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Biochemical evaluation of phenolic compounds and steviol glycoside from *Stevia rebaudiana* extracts associated with *in vitro* antidiabetic potential

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ABSTRACT

Apart from being well known to the world as herb-based sweetening additive, *Stevia rebaudiana* (*S. rebaudiana*) and its phenolic compounds are considered as a natural antidiabetic alternative to replace synthetic drugs that possess numbers of side effects. Therefore, this study was aimed to evaluate the solvent effects on the extraction of phenolic compounds and steviol glycoside identification associated with antidiabetic potential of the extracts. Total phenolic (TPC) and flavonoid (TFC) content of the extracts were quantified, while the antidiabetic activity of the extracts was determined by α-amylase and α-glucosidase inhibitory assay. As a matter of interest, TFC was found to be present at the highest concentration in ethanol extract (10.91 mg QE/g), while the presence of TPC showed no significant difference between water extract (6.65 mg GAE/g) and other organic solvents. HPLC analysis showed the abundant presence of steviol glycoside in the water extract, the principal compound suggested for treating diabetes. Furthermore, GC-MS analysis has shown the major compounds of 1-heptatriacotanol, duvatrienediol, dihydroxanthin, β-amyrin, lupenone, phytol, γ-sitosterol, agatholic acid and fatty acids were present. In relation to the antidiabetic potential, the effects of the extracts in inhibiting α-amylase and α-glucosidase activity were investigated *in vitro*. Interestingly, among all *S. rebaudiana* extracts, water extract exhibited the most significant α-amylase inhibitory activity with IC_{50} = 8.63 μg/ml, comparable to the synthetic drug, acarbose IC_{50} = 13.73 μg/ml. These findings demonstrated that phenolic recovery was highly dependent on extraction solvent and the promising water extract as the best α-amylase inhibitory potential with greatest steviol glycoside recovery.

1. Introduction

Diabetes mellitus is a chronic metabolic illness that associates with hyperglycemia or high blood glucose level due to defects of insulin secretion, action or both (Chaudhury et al., 2017). Kazi (2014) stated that many individuals were reported to be affected by diabetes in the 21st century, thus diabetes is known as the fifth leading cause of death. Type 2 diabetes mellitus (DM) which also known as non-insulin dependent diabetes mellitus is the most common form of diabetes. This type of diabetes is caused by disproportionation of insulin secretion and blood sugar absorption (Afrisham et al., 2015) which leads to higher risk of both short and long term complication which normally causes death at a younger age (Olokoba et al., 2012). Type 2 DM has gained more focused by many researchers for their management and alternative treatment as this type of diabetes is considered to be preventable and curable (Afrisham et al., 2015).

The global statistic has shown that 415 million of people around the world were reported to be diagnosed with diabetes on the last two years and 3.3 million among them are Malaysian (International Diabetes Federation, 2017). Plenty of medicinal plants have been studied for their antidiabetic potential (Kooti et al., 2016; Osadebe et al., 2014) due to the presence of carotenoids, flavonoids, polyphenols, terpenoids, alkaloids and glycosides which have been found to possess antidiabetic effects (Adekola et al., 2017; Coman et al., 2012; Etxeberria et al., 2012; Lim and Loh, 2016). Flavonoid is a large component of polyphenols and possesses many biological activities, for instance, anti-inflammatory, antitumor and antibacterial effects (Afrisham et al., 2015).

Multiple studies had found that *Stevia rebaudiana (S. rebaudiana)*

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exhibit various therapeutic benefits as they possess antioxidant, antidiabetic, antimicrobial and antitumor properties (Zaidan et al., 2018; Jayaraman and Manoharan, 2008; Kim et al., 2011; Ruiz-Ruiz et al., 2015; Shukla et al., 2009; Singla et al., 2017). *S. rebaudiana* Bertoni is a small perennial herb that originated from Paraguay and Brazil. Their leaves have been widely used around the world as a natural sweetener (Lemus-Mondaca et al., 2012) as it contains stevioside as the main compound that contributes to the sweetness properties of *S. rebaudiana* (Ceunen and Geuns, 2013). Due to the various and multiple biological benefits exerted by *S. rebaudiana* leaves, the plant properties have attracted numerous scientific and economic interests (Chatsudthipong and Muanprasat, 2009).

One therapeutic approach for inhibiting postprandial hyperglycemia is to impede glucose absorption by inhibiting carbohydrate hydrolysing enzymes, α-amylase and α-glucosidase presence in the digestive organ. Therefore, dietary modification should be given attention since it is the simplest and cheapest way of treating diabetes (Ojo, 2013). Consequently, due to *S. rebaudiana* leaf composition and health-promoting phytochemical constituents (Chatsudthipong and Muanprasat, 2009; Lemus-Mondaca et al., 2012), it is essential to establish further study on the evaluation of plant extraction approach using safe and effective solvent for human consumption as well as their bioactive compounds potential in inhibiting α-amylase and α-glucosidase enzymes. Hence, this study was aimed to evaluate the solvent effects on the extraction of phenolic compounds and steviol glycoside identification using HPLC and GC-MS associated with the antidiabetic potential of the extracts which could enhance the utilization of *S. rebaudiana* extracts for therapeutic purposes.

