Evaluation of the α-glucosidase inhibitory and free radical scavenging activities of selected traditional medicine plant species used in treating diabetes

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Abstract
Plants constitute a major ingredient in traditional or folk medicine. The therapeutic claims made on the use of these traditional medicinal plants range from simple conditions such as fevers and migraines, to more complex diseases such as cancer, metabolic syndrome and diabetes mellitus. The aqueous ethanolic extracts of five medicinal plant species; Cosmos caudatus, Leucaena leucocephala, Momordica charantia, Pereskia bleo and Averrhoa bilimbi were assessed for glucose lowering effect via the in vitro α-glucosidase inhibition assay. Their antioxidant potential, represented by their DPPH radical scavenging activity and total phenolic contents were also measured. The most potent α-glucosidase inhibitory activity was recorded for the leaf extract of C. caudatus with IC$_{50}$ of 21.90 ± 3.60 µg/mL, followed by L. leucocephala with IC$_{50}$ value of 30.80 ± 2.50 µg/mL. M. charantia, P. bleo and A. bilimbi did not show any significant inhibition of α-glucosidase. Meanwhile C. caudatus also gave the highest DPPH radical scavenging activity with IC$_{50}$ value of 272.46 ± 8.98 µg/mL, and the highest total phenolic content with a value of 0.263 ± 0.02 g GAE/g DW. The present work provides a priority list of interesting plants for further study with respect to the treatment of diabetes.

Introduction
Diabetes mellitus (DM) is a debilitating disease that has become an increasing health burden to many of its sufferers. This metabolic disorder is characterised by the loss of glucose homeostasis with disturbances of carbohydrate, fats and protein metabolism resulting from defects in insulin secretion, insulin action or both (WHO, 1999; Pietropolo, 2001; DeFronzo, 2004). Without having enough insulin, body tissues, particularly the liver, muscle and adipose tissues, fail to take and utilise glucose for blood circulation. Subsequently, this could increase the blood glucose level, a condition known as hyperglycaemia. If the blood glucose level remains high for long periods of time, it could damage body organs such as kidneys, eyes, nerves, heart and blood vessels. Complications arising from such organ failures could also lead to death (Brownlee, 2001; Weiss and Sumpio, 2006).

According to the International Diabetes Federation (IDF; 2017), about 425 million people worldwide are suffering from diabetes. This number is expected to rise to 522 million by year 2030. It was also estimated that more than 60% of the world’s diabetic population were from Asia (Guariguata et al., 2014). In Malaysia alone, approximately one for every five adults has diabetes. This statistic exceeds the Health Ministry’s 2014 projection, which predicted that the milestone would only be reached in 2020 (National Health and Morbidity Survey, 2015). About 2,100 patients were diagnosed in 2000, and this number had increased to 4,000 in 2012. A National Health and Morbidity Survey, which was carried out every five years, indicated that the prevalence of diabetes among adults has increased from 11.6% in 2006 to 15.2% in 2011. The diabetes clinical audit also showed that the usage of α-glucosidase inhibitors as a method of treatment has increased, with 4.7%, 5.9% and 6.5% for 2009, 2010 and 2011, respectively (Ministry of Health Malaysia, 2012). Despite the improved
understanding of its epidemiology in the recent decade, there are still no effective therapies to cure diabetes (Maiti et al., 2004), and a define solution for its prevention is still not forthcoming (Zhang et al., 2008). Acarbose, miglitol, voglibose and nateglinide are drugs used alone or in combination with insulin to treat this disease. Unfortunately, these medications have side effects and high rates of secondary failures (Bailey, 2000; Erasto et al., 2005; Dey et al., 2007). Furthermore, due to their high costs, these drugs cannot be afforded by a majority of people living in the rural communities of developing countries such as South Africa (Bailey, 2000). This failure leads to a greater demand for safer and more effective alternative anti-diabetic agents. Many studies have focused on the exploration of herbal remedies as treatment of DM, since this natural method of treatment promises lesser side effects and lower costs than using modern, synthetic drugs (Pushparaj et al., 2000; Gupta et al., 2005; Kim et al., 2005; Sohn et al., 2010; Petchi et al., 2014).

