



## Antioxidant and $\alpha$ -glucosidase inhibitory activities of the leaf and stem of selected traditional medicinal plants

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### Abstract

The study was aimed to determine the antioxidant and  $\alpha$ -glucosidase inhibition activities of the stem and leaf of five different traditional medicinal plants. The studied plants exhibited varied antioxidant and  $\alpha$ -glucosidase inhibition activities. The antioxidant activities of the plants were determined through their free radical scavenging capabilities using DPPH assay. The most potent antioxidant activity was demonstrated by *Neptunia oleracea* with an  $IC_{50}$  of 35.45 and 29.72  $\mu$ g/mL for leaf and stem, respectively. For  $\alpha$ -glucosidase inhibition activity, *Neptunia oleracea* exhibited potential  $\alpha$ -glucosidase inhibition activity with  $IC_{50}$  value of 19.09 and 19.74  $\mu$ g/mL for leaf and stem, respectively. The highest total phenolic content (TPC) was also marked in *Neptunia oleracea* leaf and stem with value of 40.88 and 21.21 mg GAE/g dry weight, respectively. The results also showed that *Strobilanthes crispus* collected from two different locations possessed different levels of phenolic content, antioxidant and  $\alpha$ -glucosidase inhibition activities. The study revealed that phenolic compounds could be the main contributors to the antioxidant and  $\alpha$ -glucosidase inhibition activities with R values of 78.9 and 67.4%, respectively. In addition, antioxidant and  $\alpha$ -glucosidase were positively correlated ( $R = 81.9\%$ ). *Neptunia oleracea* could be suggested as a potential natural source of antioxidant and antidiabetic compounds that can be used for the prevention or treatment of diabetes.

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### Keywords

Free radical scavenging activity

$\alpha$ -glucosidase inhibitory activity

Traditional medicinal plants

### Introduction

Free radicals are the molecular species that possess a single unpaired electron in the outermost atomic orbit. In spite of different types of free radicals, those of most concern free radicals in biological system are the reactive oxygen species (ROS) which include hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite (Valko *et al.*, 2007). Human body has antioxidant defence system to cope with the activity of free radicals formed in the body. However, the imbalance between the free radical production and the antioxidant defence arises when the free radicals are overproduced in the body (Baharun *et al.*, 2006). This may result in the accumulation of oxidative stress in the body and cause damage to macromolecules, such as lipid, protein and nucleic acid. Oxidative damage to these molecular species leads to the development of various diseases, such as cardiovascular disease, cancer, cataract, diabetes mellitus and Parkinson's disease (Lobo *et al.*, 2010; Mayne, 2003).

Diabetes mellitus is a metabolic disease in which the patient has high level of blood glucose. Generally,

there are two types of diabetes mellitus which are type 1 and type 2. Type 1 diabetes is insulin-dependent and takes place due to the insufficient insulin production, which arises from defects in the insulin gene. Free radicals play a significant part in the development of diabetes mellitus. It is currently hypothesized that oxidative stress is the common pathogenic factor contributing to insulin resistance,  $\beta$ -cell dysfunction, impaired glucose tolerance and ultimately to type 2 diabetes (Ceriello and Motz, 2004). Diabetes in turn will enhance the oxidative stress in the body of diabetic patient. The possible mechanisms leading to enhanced oxidative stress in diabetic patients include compromised antioxidant defenses, glucose autooxidation, formation of advanced glycated end products and a change in the glutathione redox status (Davison *et al.*, 2002). The enhanced oxidative stress in diabetes leads to its complication with cardiovascular disease (Jay *et al.*, 2006). Hence, consumption of antioxidant rich foods is beneficial in relieving diabetes particularly the type 2 diabetes.