2. Materials and methodology

2.1. Sample collection and preparation

S. rebaudiana dried leaves used in this study were obtained from Koperasi Warisan Munsyi, Selangor Berhad (KOWARIS), Malaysia. The sample preparation was carried out according to a method described by Kamal (2016). The dried leaves were milled using a dry blender into powdered form. Samples were then stored in opaque polyethylene airtight container, at room temperature prior to extraction.

2.2. Crude extracts preparation

The plant extracts were obtained using the maceration technique according to Kamal (2016). Twenty-five gram of powdered leaves was suspended in 250 ml of solvent (water, methanol, ethanol and acetone) separately in a conical flask. The mixture was macerated in an orbital incubator shaker at 200 rpm, for 90 min, at 40 °C. The mixture was then filtered using 110 mm pore size Whatman filter paper and the marc (damp solid material) was pressed using a vacuum pump. For the preparation of methanol, ethanol and acetone extract, the filtrate was concentrated using a rotary evaporator at a temperature of 60–65 °C to remove the solvent. While, for preparation of water extract, the filtrate was frozen at -20 °C before lyophilized in a freeze dryer at -55 °C for 96 h until the sample turned into a powdered form. All the extracted samples were stored in a sealed container at -20 °C until further use.

2.3. Total phenolic content (TPC)

TPC was measured according to Ashraf et al. (2015) method with slight modification, by performing Folin-Ciocalteu assay. 500 µl of diluted sample (1000 µg/ml) was mixed with 3.7 ml distilled water, 250 µl of Folin-Ciocalteu and 500 µl of 5% Na_2CO_3. The mixture was incubated in the dark for 1 h and the sample was prepared in triplicates. The sample solution absorbance was measured at 760 nm against a reagent blank. A standard calibration curve for gallic acid 0–100 µg/ml was prepared and each of the sample phenolic content was expressed as gallic acid equivalent (mg GAE/g dry weight).

2.4. Total flavonoid content (TFC)

TFC of samples were determined using aluminum chloride method described by Ruiz-Ruiz et al. (2015). Briefly, 0.5 ml of the sample solution (1000 µg/ml), 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride ($AlCl_3$), 0.1 ml of 1.0 M potassium acetate and 2.8 ml of distilled water were added together and the mixture was left to incubate in the dark at room temperature for 30 min. The sample was prepared in triplicates. Finally, the absorbance was measured at 415 nm against a reagent blank. A standard calibration curve for quercetin in the range of 0–200 µg/ml was prepared and each of the sample flavonoid content was expressed as quercetin equivalent (mg QE/g dry weight).

2.5. HPLC analysis of steviol glycoside

The chromatographic analysis was performed based on method described by Woelwer-Rieck et al. (2010) on Agilent 1100 series HPLC-DAD system (Agilent Technologies Deutschland GmbH, Germany) using a reverse phase Nucleosil 100 C18 column (250 × 4.5 mm) packed with 5 µm diameter particles which was controlled at a temperature of 36 °C. The mobile phase consisted of acetonitrile/water (80:20 v/v) set to a flow rate of 1.0 ml/min kept at 36 °C. The sample injection volume was 10 µl. All the extract was prepared in 7.5 mg/ml concentration. A stock standard solution of steviol glycoside compound was prepared at concentrations of 0.8, 2.5, 5.0, 7.5 and 10 mg/ml. The chromatogram peaks were confirmed by comparing its retention time with those of reference standards.

2.6. GC-MS analysis of S. rebaudiana leaf extracts

The GC-MS analysis of *S. rebaudiana* leaf extracts was carried out using Hossain et al. (2010) method by using GC-MS (Model QP5050A, Shimadzu Japan) equipped with an HP5MS fused silica capillary column (30 m × 0.25 i. d. Mm, film thickness 0.25 µm). An electron ionization system with an energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 ml/min. The injector and mass transfer line temperatures were set at 250 °C and 300 °C, respectively. The oven temperature was programmed from 50 °C to 200 °C at 8 °C/min, and then kept isothermal for 20 min and finally raised to 300 °C at 10 °C/min and held for 5 min 1 µl of diluted samples (1/100 w/v, in methanol) were manually injected in splitless mode. Identification of compounds of the leaf extracts was based on GC retention times on the HP5MS capillary column, with computer matching of mass spectra with standards.