Glucosidases, a group of digestive enzymes, break down dietary carbohydrates into simple monosaccharides, thus increasing sugar levels, particularly after meals. This process of carbohydrate digestion and absorption from the digestive tract can be delayed by the use of glucosidase inhibitors such as acarbose. Lowering the after-meal glucose levels by such inhibitors has the potential to prevent development of type 2 DM.

In the search for plants possessing anti-diabetic property, worthy of further chemical and biological investigations, five plant species used in Malay traditional medicine to treat diabetes were evaluated for their α-glucosidase inhibitory activity. Their total phenolic content and antioxidant potential were also evaluated. The selected plants consisted of the popular ‘ulam’ (traditional vegetable) species; Cosmos caudatus Kunth, Leucaena leucocephala, Momordica charantia, Pereskia bleo and Averrhoa bilimbi. Cosmos caudatus (Asteraceae) is a popular vegetable known as “ulam raja” and is recommended for use against diabetes, high blood pressure, arthritis and fever (Burkill, 1966; Abas et al., 2003; Rasdi et al., 2010) as well as for several other health uses such as longevity and aiding digestion (Ong and Norzalina, 1999; Bunawan et al., 2014). It has also been reported to have antioxidant properties (Shui et al., 2005). Some of its compounds, for example the phenolics, have been shown to exert antioxidant and anti-diabetic activities (Kerem et al., 2006; Mai et al., 2007; Ranilla et al., 2010; Kunyanga et al., 2012). Leucaena leucocephala (Fabaceae) locally known as “petai belalang” has been reported to have anti-diabetic properties (Syamsudin et al., 2010; Kuppusamy et al., 2014; Chowtivannakul et al., 2017). Lesniak and Liu (1981) reported that L. leucocephala seeds contain galactomannan and lectin galactomannan. Meanwhile, M. charantia (Cucurbitaceae) or commonly known as “peria katak” is used as anti-diabetic and anti-hyperglycaemic agent (Karananayake and Tennekoon, 1993; Ahmed et al., 2001; Ooi et al., 2012). The cucurbitane triterpenoids, momoccharin and momordicin have been identified to be responsible for the hypoglycaemic activity of the plant (Platel and Srinivasan, 1997; Singh et al., 2011). Pereskia bleo (Cactaceae), known as “jarum tujuh bilah”, contains high amounts of phenolic compounds such as epicatechin, quercetin, catechin and myricetin, which are often reported to be responsible for anti-diabetic and hypertensive properties (Shahidi, 1997; Hassanbaglou et al., 2012). Furthermore, previous studies showed that A. bilimbi (Oxalidaceae), or “belimbing buluh”, has been used to reduce blood glucose levels (Pushparaj et al., 2000). Although there have been several reports on the anti-diabetic and antioxidant activities of some of these plants, more studies that can help to further understand the value of these food plants would still be beneficial, particularly from the perspective of plant-based dietary supplements, the use of which have accelerated in recent years.

Materials and methods

Materials

Cosmos caudatus Kunth with voucher specimen SK 2511/14 was obtained from UPM Agriculture Park, while the other four species namely L. leucocephala (SK 2512/14), M. charantia (SK 2513/14), P. bleo (SK 2514/14) and A. bilimbi (SK 2515/14) were collected from the plant sources growing in Serdang and Seri Kembangan areas. Voucher specimens were deposited at the mini herbarium of Institute of Bioscience, Universiti Putra Malaysia.

Plant extraction

The respective plant materials (500 g) were each freeze-dried, pulverised into fine powder, and their weights recorded. The powdered material was soaked with 80% EtOH in water at a ratio of 1:3 weight (g):volume (mL). To facilitate extraction, the mixture was sonicated for 1 h at room temperature. This period was divided in two intervals of 30 min with a break of 15 min to avoid an increase in temperature. The extraction procedure was repeated three times, and the filtrates pooled. The pooled filtrates were filtered through a Whatman filter no. 1,
and evaporated to dryness on a rotary evaporator to yield the crude extract. All of the plant extracts were weighed and stored at -80°C prior to analysis.