Other than the oxidative damage, diabetes also might arise because of the release of glucose from carbohydrate in the diet resulting in a high postprandial blood glucose level in diabetic

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patients. The carbohydrate-hydrolyzing enzyme,  $\alpha$ -glucosidase in the digestive tract will hydrolyzes the carbohydrate, releasing glucose and cause the raised postprandial blood glucose level. Hence, the inhibition of the activity of this enzyme can effectively reduce the postprandial blood glucose level.  $\alpha$ -Glucosidase inhibitors combine with the intestinal  $\alpha$ -glucosidase and inhibit the release of glucose from the carbohydrate and hence inhibit the uptake of postprandial blood glucose. There are many synthetic drugs available to prevent or treat diabetes such as acarbose, voglibose and miglitol. However, they may usually cause hepatic disorders and other negative gastrointestinal symptoms (Murai *et al.*, 2002). Hence, antioxidant and  $\alpha$ -glucosidase inhibitors from natural source have become more preferred choice to prevent or treat diabetes. Traditional medicinal plants are one of the potential natural sources of these antioxidant and  $\alpha$ -glucosidase inhibitor. Traditional medicinal plants have been traditionally used by the society as traditional medicine either via decoction, tonic, or poultice of whole parts of the plants and the knowledge is passed down from generation to generation (Mustafa *et al.*, 2010). Recently, many of the traditional medicinal plants have gained attention worldwide due to their potential beneficial usage.

Malaysia is one of the 12 megadiversity countries of the world. With the diverse species of flora, it is expected that there are valuable medicinal plants in the tropical rainforest in Malaysia. The environmental variations in the growing area of the plants are known to affect the metabolism of the plants. Such variations include fluctuations in sunlight, water stress, temperature, intensity of rain, restrictions on nutritive components and air humidity (Ahmed *et al.*, 2012). Variations in these parameters affect both the primary and secondary metabolism of the plant and hence giving to the different bioactivity of the plants (Hong *et al.*, 2008).

Due to the great diversity of floral species in Malaysia, it is worthwhile to select the local traditional medicinal plants for the purpose of studying their antioxidant and antidiabetic activities. Therefore, in the present study, five local traditional medicinal plants have been chosen for this purpose. The aims of this study were to evaluate the antioxidant and antidiabetic activities of leaves and stems of five selected traditional medicinal plants using diphenyl-1-picrylhydrazyl (DPPH) and  $\alpha$ -glucosidase inhibition assays, respectively and to determine the effect of location on the bioactivity and efficiency of plant. The five studied traditional medicinal plants include *Mitragyna speciosa* (ketum), *Clinacanthus nutans* (belalai gajah), *Strobilanthes crispus* (pecah

beling), *Neptunia oleracea* (tangki) and *Mentha asiatica* (pudina).

## Material and Methods

### Plant materials

The plant materials were obtained from Pusat Pembangunan Komoditi Sendayan, Seremban, Negeri Sembilan and University Agriculture Park (TPU), UPM. The leaves and stems were cut into small pieces and dried in ventilated drying oven at 40°C for 24 hours. They were then ground to a fine powder (particle size 0.70 mm) and stored in air-tight containers until further use.

### Chemical reagents

Absolute methanol, sodium carbonate, Folin-Ciocalteu reagent, gallic acid, acarbose, quercetin, phosphate buffer,  $\alpha$ -glucosidase enzyme, glycine and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were supplied by Merck (Darmstadt, Germany) while p-nitrophenyl- $\alpha$ -D-glucopyranose (PNPG) was supplied by Sigma (Aldrich, Germany).

### Plant extraction

After the grinding step, the leaves and stems were extracted using absolute methanol. The extraction was performed by weighing 20 g of the ground leaves and stems, and then soaked in 500 mL of absolute methanol and subjected to sonication (at controlled temperature) in sonicator (Nexul Ultrasonic Cleaner, NXP 1002) for 1 h. The mixture was then filtered using filter paper before concentrated using rotary evaporator to yield the crude extract. The crude extracts were stored at 4°C until further analysis.

### Total phenolic content

The TPC was determined using the Folin-Ciocalteu method according to the procedure reported by Zhang *et al.* (2006) with some modifications. Briefly, a volume of 20  $\mu$ L of standards or test samples was mixed with 100  $\mu$ L of Folin-Ciocalteu reagent in 96-well plates. After 5 minutes, 80  $\mu$ L of 7.5% sodium carbonates was added to each well. The plate was then covered and kept in the dark for 30 minutes before the absorbance was measured at 765 nm using a micro-plate reader (SPECTRAMax PLUS). The analysis was performed in triplicate. The standard curve was obtained for the calculation of phenolic content of gallic acid and the results were expressed in mg GAE/g dry weight basis of sample.