2.7. α-Amylase inhibition assay

The inhibitory effect of extracts against alpha-amylase activity was determined according to the 3,5-dinitrosalicylic acid (DNSA) method described by Wickramaratne et al. (2016) using 96-wells microplate with slight modification. Briefly, leaf extract of *S. rebaudiana* was dissolved in minimum amount of methanol and was further dissolved in buffer ($Na_2HPO_4/Na_2H_2PO_4$ (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentration ranging from 12.5 to 600 µg/ml. A volume of 200 µl of *Bacillus licheniformis* α-amylase (2 units/ml) was mixed with 200 µl of extract and was incubated for 10 min at 30 °C. Then, 200 µl of the starch solution (1% in a buffer, w/v) was added to each tube and incubated for 3 min. The reaction was terminated by the addition of 200 µl DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of 3,5-dinitrosalicylic acid solution) and was boiled for 10 min in a water bath at 90 °C. The mixture was cooled to ambient temperature and was diluted with 5 ml of distilled water, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. Acarbose standard with concentrations ranging

from 12.5 to 600 µg/ml was also conducted.

Anti-α-amylase activity (inhibition %) of each sample was estimated using the equation below:

$$Inhibition(\%) = \left\{Ac - \frac{(As - Ab)}{Ac}\right\} \times 100$$

Where Ac = Absorbance of control (100% enzyme activity control subtract with 0% enzyme activity control), Ab = Absorbance of the sample blank and As = Absorbance in the presence of the sample.

2.8. α-Glucosidase inhibition assay

The inhibitory effect of extracts against alpha-glucosidase activity was measured according to the method described by Lee et al. (2014) using 96-wells microplate with slight modification. The ρ-Nitrophenyl-ρ-D-glucopyranosidase (PNPG) substrate was prepared by dissolving in 50 mM phosphate buffer (pH 6.5). The samples were prepared at a range of 15.63–1000 µg/ml and triplicate serial dilutions were performed. All samples were mixed in the 96-well microplate with 30 Mm phosphate buffer and 10 µl of α-glucosidase enzyme solution (from Maltase) and incubated at room temperature for 5 min. Then, 75 µl of PNPG was added to each well of the sample, blank substrate, negative control and positive control while the rest were loaded with 75 µl of 30 Mm phosphate buffer. The mixtures were incubated for 15 min at room temperature and stopped by using 50 µl of 2 M glycine (pH 10) for the sample, blank substrate and negative control. Quercetin standard was prepared with concentration ranging from 15.63 to 1000 µg/ml and the absorbance was measured at 405 nm.

The inhibition of α-glucosidase in percentage was calculated using the following equation:

$$Inhibition(\%) = \left\{Ac - \frac{(As - Ab)}{Ac}\right\} \times 100$$

Where, Ac = Absorbance of control (100% enzyme activity control subtracts with 0% enzyme activity control), Ab = Absorbance of the sample blank and As = Absorbance in the presence of the sample.

3. Results and discussion

3.1. Effect of extraction solvents on the yield of crude extracts

Extraction of the secondary metabolites from plant material is the major focus of investigation. The presence of phenolic families variation with different chemical structure and polarities rationalize in the use of wide range of extraction solvents (polar and non-polar types). In the present study, extraction of *S. rebaudiana* was performed using polar protic solvents (water, methanol, ethanol) and polar aprotic solvent (acetone). Table 1 shows that the extraction yields of *S. rebaudiana* decreased from water extract (27.64%) followed by methanol extract (18.80%), ethanol extract (8.98%) and acetone extract (3.36%). The extraction yield of water extract is prominently different from methanol extract by 9%. This result indicates that increasing polarity of solvent significantly enhances plant extraction yield. Nevertheless, Do et al. (2013) found that methanol is the best solvent for extraction of *Limphnophila aromatic* compared to water, ethanol and acetone, however, the extraction yield of water was only found to be slightly lower than methanol.

Table 1
Extraction yield and physical characteristics of *S. rebaudiana* in water, methanol, ethanol and acetone extracts.

Sample	Extraction yield (%w/w)	Physical characteristic
Water Extract	27.64 ± 1.82	Brown coarse solid
Methanol Extract	18.80 ± 1.13	Green coarse solid
Ethanol Extract	8.98 ± 0.31	Green coarse solid
Acetone Extract	3.36 ± 0.91	Dark green, thick semi-solid

The variation of extraction yields could be due to the difference in the solvents polarities which also play a vital role in enhancing the solubility of phytochemicals compounds (de Silva et al., 2014; Naima et al., 2015). Besides, the macromolecules such as protein and carbohydrate which are highly soluble in water might be the other contributor of compounds to be extracted in water (Zaidan et al., 2018). Furthermore, an aqueous water system which belongs to non-polluting and nontoxic properties was observed to be the best solvent for *S. rebaudiana* phytochemical extraction. According to Zhang (2015), the variability of plant extraction yield might be attributable to the different extraction solvent system with different in polarity, dispersibility and penetrability, which could selectively extract different phytochemicals. In addition, the levels of total phenolic and flavonoids in plant extracts also could possibly be influenced by the dielectric constant and chemical structure of organic solvents (Zhang, 2015). Referring to the present study, the dielectric constant of solvents used was seemed to increase from acetone (21.01), ethanol (24.6) and methanol (32.6) to water (78.54).