**Alpha-glucosidase inhibition assay**

The α-glucosidase inhibitory activity was tested following the method described by Collins et al. (1997) with slight modifications. The property was determined by measuring the release of p-nitrophenol from the substrate p-nitrophenyl-α-D-glucopyranose (PNPG) (Sigma-Aldrich, N1377-1G). Release of p-nitrophenol was observed as a formation of a yellow colour upon addition of the reaction-stopping reagent glycine (pH 10).

First, stock solutions of test extracts were prepared by dissolving 0.2 mg extract in 1 mL ethanol. Meanwhile, for use as a positive control, 0.4 mg quercetin was dissolved in 1mL ethanol (Subramaniam et al., 2008). Aliquot of 10 µL of each serial dilutions (3.125, 6.25, 12.5, 25, 50, 100, 200 ppm) made from the stock solutions (0.2 mg/mL) and for quercetin (positive control), made from the stock solution (0.4 mg/mL) were placed in each well. The substrate and enzyme were dissolved in 50 mM buffer. Next, 10 µL extract were added to 100 µL α-glucosidase type 1 from Saccharomyces cerevisiae (Sigma G5003) solution (0.02 U/well) in 30 mM phosphate buffer (pH 6.5). The sample mixture was then incubated for 5 min at room temperature (Deutschländer et al., 2009). In the meantime, 60 mg 4-nitrophenyl-α-D-glucopyranoside (PNPG), which was the substrate, was dissolved in 20 mL 50 mM phosphate buffer (pH 6.5). The sample mixture was then incubated for 5 min at room temperature (Deutschländer et al., 2009). In the meantime, 60 mg 4-nitrophenyl-α-D-glucopyranoside (PNPG), which was the substrate, was dissolved in 20 mL 50 mM phosphate buffer (pH 6.5). This solution has been reported to be comparable to that of intestinal fluid (Lee et al., 2014). The PNPG solution (75 µL) was added to each well, and the reaction mixtures were incubated for 15 min at room temperature. The reaction was stopped by adding 50 µL 2 M glycine (pH 10) to each well. The optical densities (OD’s) were then read at 405 nm using a microplate reader. The analysis was performed in triplicates.

The α-glucosidase inhibition activity of the test sample was expressed as percentage (%) inhibition and calculated using the following formula:

\[
\text{% inhibition} = \left( \frac{A_c - A_e}{A_c} \right) \times 100\%
\]

Where:

- \( A_c \) = absorbance of the reagent blank
- \( A_e \) = absorbance of the test samples

The percentage inhibition was plotted against the concentrations of each sample to determine the concentration required to inhibit 50% of the α-glucosidase enzyme (IC\(_{50}\) value) in µg/mL.

**1,1-Diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging assay**

The free radical scavenging assay was conducted according to methods described by Li and Seeram (2011) and Wan et al. (2012) with slight modification. The assay was performed in 96-well microplate and performed in triplicates. Aliquots of 50 µL of each serial dilutions (6.25, 12.5, 25, 50, 100, 200, 400 and 500 ppm), of the test samples and quercetin (positive control), made from the stock solutions 0.5 mg/mL and 0.2 mg/mL, respectively, were placed in each well. This was followed by the addition of 150 µL 1,1-diphenyl-2-picryl-hydrazyl (DPPH) which was prepared beforehand at the concentration of 59 mg/L to each well. The microplate was then incubated in the dark at room temperature for 30 min. The analysis was performed in triplicates. The absorbance of the reaction mixtures was measured at 517 nm using a microplate reader. The percentage of inhibition of each test sample was calculated from the following formula:

\[
\text{% Inhibition} = \left( \frac{A_o - A_s}{A_o} \right) \times 100\%
\]

Where:

- \( A_o \) = absorbance of the reagent blank
- \( A_s \) = absorbance of the test samples

The IC\(_{50}\) value was then determined from a plot of % inhibition versus concentration of the test samples. The IC\(_{50}\) value (µg/mL), for the free radical scavenging activity refers to the concentration at which the scavenging activity was inhibited by 50%.