### 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The assay was conducted as described by Li and Seeram (2010) and Wan *et al.* (2012). The assay

was performed by using 96-well microplate. A volume of 50  $\mu$ l aliquots of 8 serial dilutions of the tested samples and quercetin (positive control) with triplicate was put in the well. After this, 100  $\mu$ l DPPH (80 mg/L) was added to each well. The mixture was then incubated in the dark for 30 minutes. The absorbance was determined after 30 min of reaction in the dark at 517 nm with a micro-plate reader (SPECTRAMax PLUS). The scavenging capacity (SC) was calculated as  $SC \% = [(A_0 - A_s)/A_0] \times 100$  where  $A_0$  is the absorbance of the reagent blank and  $A_s$  is the absorbance of the test samples. All tests were performed in triplicate. The results expressed in  $IC_{50}$  values, which denote the concentration of sample required to scavenge 50% DPPH free radicals.

#### *$\alpha$ -Glucosidase inhibition assay*

The assay of  $\alpha$ -glucosidase inhibition activity performed as described by Kim *et al.* (2000) and Sarmadi *et al.* (2012). *p*-Nitrophenyl-*p*-D-glucopyranosidase (PNPG), was used as substrate and prepared by dissolving in 50 mM phosphate buffer (pH 6.5), which is comparable to the condition of intestinal fluid. The leaves and stems extracts were prepared at 5000 ppm and 6 serial dilutions were performed.

The extracts were mixed in the 96-well microplate and incubated at room temperature for 5 minutes. Then, 75  $\mu$ L of PNPG was added to each well of sample, blank substrate, negative control and positive control while the rest were loaded with 75  $\mu$ L of 30 mM phosphate buffer. The mixtures were incubated for 15 minutes at room temperature. The reaction mixtures were stop by using stopping agent, 50  $\mu$ L of 2M glycine (pH 10) for sample, blank substrate and negative control. The remains were added with 50  $\mu$ L of deionized water. Then the absorbance readings were measured using spectrophotometer (SPECTRAMax PLUS) at wavelength of 405 nm. The  $\alpha$ -glucosidase inhibition activity of the test sample was expressed as percentage (%) of inhibition and can be calculated as  $\% \text{ inhibition of sample} = [(a_n - a_s)/a_n] \times 100\%$  where  $a_n$  is the difference in absorbance of the negative control and all the blanks while  $a_s$  the difference in absorbance of the sample and all the blanks.

#### *Statistical analysis*

The results were expressed as mean  $\pm$  standard deviation of three replicates. ANOVA was used to execute the analysis of significant difference. MS Excel and Minitab 14 software (Version 14, Minitab Inc, State College, PA, USA) were used for statistical calculation. Pearson correlation test was also performed using Minitab 14 software. For

Pearson correlation and the  $IC_{50}$  was converted to  $1/IC_{50}$  to invert the relation between absorbance and the activity.

## **Results and Discussion**

### *Total phenolic content of local traditional medicinal plants*

Phenolic compounds are one of the most important groups of secondary metabolites present in plants. Phenolic compounds can be characterized by the possession of at least one aromatic ring carrying one or more hydroxyl groups. Due to the presence of these hydrogen-donating hydroxyl groups, phenolic compounds are known to possess high antioxidant activity (Michalak, 2006). Generally, the antioxidant activity of phenolic compounds is mainly depends on the number and position of hydrogen-donating hydroxyl groups on the aromatic ring of the phenolic compounds (Michalak, 2006). Fruits, vegetables, and medicinal herbs are the common natural sources of phenolic compounds.

The TPC of the leaf and stem of each of the plants are shown in Figure 1. The leaf of *Neptunia oleracea* contained the highest amount of phenolic compounds, whereas the leaf of *Strobilanthes crispus* (Sendayan) and *Clinacanthus nutans* possessed the least amount of phenolic compounds. The TPC of the plant leaf extracts varied from 2.64 to 40.88 mg GAE/g dry weight (DW). The leaf of *Strobilanthes crispus* (Sendayan) and *Clinacanthus nutans* contained 2.64 and 2.68 mg GAE/g DW, respectively, followed by *Strobilanthes crispus* (TPU), *Mentha asiatica*, *Mitragyna speciosa* and *Neptunia oleracea* at 6.52, 11.8, 24.02 and 40.88 mg GAE/g DW, respectively. The same trend can be observed for the stems extract of the plants. The TPC of the plant stems extracts varied from 0.055 to 21.21 mg GAE/g DW. The stems of *Strobilanthes crispus* (Sendayan) and *Clinacanthus nutans* contain 0.055 and 0.12 mg GAE/g DW, respectively, followed by *Strobilanthes crispus* (TPU), *Mitragyna speciosa* and *Neptunia oleracea* at 0.46, 0.92 and 21.21 mg GAE/g DW, respectively. The stem of *Mentha asiatica* was not analysed due to the small size of the stem and insufficiency of sample.