3.2. Determination of TPC and TFC content

Recovery amount of TPC from *S. rebaudiana* in methanol, water and ethanol extracts show significantly higher than in acetone extract. Fig. 1 elucidates that methanol extract appeared to be the highest total phenolic content of 6.96 mg GAE/g, followed by insignificant TPC in water extract (6.65 mg GAE/g) and ethanol extract (6.43 mg GAE/g), while the TPC in acetone extract (5.41 mg GAE/g) shows the lowest. This result obtained is in agreement with Azmir et al. (2013) which reported that polar solvents are preferably used for extracting soluble polyphenol presence within the plant cell vacuoles and alcohols are more efficient in the cell wall and seed degradation. Many studies also discovered that methanol and ethanol became the better choice of solvents for extracting phenolic compounds from plant materials than less polar solvents including acetone and hexane (Mohsen & Ammar, 2009; Zarena and Sankar, 2009). Besides that, the water removal method of freeze-drying used in this study had involved none usage of heat compared to the removal of other solvents, which was believed to preserve phenolic compounds in water extract. Hence, this observation proved that the polarity of solvents influences phenolic recovery. Higher polar solvent could be a better choice of solvents in recovering higher phenolic content than less polar solvent.

The result of TFC of the various extracts indicates that ethanol extract (10.91 mg QE/g) recovers the highest TFC followed by acetone (9.37 mg QE/g) and methanol (8.08 mg QE/g) extracts. Meanwhile, water extract (3.53 mg QE/g) exhibits the lowest TFC. It was observed

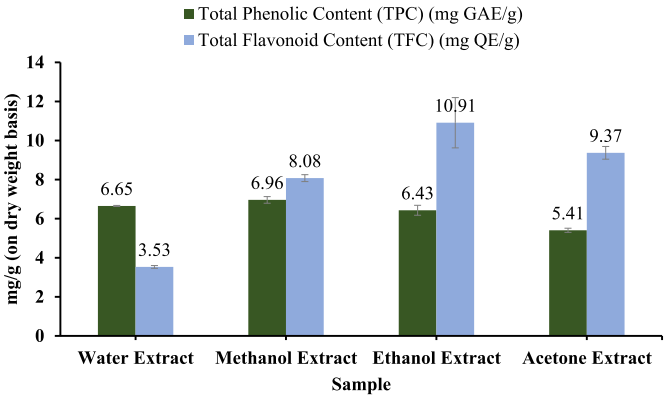

Fig. 1. The total phenolic content (TPC) and total flavonoid content (TFC) of *S. rebaudiana* extracts. TPC and TFC were expressed as mg GAE/g and mg QE/g respectively. Error bars represent mean ± standard deviation (n = 3).

that the effect of solvents on TFC is different from TPC. The results show that flavonoids were extracted better in organic solvents. This may be due to the presence of flavonoids with higher molecular weight and complex structure that more soluble in ethanol, acetone and methanol than in water. In addition, the presence of less polar flavonoids containing fewer hydroxyl group than phenolic is more favorable to be extracted in less polar organic solvents. Less polar flavonoid for instance isoflavones, flavanones and flavonols are more soluble in less polar solvents (Andersen and Markham, 2006). The result also shows that flavonoid significantly contributes to higher content than phenolic compounds. This finding might be due to the higher molecular weight of extracted flavonoids than extracted phenolic compounds. In fact, the flavonoid is the most ubiquitous group of polyphenols which might be a contributor to the result gained.

Usage of organic solvents after extraction may need further separation technique to reduce its toxicity as those solvents, for instance, generally, methanol and acetone are not safe for human consumption (Bhebhe et al., 2016). Water and ethanol are safe for human consumption (Bhebhe et al., 2016; Dai and Mumper, 2010), however, maceration using ethanol require a longer period than water. Therefore, water can be considered as the best solvent for phenolic extraction and also become the most common solvent that has been used traditionally and is easier to handle.

3.3. HPLC analysis of steviol glycosides presence in extracts

Extracts of *S. rebaudiana* leaf were analyzed using High-Performance Liquid Chromatography (HPLC) for detection of steviol glycoside presence in all extracts. Fig. 2(a–d) shows the HPLC chromatograms of steviol glycoside present in water, methanol, ethanol and acetone extract, respectively, and their corresponding peak profiles based on steviol glycoside standard compound were presented in Table 2. Steviol glycosides were found to be present in all extracts with the most abundant in water extract (97.5% area). Insignificant steviol glycoside recovery (percentage of area) in ethanol extract (71.9%) and methanol extract (69.7%) were found to be higher than in acetone extract (25.1%). The result elucidates that steviol glycoside may favor solubility in water than in organic solvents. In addition, the freeze-drying process used in removing water involves none usage of heat which may preserve steviol glycosides presence in water extract. However, Celaya & Kolb (2016) reported that stevioside and rebaudioside A which are the main steviol glycosides extracted from stevia show less soluble in water.