**Measurement of total phenolic content (TPC)**

The Folin-Ciocalteu method as described by Zhang et al. (2006), was adopted for measurement of TPC, with some minor modifications. Aliquots of 20 µL of each serial dilution (6.25, 12.5, 25, 50, 75, 100, 125, 250, 500 ppm) prepared from a stock solution (0.5 mg/mL) of the respective test extract were loaded into a 96-well microplate, alongside the same series of serial dilution of quercetin as a positive standard. Folin-Ciocalteu reagent (100 µL) was added to each well, mixed thoroughly using vortex mixer, and the mixture allowed to rest for 5 min at room temperature. This was followed by the addition of 80 µL 7.5% (w/v) sodium carbonate solution and made up to a final volume of 200 µL.
with distilled water. After thorough mixing, the plate was covered and left in the dark at room temperature. After 30 min, the absorbance of the reaction mixtures was measured at 765 nm against a blank (the solvent used for extraction) using a microplate reader. The analysis was performed in triplicates. A standard calibration curve was constructed using gallic acid solution of different concentrations (12.5, 25, 50, 75, 100, 125, 250, 500 and 1000 ppm). The TPC values were calculated from the calibration curve (y = mx + c) and the results expressed as mg gallic acid equivalent (GAE) per g dry weight of fresh sample (g GAE/g DW) basis.

Statistical analysis

The data were presented as mean ± standard deviation (SD). The statistical significance of results was evaluated using one-way ANOVA with Duncan’s post hoc test. Significant differences were based on p values where p < 0.05 was considered significantly different and vice versa. Bioassay tests were performed in triplicates.

Results and discussion

Extraction yields

The yield of plant extracts are summarised in Table 1. The leaves of C. caudatus gave the highest yield of plant extract while the fruits of A. bilimbi gave the lowest yield. The yield of plants extracts obtained from each species was in the order C. caudatus (leaf) > L. leucocephala (seed) > P. bleo (leaf) > M. charantia (fruit) > A. bilimbi (fruit). The yields of extract obtained were as expected, since leaf generally contains higher concentration of flavonoids and fat (Gulfaz et al., 2006), seed contains higher amounts of organic compounds, saponins and protein (Gulfaz et al., 2006), while fruit largely contains water (Pullins, 2000).

Alpha-glucosidase inhibitory activity

The percentage inhibition of α-glucosidase activity and the respective IC₅₀ values of the selected plant species are presented in Table 1. The extract of C. caudatus exhibited the highest percentage inhibition of 91.4%, followed by the extract of L. leucocephala with 83.5%, at test concentration of 0.2 mg/mL. The percentage inhibition activity of the test extracts was in the order C. caudatus > L. leucocephala > A. bilimbi > M. charantia > P. bleo. Table 1 also shows that the α-glucosidase inhibition of C. caudatus was comparable to that of the positive control, quercetin (95.1%) indicating that the C. caudatus extract could be as efficient as quercetin in inhibiting α-glucosidase. On the other hand, the percentage inhibition by the L. leucocephala extract was significantly lower (p < 0.05) than both C. caudatus and quercetin. Meanwhile, the remaining three plant species showed lower inhibition (8.2-37.3%) of α-glucosidase activity.

With respect to IC₅₀ values, although C. caudatus extract exhibited similar percentage of inhibition as quercetin, its IC₅₀ value was 5-fold higher (21.90 µg/mL). Therefore, C. caudatus extract could be regarded as more potent as α-glucosidase inhibitor than quercetin (109.30 ± 4.30). Meanwhile, L. leucocephala extract also exhibited better potency than quercetin with an IC₅₀ value (30.80 µg/mL, 3-fold higher; Figure 1). However, in the past, there have been variable results reporting the IC₅₀ value of quercetin with respect to α-glucosidase inhibition. Loh and Hadira (2011) reported values

<table>
<thead>
<tr>
<th>Sample (plant species and positive standard)</th>
<th>Plant part collected</th>
<th>Yield of extract (g)</th>
<th>Total phenolic content (g GAE/g DW)</th>
<th>DPPH radical scavenging activity</th>
<th>α-glucosidase inhibitory activity</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Inhibition (%)</td>
<td>IC₅₀ (µg/mL)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>[Test conc: 0.5mg/mL]</td>
<td>[Test conc. 0.2mg/mL]</td>
</tr>
<tr>
<td>Cosmos caudatus</td>
<td>Leaves</td>
<td>7.50</td>
<td>0.26 ± 0.02a</td>
<td>89.20 ± 1.21a</td>
<td>272.46 ± 8.98a</td>
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<td></td>
<td></td>
<td>72.40 ± 0.91b</td>
<td>nd</td>
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<td></td>
<td>23.10 ± 1.35b</td>
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<td></td>
<td>46.00 ± 0.14b</td>
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<td>1.04 ± 0.01b</td>
<td>nd</td>
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<td></td>
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<td></td>
<td></td>
<td>89.20 ± 2.95b</td>
<td>66.73 ± 4.82c</td>
</tr>
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</table>

