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A COMPARATIVE STUDY OF THE BIOLOGICAL ACTIVITIES OF DIFFERENT COMMERCIAL *Stevia* EXTRACTS FROM LOCAL MARKET IN MALAYSIA

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ABSTRACT

An increased awareness on consuming healthy food and beverages worldwide has led to an upsurge of interest in functional food, which includes the use of natural sweetener, the *Stevia rebaudiana* Bertoni extract. Nevertheless, limited data has been available on the biological activities of commercial *Stevia* extract available in Malaysia. Hence, the present study aims to evaluate the biological activities of commercial *Stevia* extract from local market in Malaysia, by evaluating its total phenolic (TPC) and total flavonoid content (TFC), as well as its antioxidant and anti-diabetic activities. Three independent *Stevia* extracts (Samples A, B and C) sourced from local market in Malaysia were evaluated, in comparison to the freshly prepared *Stevia* extract. The results showed a significantly lower amount of TPC in commercial *Stevia* extracts when compared to the freshly prepared *Stevia* extract (7.077 mg GAE/100 g), with sample A containing the highest TPC (6.359 mg GAE/100 g), followed by sample C (1.496 mg GAE/100 g) and sample B (0.624 mg GAE/100 g). Similar trend was observed with TFC, with sample A containing 0.937 mg QE/100 g, followed by sample C (0.264 mg QE/100 g) and sample B (0.029 mg QE/100 g) as compared to the freshly prepared *Stevia* extract (1.684 mg QE/100 g). Sample A showed the highest antioxidant activity (DPPH: 48.84%, FRAP: 2.589 µmole Fe^{2+}/g, ABTS: 28.48%), albeit lower to the freshly prepared *Stevia* extract. The commercial samples showed a higher α-amylase inhibition activity compared to the freshly prepared *Stevia* extract, but no inhibition was observed in the α-glucosidase activity. Fundamentally, the results highlight the biological activities of *Stevia* extract for functional food applications, but caution has to be exercised as all three commercial extracts have significantly different biological activities.

Key words: *Stevia rebaudiana*, commercial *stevia* extract, biological activity, antioxidant activity, α-amylase, α-glucosidase

INTRODUCTION

In recent years, there is an increased awareness on the importance of consuming healthy food and beverages worldwide. This has led to an upsurge of interest in the development of functional food that incorporate natural products from plants that are rich in phytochemicals as dietary sources for bioactive compounds (Buniowska *et al*., 2017). This includes the increased interest in the use of natural sweetener, the *Stevia rebaudiana* Bertoni extract (Korir *et al*., 2014). *Stevia rebaudiana* Bertoni, commonly known as *Stevia*, is a herbal plant that originally belongs to (*Asteraceae*) Compositae family in Paraguay (Chaturvedula *et al*., 2011). Because of its sweet-tasting constituents namely steviol glycosides that are 350 times sweeter than sugar (Purkayastha *et al*., 2016), *Stevia* extract has been widely used by the indigenous populations as their natural sweet taste for hundreds of years. Studies on *Stevia* was initiated by Dr. Moisés Santiago Bertoni upon the discovery of this plant in Paraguay in 1888 (Yadav & Guleria, 2012). In Asia, Japan was the first country to cultivate *Stevia* extract as a sweetener in the food

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and drug industries, followed by China, Malaysia, Singapore, South Korea, Taiwan, and Thailand (Kamarulzaman *et al*., 2014).

Owing to its benefits, *Stevia* extract has been granted the Generally Recognized Status (GRAS) by the Food and Drug Administration (FDA) and also approved as food additives by the Joint Expert Committee on Food Additives (JECFA) in 2009 and the European Food Safety Authority (EFSA) in 2011 with an acceptable daily intake (ADI) of 4 mg kg^{-1} body weight per day (Oehme *et al*., 2017). Currently, the rising diabetic cases have caused Malaysians to pick up the interest in this plant. As Malaysians are known for their 'sweet tooth', they have unhealthy habit in consuming much sugar in diet, which results in many health problems (Tang, 2015). Notably, this non-calorific natural sweetener can help address the rising health problems, such as obesity and diabetes.