Table 2
HPLC profile of steviol glycoside of *S. rebaudiana* in water, methanol, ethanol and acetone extracts.

Extract	Retention time (min)	Area (%)
Standard	2.95	85.6
Water	2.56	97.5
Methanol	2.84	69.7
Ethanol	2.78	71.9
Acetone	2.96	25.1

Peak chromatogram profiles of steviol glycosides presence in ethanol extract and acetone extract show additional peak which might due to the presence of impurities or other glycosides in the sample. However, the first peak from both of the extract is believed belongs to the steviol glycoside when compared to the retention time of steviol glycosides standard. Physicochemical processes which comprise of extraction, solubilization and crystallization procedure are greatly influenced by solvents (Kerton, 2013). Hence, steviol glycoside recovery from water extract has been utilized and preferred for the development of food additives (Rai et al., 2012; Uchiyama et al., 2012). Interestingly, the recovery of steviol glycosides in this study was found to be the most abundant in water extract as compared to other organic solvents, thus revealing water as a promising solvent for recovery of steviol glycoside

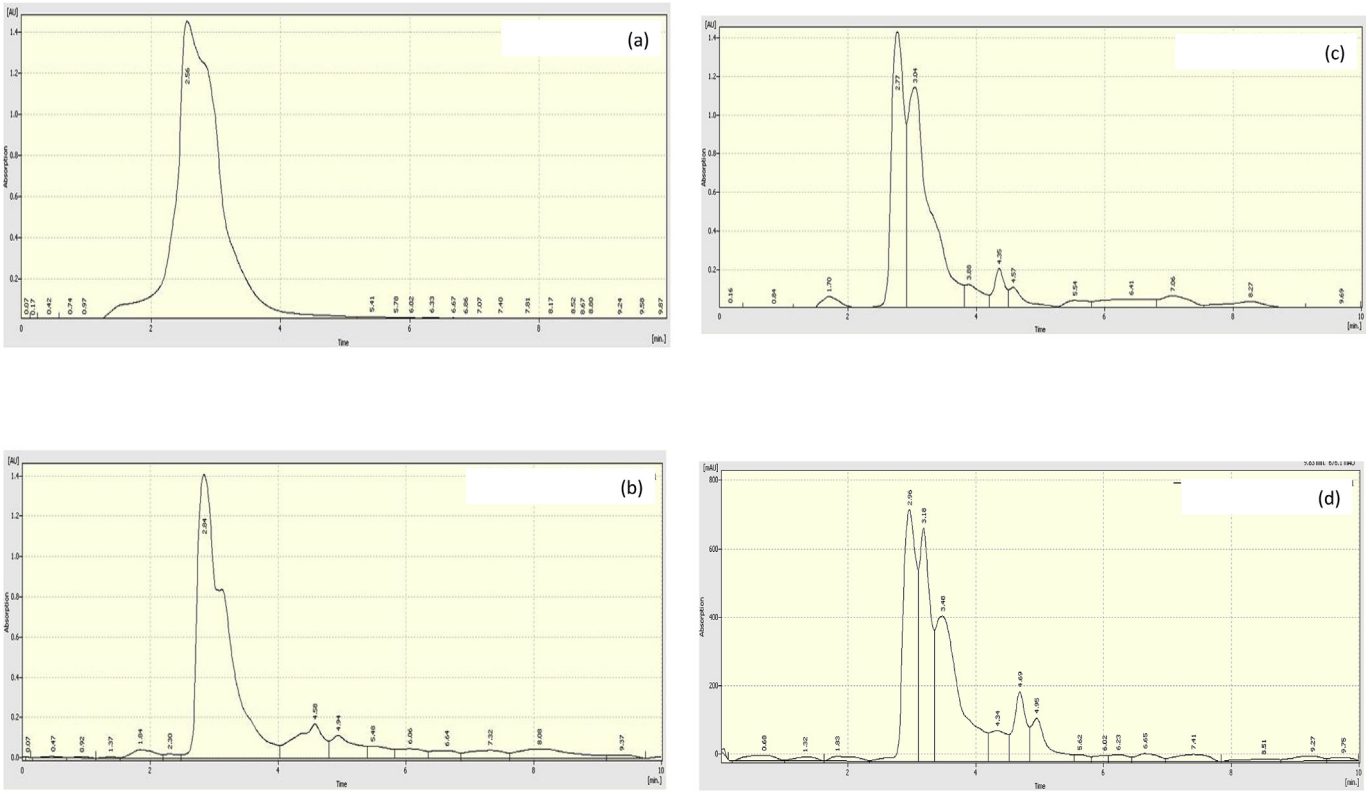

Fig. 2. HPLC chromatogram of water extract (a), methanol extract (b), ethanol extract (c) and acetone extract (d) from *S. rebaudiana*. The chromatographic analysis was performed on Agilent 1100 series HPLC-DAD system.