It was observed that the leaf and stem of each of the compared plants had significant difference ( $P < 0.05$ ) in the level of TPC. Biosynthesis of secondary plant metabolites is often goes along with translocation and storage (Hartmann, 1996). The secondary metabolites are produced in a specific organ of the plant, such as leaf and roots as a protective agent in response to environmental stress. The secondary metabolites do not accumulate in the site of synthesis for all



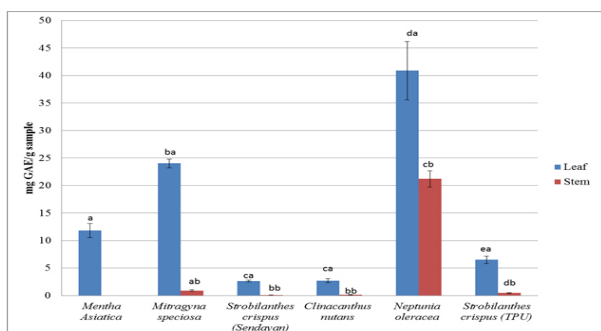


Figure 1. Total phenolic content of leaf and stem of local traditional medicinal plants. The first subscript letter is to compare the same part of every plant. The second subscript letter is to compare between parts of the same plant. Mean with different subscript letters are significantly different ( $P < 0.05$ ).

the time and will transfer to their preferred site of storage through phloem or xylem tissues (Hartmann, 1996). In this study, the leaves of the studied plants had higher amount of phenolic compounds than their stems. This could be explained by the reason that leaves are the site of biosynthesis of these phenolic compounds and they moved from leaves to the site of storage via the phloem or xylem tissues through long distance translocation. Besides, even the different parts of the fruits *Mangifera pajang* and *Artocarpus odoratissimus* studied by Abu Bakar *et al.* (2009) demonstrated different levels of the bioactivities and the chemical compounds present. Therefore it is not surprising for the stem and leaf of the plants in the present study to possess different level of phenolic compounds. The difference in TPC between different parts of *Strobilanthes crispus* had also been reported before by Ismail *et al.* (2012).

It is also interesting to note that there was different level of TPC in the same plant species collected from different location. *Strobilanthes crispus* collected from Sendayan and TPU. The plants grew at different location hence were subjected to different level of stressors. Stressors such as elevated temperature, drought stress, high irradiation, biological stress, soil composition and nutrition deficiency result in synthesis and accumulation of secondary plant products (Selmar and Kleinwächter, 2013).

In this study, variation between the TPC in the same plants with those reported by previous authors was observed. For instance, the TPC of *Neptunia oleracea* determined was around 42.88 mg GAE/g DW for leaf and 21.21 mg GAE/g DW for stem. A lower TPC (4.16 and 4.84 mg GAE/g DW, respectively) was reported by Rattanasena (2012) while a higher TPC (42.98 mg GAE/g DW) was reported by Daduang *et al.* (2011). Besides, the different TPC for the *Strobilanthes crispus* leaves was reported by Qader *et al.* (2011) and Ismail *et al.*

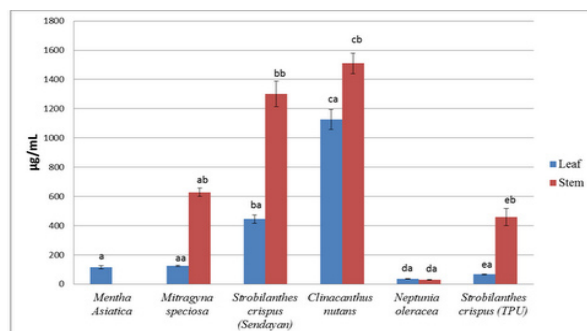


Figure 2. DPPH radical scavenging activity of leaf and stem of local traditional medicinal plants.

The first subscript letter is to compare the same part of every plant. The second subscript letter is to compare between parts of the same plant. Mean with different subscript letters are significantly different ( $P < 0.05$ ).

