Pakistan
Journal of Biological Sciences
Evaluation of Antioxidant and α-Glucosidase Inhibitory Activities of Some Subtropical Plants

12Amalia Indah Prihatini, 3Sanro Tachibana and 3Kazutaka Itoh
1United Graduate School of Agricultural Science, Ehime University, 3-5-7 Tarumi Matsuyama, Ehime, 790-8566, Japan
2Forest Research and Development Agency (FORDA), Ministry of Forestry-Republic of Indonesia, Manggala Wanabakti Building, Jl. Jend. Gatot Subroto, Jakarta, Indonesia
3Department 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 (IC50 12.7±0.5 μg mL−1), reducing power (491.1±6.3 mg QE g−1 dry extract), hydrogen peroxide (IC50 65.6±0.4 μg mL−1) and β-carotene bleaching assays (IC50 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 Dispylium racemosum had the highest activity with an IC50 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 (Sanetti 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 Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT) and Tertiary Butylated Hydroquinon (TBH) has been limited because of their
Toxicities and negative health effects (Pourmorad et al., 2006; Madhavi and Salunkhe, 1995). Well-known commercial AOs such as ascorbate, 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*, *Ficus palustris*, *Liquidambar styraciflua*, *Ternstroemia gymnanthera*, *Metasequoia 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:

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

**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 ferricyanide (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 centrifuged 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 Tween-40 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 (A0) or samples (At). The absorbance of both the
control ($A_0$) and samples ($A_t$) were measured after 120 min incubation. Furthermore, the Antioxidant Activity (AA) was evaluated with the following equation:

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

**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 IC₅₀ value of 12.7±0.5 μg mL⁻¹, followed by A. mono Maxim (27.1±0.5 μg mL⁻¹), L. styraciflua (30.5±1.5 μg mL⁻¹) and D. racemosa (33.9±1.6 μg mL⁻¹). However, the IC₅₀ 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>
<thead>
<tr>
<th>Leaf extracts</th>
<th>IC₅₀ on scavenging DPPH (μg mL⁻¹)</th>
<th>Reducing power (mg QE g⁻¹ dry extract)</th>
<th>IC₅₀ on scavenging H₂O₂ (μg mL⁻¹)</th>
<th>IC₅₀ on inhibition β-carotene bleaching (μg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>3.8±0.1†</td>
<td>-</td>
<td>52.2±0.2</td>
<td>65.9±3.9†</td>
</tr>
<tr>
<td>Quercetin</td>
<td>7.4±0.1†</td>
<td>-</td>
<td>n.a</td>
<td>n.a</td>
</tr>
<tr>
<td>E. sylvestris</td>
<td>12.7±0.5olec</td>
<td>491.1±6.3◊</td>
<td>65.6±0.4♭</td>
<td>5.1±1.9♭</td>
</tr>
<tr>
<td>A. mono Maxim</td>
<td>27.1±0.5♭</td>
<td>266.4±28.7♭</td>
<td>103.6±3.2♭</td>
<td>31.4±8.3♭†</td>
</tr>
<tr>
<td>D. racemosa</td>
<td>30.1±1.6♭</td>
<td>221.4±53.0♭</td>
<td>137.6±1.2♭</td>
<td>22.4±1.1♭†</td>
</tr>
<tr>
<td>L. styraciflua</td>
<td>39.4±1.3♭</td>
<td>47.4±2.2♭</td>
<td>148.1±1.0♭</td>
<td>41.9±2.9♭†</td>
</tr>
<tr>
<td>X. congestum</td>
<td>59.6±3.1♭</td>
<td>206.5±33.2♭</td>
<td>127.3±1.3♭</td>
<td>121.5±10.6♭†</td>
</tr>
<tr>
<td>B. microphylla</td>
<td>128.4±2.8♭</td>
<td>66.7±3.9§</td>
<td>105.9±0.5§</td>
<td>108.5±10.6§†</td>
</tr>
<tr>
<td>T. gynandraea</td>
<td>135.7±6.9♭</td>
<td>50.8±13.8§</td>
<td>427.9±17.8</td>
<td>175.0±13.8†</td>
</tr>
<tr>
<td>C. doederlea</td>
<td>148.9±1.1♭</td>
<td>76.5±8.1§</td>
<td>145.3±1.8§</td>
<td>165.9±5.4†</td>
</tr>
<tr>
<td>O. fragrans</td>
<td>168.6±9.9♭</td>
<td>73.9±8.1§</td>
<td>162.3±2.6♭</td>
<td>100.9±12.9†</td>
</tr>
<tr>
<td>M. glyptostroboides</td>
<td>215.0±14.0♭</td>
<td>58.6±7.5§</td>
<td>114.7±0.5§</td>
<td>130.2±5.3†</td>
</tr>
<tr>
<td>M. graniflora</td>
<td>235.0±12.2♭</td>
<td>86.7±14.1♭</td>
<td>209.4±0.8</td>
<td>213.9±11.2†</td>
</tr>
<tr>
<td>P. pantheri</td>
<td>349.0±3.0♭</td>
<td>52.4±17.9§</td>
<td>218.9±3.6</td>
<td>113.2±1.1†</td>
</tr>
<tr>
<td>P. thunbergii</td>
<td>794.8±24.0♭</td>
<td>50.1±17.9♭</td>
<td>151.5±1.8</td>
<td>206.6±4.2†</td>
</tr>
<tr>
<td>C. japonica</td>
<td>448.6±27.8♭</td>
<td>58.8±16.7§</td>
<td>145.3±2.6♭</td>
<td>52.7±1.2§†</td>
</tr>
</tbody>
</table>