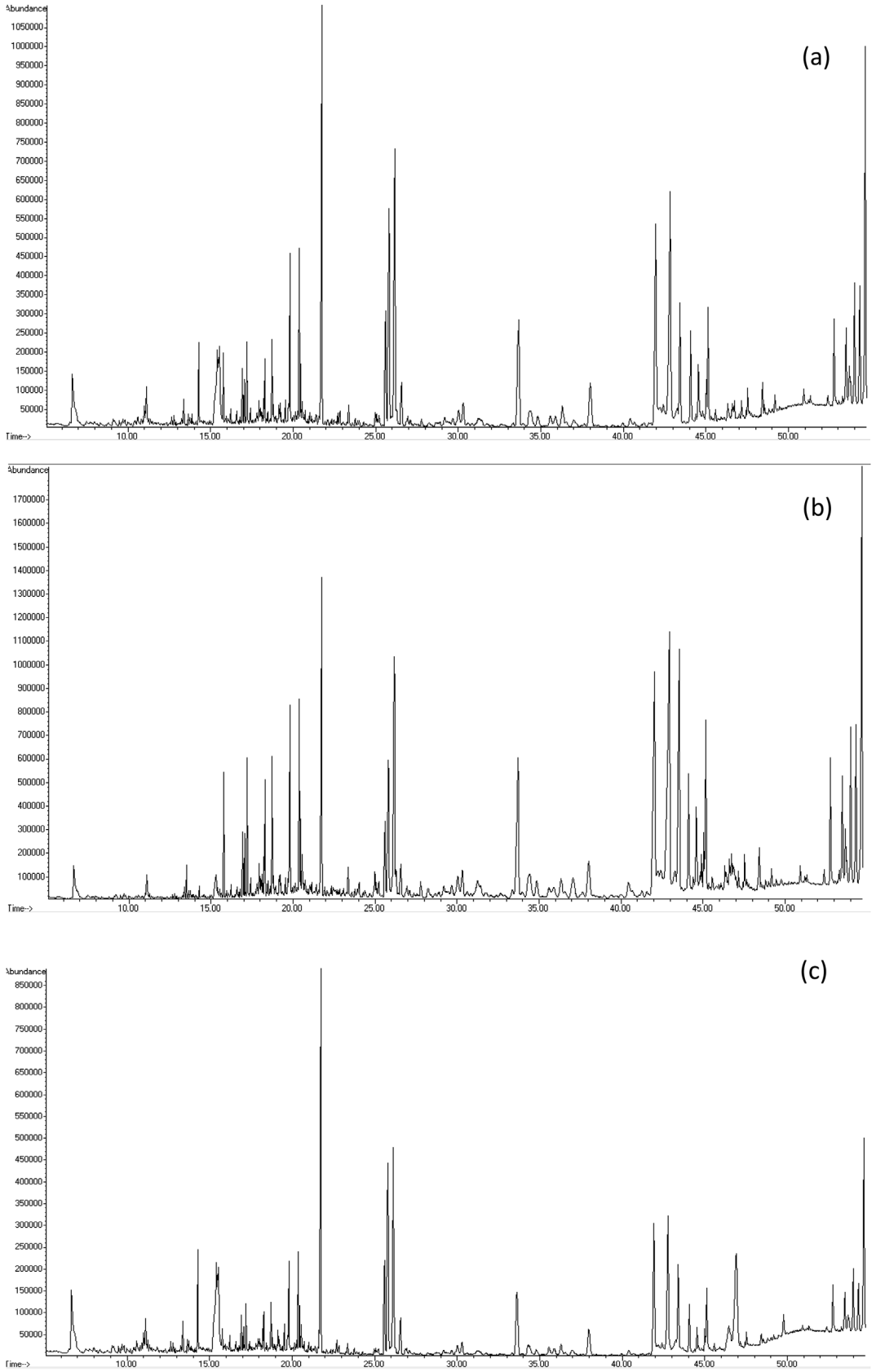

Fig. 3. GC-MS chromatogram of the constituents of methanol extract (a), ethanol extract (b) and acetone extract (c) from *S. rebaudiana*. Each chromatogram was analyzed using Agilent MSD ChemStation.

Table 3
Biological activities of major compounds found in the extracts.

Compound name	Activity	Reference
1-Heptatriacotanol	Antihypercholesterolemia	Baskaran et al. (2015)
Duvatrienediol	Wax	Chiang and Grunwald (1976)
Dihydroxanthin	Antitumor	Karmakar et al. (2015)
β-Amyrin	Anti-inflammatory	Holanda Pinto et al. (2008)
Lupenone	Anti-inflammatory	Romero-Estrada et al. (2016)
Phytol	Antidiabetic	Elmazar et al. (2013)
Agatholic acid	Resin	Bates et al. (2003)
Palmitic acid	Saturated fatty acid	Laposata (1995)
Linoleic acid	Unsaturated fatty acid	Laposata (1995)

from *S. rebaudiana* leaf.