(2012). These differences may be contributed by the differences in solvent and method of extraction, parts of plant being analyzed or even climatic condition and cultural practices, maturity at harvest and storage condition of the plants.

#### Free radical scavenging activity of the plant extracts

Antioxidants are able to stabilize or deactivate the free radicals before they cause oxidative damage to the cellular structures. Free radical scavenging is by donating hydrogen to the free radical. Thus, free radical scavenging activity can be used for determining the antioxidant capacity of plant extract. In this study, DPPH free radical scavenging activity of plant leaf and stem extracts were evaluated to determine their antioxidant properties. The free radical scavenging activity was expressed as  $IC_{50}$  value.  $IC_{50}$  is defined as the concentration of the antioxidant required to scavenge 50% of the DPPH radical.

Results of free radical scavenging activities of the leaves and stems of each plant, expressed as  $IC_{50}$  values are presented in Figure 2. The results showed that most of the studied plants exhibited potential antioxidant activity and the  $IC_{50}$  values of the leaves varied from 35.45 to 1126.63  $\mu\text{g/mL}$ . The lower the  $IC_{50}$  value, the greater is the free radical scavenging capability of the plant extract. This is because a lower concentration is required for the antioxidant compounds to exhibit 50% of inhibition on free radicals. Results of the present study showed that leaf of *Neptunia oleracea* possess the most potent free radical scavenging activity with  $IC_{50}$  of 35.45  $\mu\text{g/mL}$ , followed by the leaves of *Strobilanthes crispus* (TPU), *Mentha asiatica*, *Mitragyna speciosa* and *Strobilanthes crispus* (Sendayan) with  $IC_{50}$  of 67.63, 115.9, 127.05 and 445.36  $\mu\text{g/mL}$ , respectively. *Clinacanthus nutans* leaf possessed the lowest free radical scavenging activity with  $IC_{50}$  of 1126.63  $\mu\text{g/mL}$ . There was significant difference ( $P < 0.05$ ) in the

free radical scavenging activity of all the plant leaves, except *Mentha asiatica* and *Mitragyna speciosa*. The results of the stems also showed the same trend.

When comparing the leaf and stem of the same plant, the same pattern as the results of TPC can be seen. It was observed that the leaves and stem of the compared plants had significant difference ( $P < 0.05$ ) in the inhibition on free radicals, except *Neptunia oleracea*, which showed no significant difference ( $P > 0.05$ ) between the two parts. Both the leaf and stem of *Neptunia oleracea* had the comparable antioxidant properties and were the most active plants among all the compared plants. Since it was found that the *Neptunia oleracea* was the best for its high TPC in both the leaf and stem, it can be suggested that phenolic compounds may be the major source of antioxidant capacity of the plant (Qader et al., 2011). The TPC and the antioxidant activity were positively correlated with R value of 78.9%. For all the other plants, the leaves exhibited higher antioxidant activity than the stems. It showed that the leaves possess higher amount of antioxidant compounds than the stem. It can be explained that the leaves are the site of biosynthesis of these antioxidant compounds and these synthesized compounds are translocated from site of biosynthesis to the preferred site of storage via xylem or phloem in the stem (Hartmann, 1996).

On the other hand, it was observed that the *Strobilanthes crispus* collected from Sendayan and TPU were significantly different in their free radical scavenging activities. The difference in the antioxidant activities can occur due to the different type and amount of antioxidant compounds present in the plants. The different type and amount of antioxidant compounds present in the plants can be affected by the environmental condition. Factors such as deficiency of nutrients in the soil, increased intensity of sunlight, pest infestation, and drought stress will increase the synthesis and accumulation of secondary products in the plant (Selmar and Kleinwächter, 2013). Different stages of growth of the plants will also have the effect on the bioactive compounds and bioactivity of the plants (Ismail et al., 2012).

The results obtained from this study showed some disagreement with those previously reported by others. For instance, Parthasarathy et al. (2009) reported a greater DPPH free radical scavenging activity for *Mitragyna speciosa* leaf with  $IC_{50}$  of 37.08  $\mu\text{g}/\text{mL}$ . A lower DPPH free radical scavenging activity for *Neptunia oleracea* and *Strobilanthes crispus* were reported by Rattanasena (2012) and Qader et al. (2011), respectively. These differences can be justified by the variation in solvent and method of

extraction used, parts of plant being analyzed, or even climatic condition and cultural practices, maturity at harvest and storage condition of the plants.