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 IC₅₀ value of 65.6±0.4 µg mL⁻¹. The other extracts that 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 IC₅₀ 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 IC₅₀ of 5.1±1.9 µg mL⁻¹. *D. racemosum*, *A. mono Maxim* and *L. styraciflua* also had high activities with IC₅₀ 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 IC₅₀ 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 (355.8±28.6 mg GAE g⁻¹ dry extract) followed by *A. mono Maxim* (179.5±11.5 mg GAE g⁻¹ dry extract).
Fig. 2(a-d): Lineweaver-Burk plots of yeast α-glucosidase and PNPG in the presence of the following extracts: (a) L. styrciflora, (b) E. sylvestris, (c) A. mono Maxim and (d) D. racemosum

Table 2: Total phenolic content of the extracts

<table>
<thead>
<tr>
<th>Leaf extracts</th>
<th>Total phenolic content</th>
<th>dry extract</th>
<th>dry extract</th>
<th>dry extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. sylvestris</td>
<td>353.8±28.6</td>
<td>294.9±24.5</td>
<td>663.0±52.3</td>
<td></td>
</tr>
<tr>
<td>A. mono Maxim</td>
<td>179.5±11.9</td>
<td>145.9±9.8</td>
<td>345.6±20.9</td>
<td></td>
</tr>
<tr>
<td>D. racemosum</td>
<td>127.7±9.3</td>
<td>101.5±7.9</td>
<td>251.3±16.9</td>
<td></td>
</tr>
<tr>
<td>L. styrciflora</td>
<td>99.9±11.2</td>
<td>76.4±8.8</td>
<td>186.0±21.1</td>
<td></td>
</tr>
<tr>
<td>X. congestum</td>
<td>174.7±13.5</td>
<td>135.3±10.6</td>
<td>327.3±25.5</td>
<td></td>
</tr>
<tr>
<td>B. microphylla</td>
<td>152.0±9.9</td>
<td>122.4±8.5</td>
<td>295.4±18.5</td>
<td></td>
</tr>
<tr>
<td>T. gymnathera</td>
<td>19.2±0.8</td>
<td>13.1±0.3</td>
<td>33.6±1.8</td>
<td></td>
</tr>
<tr>
<td>C. deodara</td>
<td>36.5±5.6</td>
<td>26.5±4.7</td>
<td>135.5±10.4</td>
<td></td>
</tr>
<tr>
<td>O. fragrans</td>
<td>84.5±0.9</td>
<td>64.3±0.0</td>
<td>156.9±10.0</td>
<td></td>
</tr>
<tr>
<td>M. glyptostroboides</td>
<td>74.3±2.4</td>
<td>55.9±2.9</td>
<td>153.9±4.3</td>
<td></td>
</tr>
<tr>
<td>M. graniflora</td>
<td>63.2±8.0</td>
<td>46.2±7.4</td>
<td>133.3±15.7</td>
<td></td>
</tr>
<tr>
<td>P. sphaerocarpa</td>
<td>27.9±0.5</td>
<td>19.7±0.4</td>
<td>50.5±0.9</td>
<td></td>
</tr>
<tr>
<td>P. thunbergii</td>
<td>13.3±1.8</td>
<td>8.2±1.4</td>
<td>22.4±3.5</td>
<td></td>
</tr>
<tr>
<td>C. japonica</td>
<td>40.6±0.2</td>
<td>26.8±0.1</td>
<td>92.0±0.3</td>
<td></td>
</tr>
</tbody>
</table>

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±19.8 mg QE g⁻¹ dry extract), *X. congestum* (135.3±16.0 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 IC₅₀ value, which indicated the concentration required to scaveng 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 significance 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 Fe²⁺ to Fe³⁺ 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.

In order 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 H₂O₂ 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 linoleate-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 gallicotannin 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 IC₅₀ value, which reflected the required concentration for 50% inhibition; higher inhibitory activity was represented by a lower IC₅₀.
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. cerevisiae 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 is 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 (Sajeeeth 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/IC50 values because they paralleled with antioxidant activity (Li et al., 2009a). However, the calculation of correlation coefficients (R²) 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 R² = 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 (Sanчети et al., 2011). However, the analysis performed in the present study showed a weak correlation with R² = 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](image)
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 Kutian, 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. styrcificua. These results indicated that these plants can be considered for diabetic treatments because an ideal anti-diabetic agent 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. styrcificua. 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.

ACKNOWLEDGMENTS

The authors are thankful to Dr. Rizza Triana Dewi for kindly help in completion of this study. We also thank our colleagues for critical reading of the manuscript.

REFERENCES