3.4. GC-MS analysis of S. rebaudiana leaf extracts

GC-MS chromatograms (Fig. 3(a–c)) represent chemical compounds present in organic solvents of methanol, ethanol and acetone of *S. rebaudiana* leaf extracts, respectively. Identification of compounds was based on a comparison of relative retention indices with mass spectra library data standards (Basak and Candan, 2010). The major compounds found in most of the extracts are agatholic acid, 1-heptatriacotanol, duvatrienediol, dihydroxanthin, β-amyrin, lupenone, phytol, palmitic acid and linoleic acid which belong to alcoholic (polar), terpene hydrocarbon (non-polar), terpene alcohol (polar), saturated and unsaturated fatty acids. In relation to the major compounds present, Table 3 shows the potential biological activities exerted by corresponding major phytochemical identified in the extracts.

3.5. α-Amylase and α-glucosidase inhibitory activity of leaf extracts

The α-amylase and α-glucosidase inhibitory potential of all the extracts were compared as shown in Table 4. Results were expressed as percentage inhibitory activity and the efficacy of the extracts was compared based on their IC_{50} values. Acarbose was used as a standard drug to compare the α-amylase inhibitory effects, while quercetin was used as a standard for α-glucosidase inhibitory effects. As shown in Table 4, the IC_{50} value calculated from the standard curve of acarbose (12.5–600 µg/ml) on the inhibitory effects toward α-amylase activity was found to be 8.63 µg/ml. Among all the *S. rebaudiana* leaf extracts, water extract has shown the lowest IC_{50} value with 13.73 µg/ml, comparable with the standard acarbose (8.63 µg/ml), while the other extracts did not show any significant inhibition in α-amylase activity with IC_{50} showing much greater than 600 µg/ml *The* IC_{50} value of α-amylase inhibition by methanol, ethanol and acetone extracts were not possible to be determined due to the nearly no inhibition activity exerted by all the extracts. Interestingly, it could be seen that water was found to be the best solvent for displaying antidiabetic activity through inhibition of α-amylase activity.

According to Fig. 4 (a), the α-amylase inhibitory activities of the extracts were observed did not exert concentration-dependent manner. This may be due to the interaction between phenolic or non-phenolic bioactive compound that responsible for inhibition of α-amylase with

Table 4
α-Amylase and α-Glucosidase inhibitory activities of *S. rebaudiana* leaf extracts.

Sample	α-Amylase IC_{50} (µg/ml)	α-Glucosidase IC_{50} (µg/ml)
Water extract	13.73	ND
Methanol extract	> 600	ND
Ethanol extract	> 600	ND
Acetone extract	> 600	ND
Acarbose	8.63	–
Quercetin	–	2.33

ND: Non-determined.