Due to its chemical structure and health-promoting phytochemical components (Lemus-Mondaca *et al*., 2012; Yildiz-Ozturk *et al*., 2015), *Stevia* has been deemed suitable to replace sucrose in food and beverages, besides being used as functional food ingredients (Šic Žlabur *et al*., 2013). The interest in commercial *Stevia* extract has been high for a long time. In Malaysia, the awareness towards *Stevia* based products is increasing, with more than 60% are using *Stevia* as sugar substitute (Kamarulzaman *et al*., 2014). Nevertheless, limited data has been available on the biological activities of commercial *Stevia* extract available in Malaysia. Hence, the present study is focusing on assessing the biological activities of commercial *Stevia* extract from local market in Malaysia, by evaluating its total phenolic and total flavonoid content, as well as its antioxidant and anti-diabetic activities, with a view to investigate whether this commercial *Stevia* extract is capable to provide the biological activities that are similar or comparable to the freshly prepared extract from dried *Stevia* leaves.

MATERIALS AND METHODS

Materials

Folin-Ciocalteu phenol reagent, sodium carbonate (Na_2CO_3), sodium acetate buffer (pH 3.6), aluminium chloride ($AlCl_3$), gallic acid, quercetin, ferric chloride hexahydrate ($FeCl_3.6H_2O$), sodium phosphate monohydrate, α-glucosidase enzyme, glycine and acarbose were purchased from Merck (Darmstadt, Germany). ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), DPPH (2,2-diphenyl-1-(2,4,6-triphenyl-hydrazyl)), 2,4,6-Tripyridyl-S-Triazine (TPTZ), -nitrophenyl-α-D-glucopyranose (PNPG), ascorbic acid, starch and α-amylase were purchased from Sigma Aldrich (St Louis, MO, USA). Potassium persulphate 99% and 3,5-Dinitrosalicylic acid (DNS) were purchased from R&M Chemicals (Essex, UK), while sodium phosphate dehydrate was supplied by GmbH (Rheinland, Germany). All other chemicals and reagents used were of analytical grade.

Sample preparation

Three independent commercial *Stevia* extracts in liquid form (Sample A, B and C) were obtained from the local market in Malaysia through an online vendor. The samples used were of different brands. All of the three brands contain similar ingredients, which is *Stevia* extract prepared by reverse osmosis water. These samples were chosen based on their availability in the market and were used as purchased without further purification. The control sample used was freshly prepared *Stevia* water extract.

Stevia rebaudiana Bertoni dried leaves were obtained from Koperasi Warisan Munsyi, Selangor Berhad (KOWARIS, Malaysia). The dried *Stevia* leaves were ground into powdered form by using a dry blender (Panasonic, Malaysia). The powdered leaves were kept in opaque sealed container, at room temperature for further analysis. The freshly prepared *Stevia* extract was prepared using maceration technique (Kamal, 2016). Accurately, 40 g of powdered leaves was added with 500 mL of distilled water. The mixtures was macerated in orbital incubator shaker at 200 r.p.m, for 90 min, at 40°C. The mixture was then strained and the marc (damp solid material) was pressed by using vacuum pump and lyophilised for further analysis.

Determination of total phenolic content (TPC)

Estimation of total phenolic content was conducted using method from Rao-Narsin *et al*. (2014) with minor modification, using Folin-Ciocalteu reagent. The assay was based on the reduction of phosphor-wolframate-phosphomolybdate complex by phenolic compounds to a blue reaction product (Chaovanalikit & Wrolstad, 2004). Sample solution was measured at absorbance 760 nm. The standard curve was obtained using gallic acid as standard (0-100 µg/mL) and the results were expressed as mg gallic acid equivalent (GAE/100 g fresh weight).

Determination of total flavonoid content (TPC)

Total flavonoid content of the extracts was performed by aluminium chloride ($AlCl_3$) colourimetric method (Woisky & Salatino, 1998) with slight modifications. Absorbance at 415 nm was measured for sample solution against a reagent blank using UV-Vis spectrophotometer (Lambda 25, Perkin Elmer, USA). The standard curve was attained

using quercetin solution at concentrations of 0-100 µg/mL. Referring to quercetin standard curve, the results were expressed as mg quercetin/g fresh weight, which was prepared under the same conditions. The total flavonoid were expressed as mg quercetin equivalents (QE/100 g fresh weight).