Different superscript letters indicate significant differences (p < 0.05) for the specific values using Duncan’s test. Values are expressed as mean ± standard deviation (n = 3). nd: not detected. The weight for all plant materials were 500 g. The test concentrations for quercetin for DPPH radical scavenging activity and α-glucosidase inhibitory activity assays were 0.2 mg/mL and 0.4 mg/mL, respectively.
ranging from 12.6 to 253.3 µg/mL. On the other hand, Javadi et al. (2014) reported a value of 29.8 µg/mL, which was similar to the results obtained in the present work. Nevertheless, based on the results obtained in the present work, it could be concluded that both C. caudatus and L. leucocephala were still excellent inhibitors of α-glucosidase, if not better than quercetin.

Previous studies on C. caudatus have reported the presence of flavonoids (catechin, kaempferol, myricetin, naringenin, quercetin and various quercetin glycosides) and phenolic acids (Fuzzati et al., 1995; Abas et al., 2003; Shui et al., 2005; Andarwulan et al., 2010; Mustafa et al., 2010; Mediani et al., 2012; Mediani et al., 2013; Javadi et al., 2015). These classes of compounds together with their glycosides have been reported previously to be effective inhibitors of α-glucosidase (Jung et al., 2006; Andrade-Cetto et al., 2008).

According to Syamsudin et al. (2010), the seeds of L. leucocephala have been reported to contain glycosidic compounds with monosaccharide galactose clusters and many other polysaccharides. In another study on the seed kernels, it was also indicated that the seeds contained mainly galactomannan and lectin galactomannan (Lesniak and Liu, 1981). Galactomannan are characteristic of the Leguminosae family (Ali et al., 1995). In ethnomedicine, the seed kernels are the plant part of L. leucocephala recommended for use against DM. The results obtained in the present work have shown that the seed kernels did have significant inhibitory properties against α-glucosidase, and it is very likely that the polysaccharides are the responsible constituents for the medicinal effect. This possibility has also been proposed by other researchers (Patel et al., 2012).

Figure 1. α-Glucosidase inhibition activity of (A) Cosmos caudatus, (B) Leucaena leucocephala, and (C) quercetin at different concentrations. Error bars represent standard deviation (n = 3).
Contrary to initial expectation, based on their traditional use for DM, the other three medicinal plant species did not show any significant inhibitory effect on α-glucosidase. Both *M. charantia* (Platel and Srinivasan, 1997; Chaturvedi, 2012) and *A. bilimbi* (Chin, 1992; Pushparaj *et al.*, 2000) have been shown in previous in vivo studies as having anti-diabetic properties. The absence of bioactivity towards α-glucosidase in the present work might mean that the anti-diabetic effect did not proceed via this enzymatic pathway. It could likely be that the metabolites in these plants have other mechanism(s) for reducing blood glucose level, for example they might be acting as insulin mimetics (Patel *et al.*, 2012). Another possibility is that the extracts displayed bioactivity in vivo as a result of the metabolism of inactive compounds into active compounds (Farnsworth, 1993; Deutschländer *et al.*, 2009).

**Total phenolic contents**

The TPCs of the tested extracts varied from 0.13 to 0.26 g GAE/g dry weight (DW). *Cosmos caudatus* contained the highest amount of phenolics with 0.26 g GAE/g DW, while for the other four species the TPC were 0.13-0.14 g GAE/g DW. It was observed that the TPC of *C. caudatus* leaves was significantly different (p < 0.05) when compared to the other plant extracts. Meanwhile, no significant differences were observed between the TPCs of the extracts of *L. leucocephala*, *M. charantia*, *P. bleo* and *A. bilimbi*.