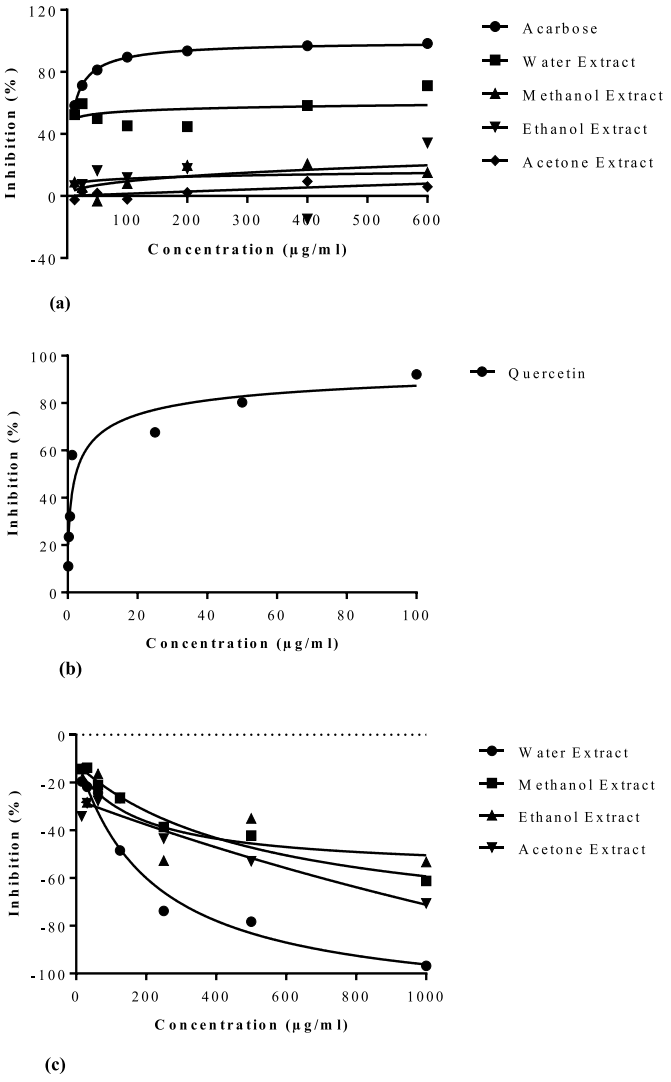

Fig. 4. (A): Dose-response curve of concentration (µg/ml) versus % inhibition α-amylase of extracts plotted using GraphPad Prism software.
Fig. 4 (b): Dose-response curve of concentration (µg/ml) versus % inhibition α-glucosidase of quercetin plotted using GraphPad Prism software.
Fig. 4 (c): Dose-response curve of concentration (µg/ml) versus % inhibition of α-glucosidase of extracts plotted using GraphPad Prism software.

solvents that affect the stabilization in the assay. The results obtained in this study were in contrast with previous antidiabetic studies on *S. rebaudiana* extracts by Ruiz-Ruiz et al. (2015) and Singla et al. (2017) thus indicating the concentration-dependent manner though water extract in this study exert better α-amylase inhibition activity than those previous study which is IC_{50} = 198.40 µg/ml and 833.68 µg/ml, respectively. A similar finding was also found by Singla et al. (2017) indicating that water extract (IC_{50} = 833.68 µg/ml) exert better α-amylase inhibition than less polar extract petroleum ether, methanol and methanol-water extracts. Besides, a study by Khan et al. (2016) reported that water extract of *Phoenix dactylifera* L. possessed much higher α-amylase inhibition properties than lower polar organic solvents (methanol, ethanol and acetone).

The result of water extract possesses high α-amylase inhibitory activity is also in line with high TPC and steviol glycosides contents recovered in this study. This observation is also in accordance with Ceunen and Geuns (2013), Jaitak et al. (2009) and Jaitak et al. (2008) which the best α-amylase inhibitory activity shown by water extract is known attributable to the presence of steviol glycosides. Moreover, it has been discovered that *S. rebaudiana* extract, steviol and stevioside

possess antidiabetic potential by acting directly on β-cells and generate insulinotropic, glucagonostatic effects and α-amylase inhibiting effects (Assaei et al., 2016; Jeppesen et al., 2002; Sireesh et al., 2015; Yang and Kong, 2016). As shown in Table 4, the IC_{50} value calculated from the standard curve of quercetin (15.6–1000 μg/ml) on the inhibitory effects toward α-glucosidase activity was found to be 2.33 μg/ml. Quercetin is used as a standard as it exhibits stronger inhibitory effects on α-glucosidase compared to acarbose (Li et al., 2009) (Fig. 4 (b)). However, in contrast to TPC and α-amylase inhibitory activity, all the extracts showed no inhibition toward α-glucosidase activity (Fig. 4 (c)). The result also elucidates that the IC_{50} values of all the extracts toward α-glucosidase activity were extremely low and showed negative inhibition percentage. The result obtained in this study is in contract with Ruiz-Ruiz et al. (2015) study which aqueous extract exert concentration-dependent inhibition towards α-glucosidase activity.

According to Rasouli et al. (2017), most docked phenolic compounds with a high binding affinity towards interaction with α-amylase active site exhibit higher docking energy which contributes to inhibitory activity compared to exhibit docking energy with α-glucosidase. The study also found that there are different polyphenols properties responsible for inhibiting α-amylase and α-glucosidase. Therefore, the primary structure of polyphenols also may change the inhibitory effects on α-amylase and α-glucosidase activity. Furthermore, it has been reported that plant-derived phenolic compounds have the ability to inhibit digestive enzyme α-amylase and α-glucosidase, thus lowering the blood sugar level and are effective as an antidiabetic agent (Kazi, 2014). The antidiabetic activity performed in plant is often due to the presence of phenolic acids, phenolic diterpenes, flavonoids, steroids, triterpenoids, alkaloids and other nitrogen compounds (Perez et al., 1998) which can be classified as polar and non-polar compounds, conferring higher antidiabetic activities performed by polar extract (Khan et al., 2016; Singla et al., 2017).

4. Conclusions

Conclusively, type of solvents for extracting plant bioactive compounds significantly influence the TPC, TFC, steviol glycosides recovery and antidiabetic activity of *S. rebaudiana* extracts. The presence of TPC, TFC and steviol glycoside were revealed to contribute the α-amylase inhibition activity in *S. rebaudiana* extracts. Among all of *S. rebaudiana* extracts, water extract was found to be the best solvent for both phenolic compounds and steviol glycoside recovery with promising α-amylase inhibition activity. Thus, water extract of *S. rebaudiana* has proven its potential in lowering blood glucose level by impeding digestion of dietary carbohydrates through inhibition of α-amylase activity. Further investigation is suggested for isolation bioactive constituent and their possible mechanism in managing and preventing diabetes mellitus.

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