Determination of total antioxidant activity

DPPH assay

The free radical scavenging activity of the extracts was investigated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method as outlined by Vongsak *et al.* (2013). 3 mL of DPPH (0.1 mM) was mixed with 0.2 mL of the extracts in methanol solution. After incubation at 37°C for 30 min, the absorbance readings were taken at 517 nm (Lambda 25 Spectrophotometer, Perkin Elmer, USA). Ascorbic acid was used as the standard reference. The DPPH radical scavenging effect was calculated as inhibition of percentage according to the following equation:

$$\%\ of\ inhibition = \frac{Abs\ control - Abs\ sample}{Abs\ control} x\ 100 \quad \text{– Equation 1}$$

Ferric reducing ability of plasma (FRAP) measurements

The ability to reduce ferric ions was measured according to Norhaiza *et al*. (2009). A FRAP reagent solution was produced by mixing 10 mM of 2,4,6-tripyridyl-s-triazine (TPTZ), 20 mM of ferric chloride ($FeCl_3.6H_2O$), and 300 mM of sodium acetate buffer ($C_2H_3NaO_2.3H_2O$) (pH 3.6) at a volume ratio of 1:1:10, and used immediately. Extracts (20 µL) were added to 180 µL of the FRAP reagent in 96 microplate well. The mixture was incubated at 37°C for 30 min. The absorbance at 595 nm was determined with Infinite® F50 microplate reader (TECAN, Switzerland). Ascorbic acid was used as the standard reference. Aqueous solutions of $FeSO_4 \times 7H_2O$ (0–1000 µmol/L) were used for calibration. The antioxidant capacity was calculated based on the ability of the samples to reduce ferric ion and was expressed as µmole Fe^{2+}/g fresh weight.

ABTS assay

The ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6- sulphonic acid) assay was carried out based on the method described by Mandana *et al*. (2012) with minor modification. The 7 mM ABTS and 2.45 mM potassium persulphate were mixed to prepare the ABTS solution. Then, the ABTS solution was incubated at room temperature in the dark as ABTS solution can easily be reduced under light, for 16 hr to ensure ABTS would completely react with potassium persulphate. The mixture was then diluted with 80% (v/v) methanol to obtain an absorbance of 0.700 at 734 nm (Lambda 25 Spectrophotometer, Perkin Elmer, USA). The ABTS solution (3 mL) was mixed with 0.2 mL of extracts. The blank solution was prepared using methanol instead of extracts and absorbance readings were taken at 734 nm following 10 min of incubation. Ascorbic acid was used as the standard reference. The ABTS radical scavenging activity (%) was measured using Equation 1 (Gan & Latiff, 2011).

Determination of anti-diabetic activity

In vitro α-amylase inhibitory assay

The α-amylase inhibition assay was performed according to the method described by Wickramaratne *et al.* (2016) with modifications. Briefly, 200 µL of the extracts was added to 200 µL of 0.02 M of sodium phosphate buffer (pH 6.9) containing α-amylase solution (2 units/mL). This solution was pre-incubated at 37°C for 10 min, after which 200 µL of 1% of starch solution in 0.02 M of sodium phosphate buffer (pH 6.9) was added. The mixture was then further incubated at 37°C for 10 min. The reaction was terminated by adding 200 µL of 3, 5- dinitrosalicylic acid (DNS) reagent and then placed in a boiling water bath for 5 min. The reaction mixture was cooled to room temperature and diluted further with 5 mL of distilled water, in which the absorbance was measured at 540 nm (Lambda 25 Spectrophotometer, Perkin Elmer, USA). A control was prepared using the same procedure by replacing the extract with distilled water. Acarbose was used as the standard reference. The α-amylase inhibitory activity was calculated as percentage of inhibition according to the following equation:

$$Inhibition\ of\ \alpha\text{-}amylase\ activity\ (\%) = \frac{Abs\ control - Abs\ sample}{Abs\ control} x\ 100 \quad \text{– Equation 2}$$

In vitro α-glucosidase inhibitory assay

Samples were analysed by the Institute of Bioscience, Universiti Putra Malaysia for α-glucosidase inhibition assay. The assay of α-glucosidase inhibition activity was performed as described by Lee *et al*. (2014) with some modifications. The release of ρ-nitrophenol from ρ-nitrophenyl-α-d-glucopyranose was measured to determine the α-glucosidase inhibitory activity. The released ρ-nitrophenol resulted in a yellow colour development upon addition of the stop reagent, glycine (pH 10). The ρ-nitrophenyl-ρ-D-glucopyranosidase (PNPG) substrate was prepared by dissolving it in 50 mM of phosphate buffer (pH 6.5), which is comparable to the condition of intestinal fluid. All samples were mixed in the 96-well microplate with 30 mM of phosphate buffer and 10

μL of alpha-glucosidase enzyme solution (from Maltase) and incubated at room temperature for 5 min. Then, 75 μL of PNPG was added to each sample, followed by incubation for 15 min at room temperature. The reaction was terminated by using 50 μL of 2 M glycine (pH 10) and the absorbance readings were measured using spectrophotometer (TECAN Infinite F200 PRO) at wavelength of 405 nm. A control was prepared using the same procedure by replacing the extract with distilled water. Quercetin was used as the standard reference. The α-glucosidase inhibition activity of the test sample was expressed as percentage of inhibition according to Equation 2.

