 = 0.793$, as shown in Fig. 3. This result implied that 79.3% of the antioxidant activity observed in this study could be attributed to the presence of phenolic compounds. However, other secondary metabolites may account for an approximately 20.7% of the antioxidant activity. This result is consistent with the findings of several studies in which other metabolites such as vitamins, volatile oils, carotenoids, proteins, terpenoids and alkaloids were also assumed to have contributed to antioxidant activity (Basma *et al*., 2011; Srivastava *et al*., 2006; Hassimotto *et al*., 2005). On the other hand, phenolic compounds have also been considered to contribute to α-glucosidase inhibitory activity (Sancheti *et al*., 2011). However, the analysis performed in the present study showed a weak correlation with $R^2 = 0.235$ as shown in Fig. 3. This result suggests that only 23.5% of the

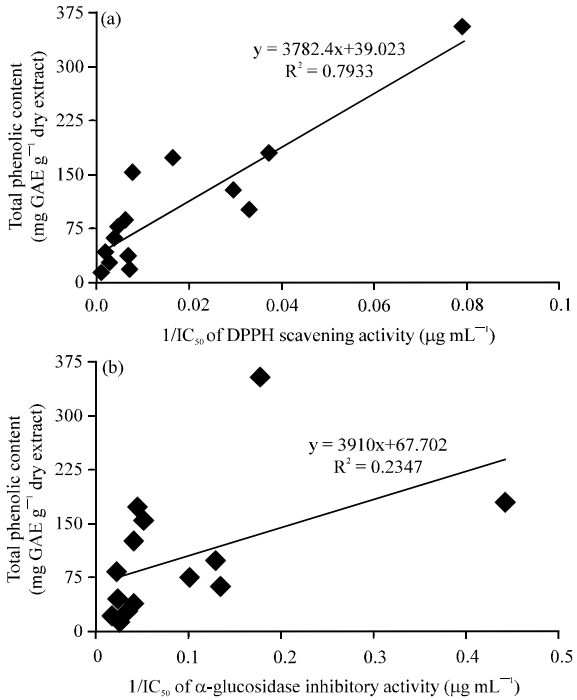

Fig. 3(a-b): Correlation between total phenolic content with (a) DPPH scavenging activity and (b) α-glucosidase inhibitory activity

inhibitory activity of α-glucosidase observed in this study could be attributed to the phenolic contents. Based on this result, the inhibitory activities of the plant extracts used in this study against α-glucosidase may have been caused by other functional groups beside phenolic groups, as has been suggested by other previous studies (Srivastava *et al*., 2006; Harish and Shivanandappa, 2006; Sabu and Kuttan, 2002). Furthermore, antagonistic or synergetic interactions between phenolics and other compounds may have contributed to the activities of the extracts.

However, the potential plant extracts that strongly inhibited α-glucosidase were also had high antioxidant activities such as *D. racemosum*, *E. sylvestris*, *A. mono Maxim* and *L. styraciflua*. These results indicated that these plants can be considered for diabetic treatments because an ideal anti-diabetic agents should be able to reduce blood glucose level as well as possessing antioxidant activities (Shibano *et al*., 2008).

CONCLUSION

It was evaluated that four extracts exhibited high activities both on antioxidant and α-glucosidase inhibition assays. The active extracts were *E. sylvestris*, *D. racemosum*, *A. mono Maxim* and *L. styraciflua*. Their strong activities on antioxidant and α-glucosidase inhibition assays are important for the consideration of the potencies of the plants on antioxidant compounds and diabetic treatment. Among them, the *E. sylvestris* extract had the highest potency as an antioxidant source, whereas the *D. racemosum* extract had the strongest potency as source of α-glucosidase inhibitor. The high activities of *E. sylvestris* on antioxidant assays were considered because of the high content of total phenolics. Studies on isolation of the compounds responsible for the antioxidant and α-glucosidase inhibitory activities of the extract especially for *E. sylvestris* as well as *D. racemosum* are warranted.

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