#### *$\alpha$ -Glucosidase inhibitory activity of the plant extracts*

The  $\alpha$ -glucosidase enzyme is one of the key enzymes involved in dietary carbohydrate digestion in human. It hydrolyzes the carbohydrate, releasing glucose and cause the raised postprandial blood glucose level. Inhibitions to this enzyme can effectively descent the postprandial blood glucose level. This is especially beneficial for diabetic patients. There are many synthetic drugs available to prevent or treat diabetes such as acarbose, voglibose and miglitol. However, they usually can cause hepatic disorders and other negative gastrointestinal symptoms (Murai et al., 2002). Hence,  $\alpha$ -glucosidase inhibitors from natural source have become the more preferable as means to prevent or treat diabetes. In this study, the  $\alpha$ -glucosidase inhibitory activity of the plant leaf and stem extracts were evaluated and the results were shown in Table 1.

The results showed that for both the leaf and stem of *Neptunia oleracea* possessed the most potent  $\alpha$ -glucosidase inhibitory activity. For the leaf, the inhibitory on  $\alpha$ -glucosidase by *Neptunia oleracea* was as high as 84.16% at concentration of 39.06  $\mu\text{g}/\text{mL}$ . Meanwhile, *Strobilanthes crispus* (Sendayan), *Mitragyna speciosa*, *Strobilanthes crispus* (TPU), *Clinacanthus nutans* and *Mentha asiatica* only showed inhibition values of 46.69, 20.83, 14.02, 13.57 and 9.79%, respectively at concentration of 5000  $\mu\text{g}/\text{mL}$ . In contrast to the results of TPC and DPPH radical scavenging activity, the trend for the results of  $\alpha$ -glucosidase inhibitory activity in stems were not the same as those of leaf. The inhibitory on  $\alpha$ -glucosidase by *Neptunia oleracea* stem was as high as 90.66% at concentration of 39.06  $\mu\text{g}/\text{mL}$ , followed by *Mitragyna speciosa*, *Clinacanthus nutans*, *Strobilanthes crispus* (TPU) and *Strobilanthes crispus* (Sendayan) with percent inhibition of 29.64, 17.67, 14.44 and 0.91%, respectively at concentration of 5000  $\mu\text{g}/\text{mL}$ . As shown in the table 1, only the  $IC_{50}$  of *Neptunia oleracea* leaf and stem can be determined. There was significant difference among all the studied plants by comparing both the leaf and stem.

The high inhibition activity of *Neptunia oleracea* on  $\alpha$ -glucosidase might be contributed by the high content of phenolic compounds in the plant as a remarkably high content of phenolic compounds was also reported in this study. The phenolic compounds might be the contributor to  $\alpha$ -glucosidase inhibitory activity. In this study, the TPC and the  $\alpha$ -glucosidase

Table 1. Percentage of  $\alpha$ -glucosidase inhibition and the  $IC_{50}$  values

Plant		Highest concentration ( $\mu\text{g/mL}$ )	Percentage of Inhibition	$IC_{50}$ Values ( $\mu\text{g/mL}$ )
<i>Mentha asiatica</i>	Leaf	5000	9.79 $\pm$ 1.0 <sup>a</sup>	ND
	Leaf	5000	20.83 $\pm$ 0.5 <sup>ba</sup>	ND
<i>Mitragyna speciosa</i>	Stem	5000	29.64 $\pm$ 0.6 <sup>ab</sup>	ND
<i>Strobilanthes crispus</i> (Sendayan)	Leaf	5000	49.69 $\pm$ 1.7 <sup>ca</sup>	ND
	Stem	5000	0.91 $\pm$ 0.06 <sup>bb</sup>	ND
<i>Clinacanthus mutans</i>	Leaf	5000	13.57 $\pm$ 1.2 <sup>da</sup>	ND
	Stem	5000	17.67 $\pm$ 1.9 <sup>db</sup>	ND
<i>Neptunia oleracea</i>	Leaf	39.06	84.16 $\pm$ 8.8 <sup>ca</sup>	19.09 $\pm$ 3.2
	Stem	39.06	90.66 $\pm$ 1.4 <sup>db</sup>	19.74 $\pm$ 1.4
<i>Strobilanthes crispus</i> (TPU)	Leaf	5000	14.02 $\pm$ 0.3 <sup>da</sup>	ND
	Stem	5000	14.44 $\pm$ 0.04 <sup>ca</sup>	ND

Values are the means  $\pm$  standard deviation of three replicates.