Statistical analysis

All results were expressed as mean ± SD (n=3). Statistical analyses of data presented were conducted using One-way ANOVA, with Tukey's test applied for comparison of means (GraphPad Prism version 7, GraphPad Software Inc., California, IL, USA). A p value of ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Determination of total phenolic and total flavonoids content

Three commercial *Stevia* extracts were chosen as the test samples (Samples A, B and C). The TPC of the extracts was determined by using Folin-Ciocalteu method as described in Section 3.2. Folin-Ciocalteu reagent reacts with phenolic compounds and obtains a complex to be measured at 760 nm (Khan *et al*., 2016). The results obtained showed that the TPC in samples A, B and C were significantly lower when compared to the freshly prepared extract (Figure 1A). The freshly prepared extract was observed to contain 7.077 mg GAE/100 g, which is in accordance with other studies that have reported high levels of phenolic compounds in *Stevia* water extracts (Muanda *et al*., 2011; Shukla *et al*., 2012), with an average polyphenol concentration of 4.15% by weight of dried Stevia leaf (Kaushik *et al*., 2010). Sample A was found to have the highest TPC (6.359 mg GAE/100 g) followed by sample C (1.496 mg GAE/100 g), while sample B was found to have the lowest TPC (0.624 mg GAE/100 g). Dried *Stevia* leaves extract has been shown to contain myriads of phenolic acid compounds, which include chlorogenic, caffeic and trans-ferulic acids (Karaköse *et al*., 2015).

A similar trend was also observed in the TFC of the commercial *Stevia* extract, albeit a lower amount compared to the TPC. Flavonoids are the largest and the most studied group of plant phenols with significant therapeutic activity (Gaweł-Bęben *et al*., 2015). Sample A was found to possess the highest amount of TFC (0.937 mg QE/100 g), followed by sample C (0.264 mg QE/100 g), while sample B (0.029 mg QE/100 g) was observed to have the lowest TFC (Figure 1B). *Stevia* extract has been reported to contain flavonoid compounds, such as flavonol (quercetin) and flavone (luteolin and apigenin) derivatives (Belščak-Cvitanović *et al*., 2015).

Total antioxidant activity

Various antioxidants are present in plants, hence there are no designated method to measure their antioxidant capacity. Several methods have been occasionally employed to evaluate the total antioxidant activity in plants, which include the Trolox equivalent antioxidant capacity (TEAC), total radical absorption potentials, oxygen radical absorption capacity and the ferric reducing ability of plasma (FRAP) assays (Tadhani *et al*., 2007). In the present study, the total antioxidant activity of both *Stevia* water extract and commercial samples

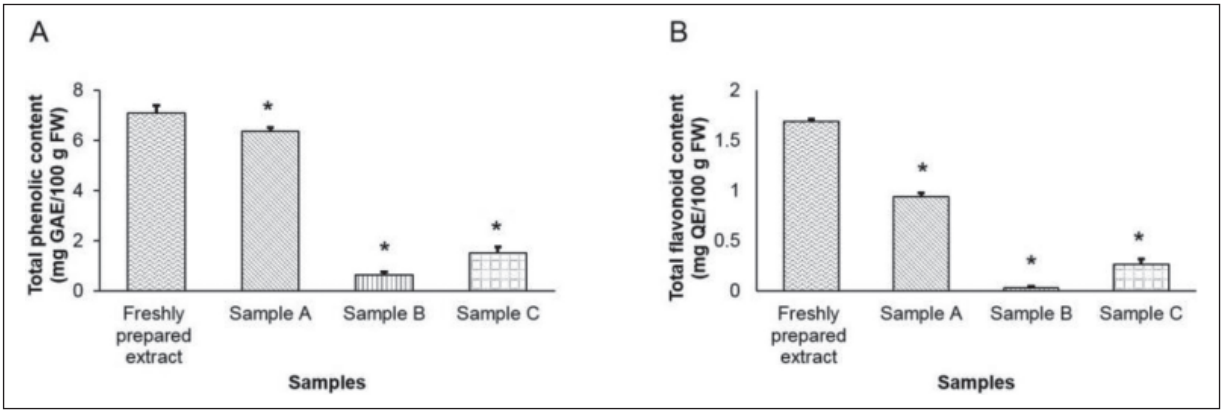

Fig. 1. (A) Total phenolic content and (B) total flavonoid content of freshly prepared extract and commercial stevia extract: Sample A, B and C as determined by Folin-Ciocalteu method. Data represent mean ± SD (n=3). *indicates significant difference to the freshly prepared extract at $p < 0.05$ Tukey's range test.