The TPC results (0.26 g GAE/g DW) for *C. caudatus* leaves, were very different from those reported by Reihani and Azhar (2012) and Dian-Nashiela *et al.* (2015), who reported much lower TPCs for *C. caudatus* i.e., 0.031-0.033 g GAE/g DW and 0.018-0.066 g GAE/mL, respectively. This could be due to the differences in methods of extraction (i.e., air dried, freeze dried, oven dried). In the previous two studies, the plant material was dried at elevated temperatures i.e., 45°C and 50°C, respectively. Whereas, in the present work, the leaves of *C. caudatus* were dried at room temperature until constant weights were achieved. Nevertheless, the TPC of the *C. caudatus* in the present work was still comparable to the results obtained by Mediani *et al.* (2012; 2013) who reported the TPCs of 0.19-0.22 g GAE/g DW. The extracting solvents used in Mediani *et al.* (2013) study were either EtOH:water (80:20) or MeOH:water (80:20).

Several studies have reported that the phenolic compounds in plants significantly contributed to their antioxidant properties (Wu *et al.*, 2004; Shan *et al.*, 2005; Wong *et al.*, 2006; Maizura *et al.*, 2011). Phenolic compounds, an important group of secondary metabolites present in plants, are characterised by the presence of at least one aromatic ring carrying one or more hydroxyl groups. The high antioxidant activity
is due to the presence of these hydrogen-donating hydroxyl groups, (Michalak, 2006). For example, an increase in the number of hydroxyl groups on ring B of a flavonoid scaffold will increase the antioxidant activity of the flavonoid (Seeram and Nair, 2002). Recognising the importance of phenolic compounds to the antioxidant potential of a plant, and due to their high TPCs, the extracts were further subjected to the DPPH free radical scavenging assay.

**DPPH free radical scavenging activity**

The percentage inhibition of free radical scavenging activity of the selected plant species are presented in Table 1, together with their respective IC$_{50}$ values. The results showed that *C. caudatus* extract at a test concentration of 0.5 mg/mL had the highest percentage of DPPH radical scavenging activity (89.2%) whereas significantly (p < 0.05) lower inhibitions (14.0 to 24.7% inhibition) were exhibited by the other test extracts. The DPPH free radical scavenging activity of the test extracts were in the order *C. caudatus* > *P. bleo* > *L. leucocephala* > *M. charantia* > *A. bilimbi*. The difference in the antioxidant activities can occur due to the different types and amounts of antioxidant compounds present in the plant, which can be further influenced by environmental conditions. Factors such as deficiency of nutrients in the soil, increased intensity of sunlight, pest infestation and drought stress will also increase the synthesis and accumulation of secondary products in the plant (Selmar and Kleinwächter, 2013; Lee et al., 2014). The inhibitory activity of *C. caudatus* was comparable to that of the positive control, quercetin (89.2%), indicating that the *C. caudatus* extract could be as efficient as quercetin in scavenging free radicals. However, the IC$_{50}$ value for *C. caudatus* was significantly lower (p < 0.05), with only 272.46 µg/mL in comparison to 66.73 µg/mL for quercetin (Figure 2). Therefore, although *C. caudatus* extract seemed to be a good scavenger of free radicals, its bioactivity was not as potent as quercetin. Nevertheless, it should be noted that *C. caudatus* was tested in the form of an extract where the bioactive constituents could be present in low concentrations, hence the lower potency.

In general, extracts with high phenolic contents have been reported to exhibit high antioxidant activity (Zheng and Wang, 2001; Cai et al., 2004; Shan et al., 2005; Wong et al., 2006; Bolling et al., 2010; Maizura et al., 2011). The findings of the present work are in agreement with these reports, in which the EtOH:water (80:20) extract of *C. caudatus* with the highest TPC also exhibited the highest antioxidant activity, while the other plant extracts with lower TPC values exhibited lower antioxidant activity.