The first subscript letter is to compare the same part of every plant. The second subscript letter is to compare between parts of the same plant. Mean with different subscript letters are significantly different ( $P < 0.05$ ).

ND = not determined.

inhibition activity were positively correlated with R value of 67.4%. There was also strong positive correlation between antioxidant and  $\alpha$ -glucosidase inhibition activities with R value of 81.9%. The positive relationships between the quantity of phenolic compounds and  $\alpha$ -glucosidase inhibitory activity of 28 edible plants in Vietnam had been reported by Mai *et al.* (2007). This positive relationship might be found in *Neptunia oleracea* in this study as well. Besides, several flavonoids, including luteolin, amentoflavone, luteolin 7-O-glucoside and daidzein had been reported to exhibit  $\alpha$ -glucosidase inhibition (Kim *et al.*, 2000). Nonetheless, Tunsaringkarn *et al.* (2009) reported a 0%  $\alpha$ -glucosidase inhibition for the *Neptunia oleracea* extract at a concentration of 1 mg/ml. That was a great difference between the results obtained from this study and their results. This difference in the activity can arise because of the usage of different extraction solvent and method of extraction

When comparing the leaf and stem of the same plant, the same as the results of TPC and free radical scavenging activity, it was observed that the leaves and stem of the compared plants had significant difference ( $P < 0.05$ ) in the inhibition  $\alpha$ -glucosidase, except *Strobilanthes crispus* (TPU), which showed no significant difference ( $P > 0.05$ ) between the two parts. Unlike the results of TPC and DPPH radical scavenging activity, stem of some plants showed greater inhibition activity on  $\alpha$ -glucosidase than their leaves. It was expected that there were other compounds besides phenolic compounds present in the stems of these plant that possess  $\alpha$ -glucosidase inhibition activity. The type of alpha-glucosidase inhibitor drugs available today, such as acarbose and myglitol are carbohydrate compound. Hence, besides phenolic compounds, it was predicted that the compounds present in the stem of the plants could be some carbohydrates that are act as competitive inhibitor of alpha-glucosidase enzyme (Sugiwati *et al.*, 2009).

*Strobilanthes crispus* collected from Sendayan

and TPU exhibited different activities on  $\alpha$ -glucosidase inhibition. Due to the different environmental condition, the type and amount of secondary metabolites can be different in the plants (Selmar and Kleinwächter, 2013). The *Strobilanthes crispus* from Sendayan showed greater  $\alpha$ -glucosidase inhibitory activity instead of that collected from TPU. This result was different with those of TPC and DPPH radical scavenging activity which demonstrated better activity in *Strobilanthes crispus* collected from TPU. Although the TPC in *Strobilanthes crispus* collected from TPU was reported to be higher than that from Sendayan, the type and quality of phenolic compounds might be different. The phenolic compounds found in *Strobilanthes crispus* collected from TPU might be effective in antioxidant activity but not in inhibition of  $\alpha$ -glucosidase.

## Conclusion

The results of the current study showed that leaves and stems of the five plants tested consisted of wide range of phenolics compounds and exhibit potential antioxidant and  $\alpha$ -glucosidase inhibitory activities. The leaves and stems of some of the plants were different in their bioactivities. The study revealed that phenolic compounds could be the main contributors to the antioxidant and  $\alpha$ -glucosidase inhibition activities with R values of 78.9 and 67.4%, respectively. It was also shown that plants from different locations exhibited different bioactivities. It is encouraging to note that *Neptunia oleracea* possess remarkable phenolics content, antioxidant and antidiabetic activities. The results suggested that phenolic compounds are the major contributor to the antioxidant and  $\alpha$ -glucosidase inhibitory capabilities of this plant. Consequently, this plant can be suggested as a potential natural source of antioxidant and antidiabetic compounds for the prevention or the treatment of diabetes and its complications. However, the use of this plant as an alternative remedy for diabetes requires more extensive studies or the optimum extraction and isolation of the bioactive compounds and its safety and efficacy evaluation on human subjects.

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