were evaluated by using three different approaches, namely the DPPH, ABTS and FRAP assays.

DPPH radicals are widely employed as a model system that investigates the antioxidant of various natural compounds by their ability to scavenge DPPH radicals and by monitoring the colour changes in the reaction at 517 nm of wavelength. The percentage inhibition of DPPH radicals with freshly prepared *Stevia* water extract was found to be 68.79%, which is significantly higher than the commercial samples, which was evidenced with the percentage of DPPH inhibition; sample A (48.84%), sample C (7.33%) and sample B (1.36%) (Figure 2A).

Another approach to measure the total antioxidant is also based on electron transfer and reduction of a coloured oxidant; the ABTS assay. ABTS assay monitors the generation of a blue/green ABTS radicals that can be reduced by antioxidants found in plants (Floegel *et al*., 2011). Similar trend was also observed, with the freshly prepared *Stevia* water extract was found to have a significantly higher percentage of inhibition (43.03%) from the commercial samples, albeit a lower inhibition was observed when compared to the DPPH assay. As for the commercial samples, the highest percentage of inhibition was found in sample A (28.48%), followed by sample B (10.31%) and sample C (4.47%) (Figure 2B).

Both DPPH and ABTS assays are convenient in most applications, they, however, depend on the scavenging activity of antioxidants towards non-physiological radicals (Floegel *et al*., 2011). The third approach, which is the FRAP assay is different among others as no free radicals are involved in this assay as the antioxidant capacity was estimated by monitoring the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by the compounds of interest (Belščak-Cvitanović *et al*., 2015). The commercial samples showed a significantly lower FRAP value when compared to the freshly prepared *Stevia* water extract (4.641 µmole Fe^{2+}/g). Like DPPH assay, the antioxidant capacity measured by FRAP assay showed that the FRAP value for sample A (2.589 µmole Fe^{2+}/g) was significantly higher than sample C (0.307 µmole Fe^{2+}/g) followed by sample B (0.101 µmole Fe^{2+}/g) (Figure 2C).

Even though all three assays conducted in the present study employed different mechanisms, the total antioxidant activity of the freshly prepared *Stevia* water extract was anonymously higher when compared to the commercial samples, which is in accordance with the results obtained for TPC and TFC in section 4.2 (Muanda *et al*., 2011). The