**Correlation between TPC, DPPH and α-glucosidase inhibitory activity**

The relationship between the TPCs and the biological activities of the bioactive *C. caudatus* extract was further evaluated using Pearson’s Correlation analysis (Mediani et al., 2012; Ado et al., 2014; Noriham et al., 2015). The correlations are shown in Table 2. The analysis showed a strong positive correlation between their TPC and DPPH radical scavenging activities ($r = 0.954$, $p < 0.01$). This indicates that the antioxidant activity of *C. caudatus* extract could be explained by its phenolic constituents. Similar significantly positive correlations between the antioxidant property of a plant with its TPC have also been reported in other studies (Gao et al., 2000; Sreelatha and Padma, 2009; Mossa and Nawwar, 2011; Manaharan et al., 2012; Reihani and Azhar, 2012). Redox properties and presence of hydrogen donors and singlet oxygen quenchers have been proposed to be the causal factors for this phenomenon (Sreelatha and Padma, 2009).

<table>
<thead>
<tr>
<th>TPC vs. DPPH</th>
<th>TPC vs. α-glucosidase</th>
<th>DPPH vs. α-glucosidase</th>
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<tbody>
<tr>
<td>$r$ value</td>
<td>0.954</td>
<td>0.946</td>
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<tr>
<td>$p$ value</td>
<td>0.01</td>
<td>0.01</td>
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</table>

Significant positive correlation was also shown for the relationship between the TPC and the α-glucosidase activity of the *C. caudatus* extract. While there had been many reports on the linear correlation between TPC and DPPH radical scavenging of plant extracts, reports on positive correlation between TPC and α-glucosidase activity are less common. Previously, positive relationships have been reported between the quantity of phenolic compounds and the α-glucosidase inhibitory activity of 28 edible plants in Vietnam by Mai et al. (2007). Moreover, polyphenolic compounds in plants have been shown through *in vitro* assays to inhibit the activities of digestive enzymes due to their carbohydrate-hydrolysing and protein-binding abilities (Bothon et al., 2013).

Highly significant, positive correlation ($r = 0.886$) was also observed between the DPPH free radical scavenging and the α-glucosidase inhibition activities of *C. caudatus* extract. Similar correlations have also been reported previously by other researches. For example, Pinto et al. (2009) reported strong
correlation between α-glucosidase inhibitory and the DPPH radical scavenging property of activities of Ginkgo biloba L. leaf extract. In their report, they concluded that the correlation was due to the high TPC of the plant species.

Previous reports showed that C. caudatus extracts contained common flavonoids such as quercetin (Andarwulan et al., 2010; Mediani et al., 2013), catechin (Javadi et al., 2015) and kaempferol (Andarwulan et al., 2010). Other studies by Fuzzati et al. (1995), Abas et al. (2003), Shui et al. (2005), Mustafa et al. (2010), Sukrasno et al. (2011) and Mediani et al. (2012) showed the presence of cryptochlorogenic acid, neo-chlorogenic acid, myricetin, catechin, quercetin, quercetin 3-O-rhamnoside, quercetin 3-O-β-arabinofuranoside, quercetin 3-O-β-glucoside, proanthocyanidin and naringenin. These classes of compounds (i.e., phenolics and flavonoids) together with their glycosides, have been reported previously to be effective inhibitors of α-glucosidase (Jung et al., 2006; Andrade-Cetto et al., 2008). Meanwhile, the potent antioxidant property of C. caudatus could also be due to presence of both phenolics and carotenoids. Cosmos caudatus have been reported to contain the carotenoids, lutein and β-carotene (Fatimah et al. 2012). Both carotenoids have powerful antioxidant property, helping the body scavenge free radicals, thereby limiting damages to cell membranes, DNA and protein structures in the cell. Research showed that dietary intake of foods high in β-carotene is well correlated to a lowered risk of contracting chronic conditions such as cardiovascular disease, stroke and lung cancer (Keli et al., 1996; Geleijnse et al., 1999; Cutler et al., 2008).

Conclusion

Out of the five plants studied, C. caudatus was shown to be the most potent α-glucosidase inhibitor, followed by L. leucocephala. The α-glucosidase activities of the aqueous ethanolic extracts of both plants were more potent than that of quercetin. It is likely that the high α-glucosidase inhibitory and antioxidant properties of C. caudatus were due to its high polyphenolic content. Additionally, follow-up tests with pure polyphenolic compounds may provide confirmatory evidence of the biological activity of the selected plants. Both plants could be promising sources of dietary supplements in managing diabetes, and thus merit further chemical and pharmacological investigations to ascertain the bioactive constituents and the in vivo anti-diabetic property, respectively.

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