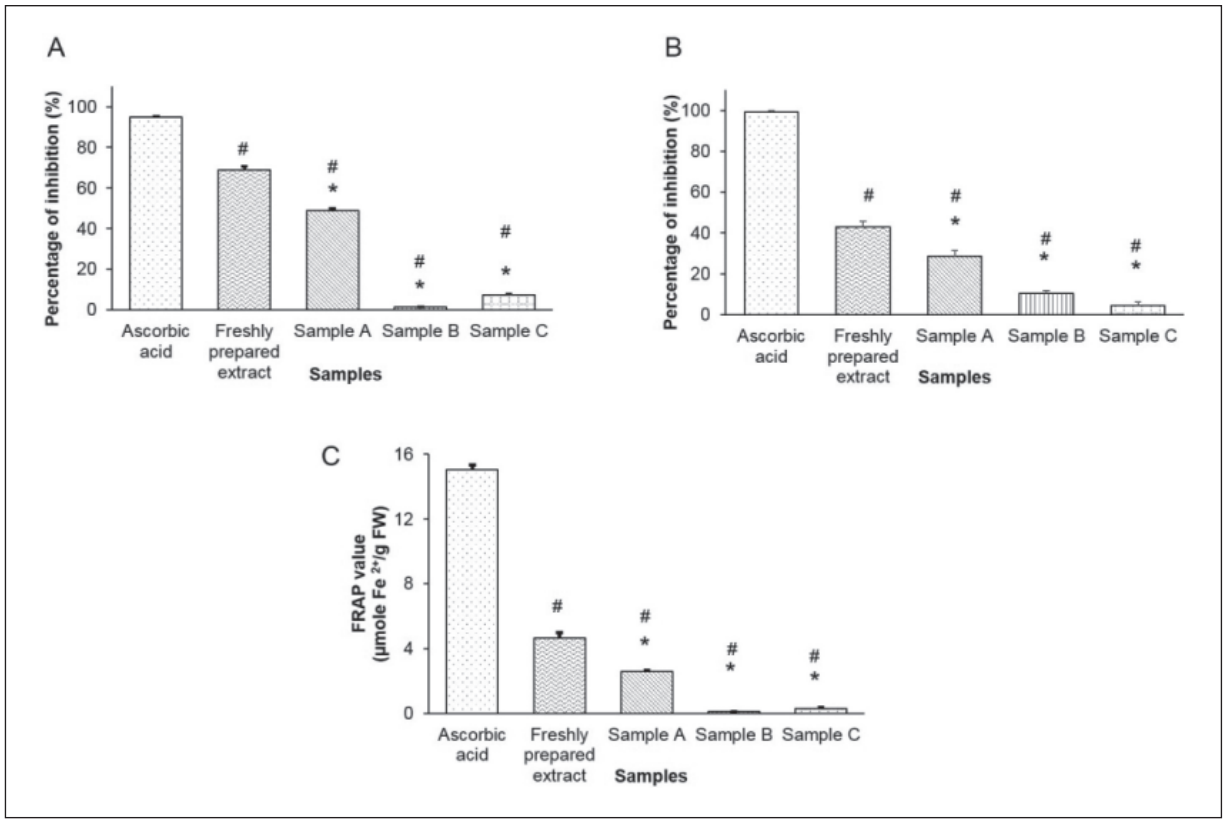

Fig. 2. Total antioxidant activity of freshly prepared extract and commercial stevia extract: Sample A, B and C as determined by (A) DPPH, (B) ABTS and (C) FRAP assays. Data represent mean ± SD (n=3). *indicates significant difference to the freshly prepared extract and # indicates significant difference to ascorbic acid at $p < 0.05$ Tukey's range test.

correlation between total polyphenols and antioxidant activity are in general good, but it is dependent on the nature of the sample and on the implication of other compounds that can interfere with the measurement of the antioxidant activity (Moure *et al*., 2001). The DPPH, FRAP and ABTS assays showed no difference in the total antioxidant activity of all *Stevia* samples, but discrepancy was observed for Sample B and C in ABTS assay. Similar trend was observed throughout the determination of the total antioxidant capacity, with freshly prepared *Stevia* water extract was shown to have the highest activity, followed by sample A, sample C and B in DPPH and FRAP assays. However, sample B showed a higher total antioxidant activity in ABTS assay when compared to sample C. The reason for the observed difference could be attributed to the different mechanisms employed by all three assays. Taking into account the diverse bioactive composition of each commercial sample, attributed to the use of various plant materials and their phytochemical constituents, the prevalent antioxidant capacity of sample B may be the consequence of specific compounds that interacted more potently with ABTS radicals. Previous study has also suggested the probable involvement of other compounds that could potentially act as antioxidants, particularly any nitrogenous and oxygenated heterocyclic compounds that undergo Maillard reaction during food processing (Payet *et al*., 2005). However, further analysis has to be conducted in order to identify the phytochemical constituents that contribute to the antioxidant capacity of all the samples used in the present study.

Anti-diabetic activity

Diabetes mellitus is attributed to the deficiency in insulin secretion and decreased responsiveness of organs towards secreted insulin. One of the therapeutic approaches to manage diabetes is through the inhibition of α-glucosidase and α-amylase activities, which are the enzymes responsible in hydrolysing carbohydrate. Inhibition towards both α-glucosidase and α-amylase activities can significantly result in the reduction of postprandial elevation of blood glucose (Chen & Kang, 2013). Hence in the present study, the ability of all *Stevia* samples as anti-diabetic agent was evaluated based on their capacity to impose inhibition against α-amylase and α-glucosidase activities.

Based on the results obtained, all *Stevia* samples were able to inhibit α-amylase activity (Figure 3). The freshly prepared extract was shown to be

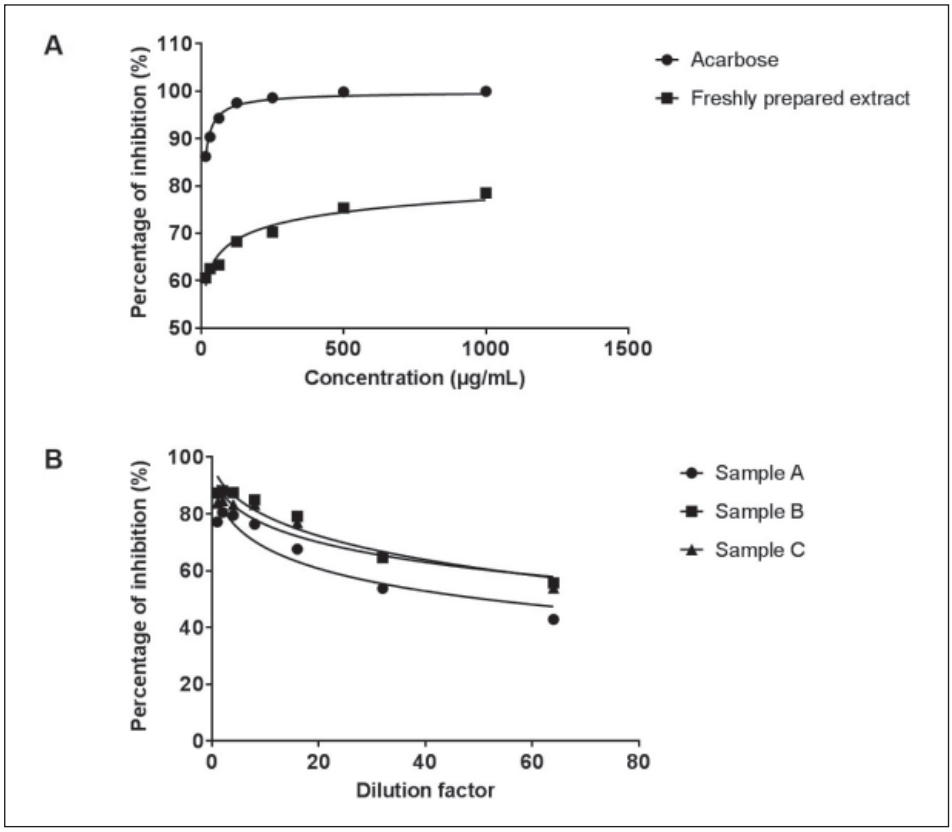

Fig. 3. The anti-diabetic activity of (A) acarbose as standard reference and freshly prepared extract and (B) commercial stevia extract as determined by the percentage of inhibition towards α-amylase enzyme. Data represent mean ± SD (n=3).

Table 1. The IC_{50} values of acarbose, freshly prepared extract and commercial samples; Sample A, B and C against α-amylase activity. * denotes significant difference to acarbose at $p < 0.05$ (n=3)

Samples	IC_{50}
Acarbose	1.736 (µg/mL)
Freshly prepared extract	2.679 (µg/mL)*
Sample A	IC_{50} = 1:5 dilution
Sample B	IC_{50} = 1:8 dilution
Sample C	IC_{50} = 1:8 dilution

capable of inhibiting the α-amylase activity with IC_{50} value of 2.679 µg/mL, which was significantly higher than the standard acarbose (1.736 µg/mL) as shown in Table 1. Contrary to this, the inhibition of the commercial samples towards α-amylase was surprisingly better than the freshly prepared stevia extract (Figure 3B), with the maximum inhibition activity observed to be >80% for commercial sample B (Figure 3A). However, all commercial samples were observed to have a declined inhibition capacity towards α-amylase once the maximum capacity was reached, which was probably due to the saturation of the enzyme. The IC_{50} of sample A was lower (IC_{50}=1:5 dilution) when compared among others, then followed by both sample B (IC_{50}=1:8 dilution) and sample C (IC_{50}=1:8 dilution). The IC_{50} values of commercial samples were reported in dilution because (Table 1) there was no information on the concentration provided on the labels of the commercial samples.

Surprisingly, commercial sample with the lowest TPC and TFC, as well as antioxidant capacity; sample B exhibited the best inhibition activity towards α-amylase. Previous study has claimed that there is no correlation between the TPC and antioxidant activity with the capacity of an extract to inhibit α-amylase activity. In addition, they also proposed that water extract samples may contain some non-phenolic water-soluble compounds, such as alkaloids and other nitrogenous compounds that are able to act as inhibitors of the α-amylase enzyme (Khan *et al*., 2016). Nevertheless, previous study has documented the presence of steviol glycosides compound in *Stevia* water extract (Shamsi *et al*., 2018), which is known to attribute to the capability of this extract in inhibiting α-amylase activity (Ruiz-Ruiz *et al*., 2015a).

Figure 4 shows the inhibition of all *Stevia* extracts on α-glucosidase inhibition activity. Based on the results obtained, in contrast to the α-amylase inhibition assay, all samples; the freshly prepared *Stevia* extract (Figure 4B) and the commercial samples (Figure 4C) showed no comparable inhibition activity towards α-glucosidase as compared to the standard, quercetin (Figure 4A) at the concentration range similar to the one used in α-amylase inhibition assay. This could be due to the compounds that inhibit α-amylase enzyme are not compatible to α-glucosidase enzyme, resulting in no inhibition towards α-glucosidase activity. Previous study has also found that *Stevia* has shown a slightly weak inhibition towards α-glucosidase activity compared to α-amylase activity (Ruiz-Ruiz *et al*., 2015b). However, surprisingly sample B was observed to be able to inhibit the α-glucosidase activity at a lower inhibition capacity (<50%) at higher concentrations (Figure 4C). Similar observation was also observed with commercial sample C when presented undiluted. Further analysis has to be conducted in order to identify the type of compounds present in both samples B and C, which contributes to the capacity of both samples to inhibit α-amylase and α-glucosidase activities.

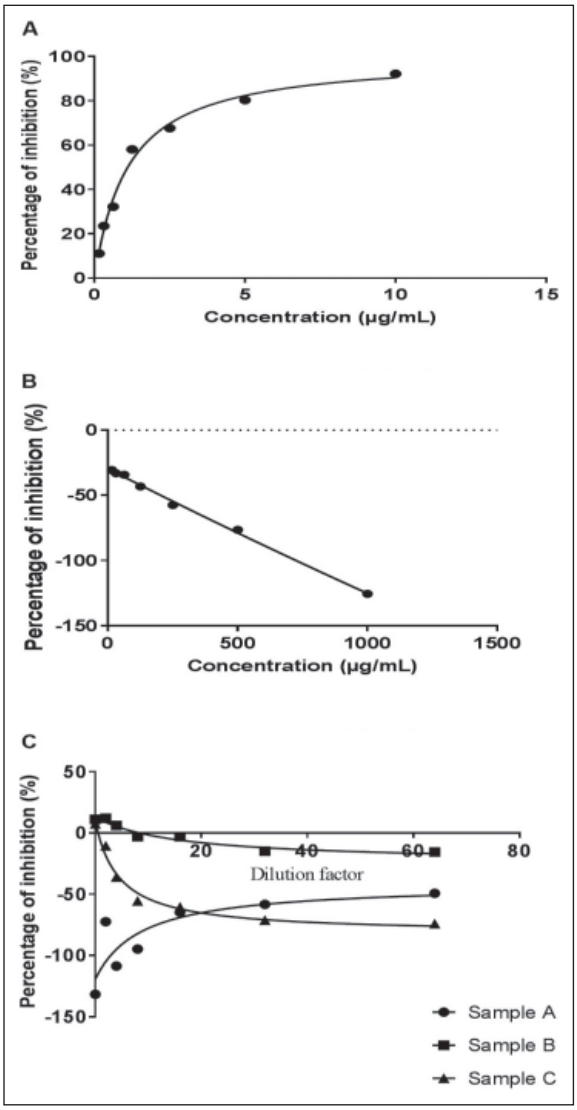

Fig. 4. The anti-diabetic activity of (A) quercetin as standard reference, (B) freshly prepared extract and (C) commercial stevia extract: ● Sample A, ■ Sample B and ▲ Sample C as determined by the percentage of inhibition towards α-glucosidase enzyme. Data represent mean ± SD (n=3).

CONCLUSION

The present study showed that the phenolic and flavonoid compounds, as well as antioxidant activity of all commercial *Stevia* samples to be significantly lower when compared to the freshly prepared *Stevia* extract. However, the anti-diabetic activity of all commercial *Stevia* extracts showed a significantly higher activity compared to the freshly prepared *Stevia* extract. Both samples B and C were able to inhibit the activity of α-glucosidase at a lower capacity (<50%) when presented at higher concentrations, which warrants further investigation. Fundamentally, the present study highlights the biological activities of commercial *Stevia* extract in Malaysia, which is paramount for its development in functional food applications, but caution has to be exercised as all three commercial extracts have significantly different biological activities due to the different raw materials and manufacturing processes.

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