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FULL PAPER

Antidiabetic and antioxidative effects of *Lepisanthes fruticosa* fruit seed extract in type 2 diabetic experimental rats

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Abstract

Lepisanthes fruticosa (Roxb) Leenh or locally known as *ceri Terengganu* is an underutilised fruit species from the Sapindaceae family. The species was previously identified as a potent antioxidant source, but scientific information is still lacking and limited to *in vitro*. Therefore, the present study aimed to investigate the *in vivo* antidiabetic and antioxidative effects of *L. fruticosa* fruit seed extract (LFSE) in a high-fat diet, streptozotocin (HFD/STZ)-induced *Sprague Dawley* rats. After 8 weeks of HFD feeding, a low-dose STZ (35 mg/kg body weight) was injected to induce type 2 diabetes mellitus (T2DM). Two dosages of LFSE (300 and 600 mg/kg body weight) were orally administered to diabetic rats for 4 weeks. High dosage LFSE (600 mg/kg body weight) showed a more pronounced effect in anti-hyperglycemic activities as indicated by both sub-chronic and acute studies. The glucose-lowering ability of LFSE was supported by its improved serum insulin level (40%) as compared to diabetic control. LFSE (600 mg/kg) also resulted in a significant ($P<0.05$) increase in superoxide dismutase (SOD) (20%) and catalase (CAT) (43%) activities, with a significant ($P<0.05$) decrease in lipid peroxidation (28%) as compared to diabetic control. With regard to lipid profile, low dosage LFSE (300 mg/kg) led to a significant ($P<0.05$) improvement in high-density lipoprotein cholesterol (HDL-c) (39%). Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of LFSE showed the presence of various phytochemicals with antidiabetic and antioxidant properties, with α-kojibiose, genistein-7,4'-di-O-β-D-glucoside and soyacerebroside II being the predominant ones. The findings may suggest that *L. fruticosa* fruit seed extract is potential in reducing hyperglycaemia and enhancing antioxidant status in HFD/STZ-induced diabetic rats. Therefore, it can be concluded that *L. fruticosa* fruit species may be considered as a promising candidate for a new and safe alternative remedy for the prevention and treatment of T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is the major contributor to diabetic incidents and has become among the most serious health challenges affecting the world population. It is the most common condition and accounts for about 90-95% of all diabetic cases (Irondi *et al.,* 2015; Chinsembu, 2018). The disease is characterised by chronic hyperglycaemia resulting from impaired insulin secretion and/or insulin resistance or insensitivity (Adam *et al.,* 2012; Tang *et al.,* 2017). The inability of the pancreas to produce sufficient insulin and/or failure of the body's cells to effectively utilise

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glucose may result in an abnormal blood glucose level in the bloodstream, instead of being taken into and used by insulin-responsive cells such as muscle, liver and adipocytes (Adam *et al.,* 2012).

The risk of developing T2DM increases with age, obesity, poor diet and physical inactivity (Choudhury *et al.,* 2018), all of which strongly contribute to insulin resistance. In obese diabetics, insulin resistance results in the failure of pancreatic β-cells to secrete a sufficient amount of insulin due to the excessive deposition of fats in non-adipose tissue, leading to β-cell dysfunction, and consequently T2DM (Retnasamy and Adikay, 2014). If uncontrolled, the metabolic disorder may lead to the pathogenesis of other chronic diseases such as cancers, neurodegenerative and cardiovascular diseases (CVD) (Martín and Ramos, 2016). Owing to the complexity of this disease, a suitable animal model that similarly reflects the human condition is vital (Gheibi *et al.,* 2017). The combination of a high-fat diet (HFD) and streptozotocin (STZ) is a valid experimentally-induced animal model of T2DM to mimic the pathology of T2DM in humans (Skovsø, 2014).

Fruits rich with natural antioxidants possess a significant antidiabetic potential and have been widely used in the prevention and treatment of DM and its associated complications (Arulselvan *et al.,* 2014). According to World Health Organisation (WHO), natural sources are excellent candidates for oral antidiabetic therapy as they are effective, non-toxic, and have poor or no side effects (Gargouri *et al.,* 2016). *Lepisanthes fruticosa* (Roxb) Leenh or locally known as *ceri Terengganu,* is an underutilised fruit species from the Sapindaceae family. The species can be found in Malaysia, Myanmar, Thailand, Indonesia and the Philippines, and has long been used as a food source and traditional remedy by rural folks (Mirfat *et al.,* 2017). It was discovered that *L. fruticosa* fruit was a promising source of antioxidants in comparison to a number of underutilised fruits and some popular fruits such as guava, mango and orange. The antioxidant activity and total phenolic content of the fruit pulp were found the highest at the unripe stage (Mirfat *et al.,* 2017). A preliminary phytochemical identification using high-performance liquid chromatography (HPLC) found the presence of 4-hydroxybenzoic acid in the fruit pulp extract, which was previously reported to have free radical scavenging activity (Nur Yuhasliza *et al*., 2018). In an *in silico* modelling, 5,6,7,4'-Tetrahydroxyflavanone6,7-diglucoside, 5,7,4'-Trihydroxy3,6,8,2',5'-pentamethoxyflavone, distemonatin, quercetin 3-galactoside-7-xyloside and cyanidin-3-O-rutinoside were suggested as promising compounds with medicinal benefits (Lina *et al*., 2018). *In vitro* antidiabetic study of aqueous extract of the unripe fruit, pulp showed that *L. fruticosa* possessed strong α-glucosidase inhibition and insulin secretion activity (Mirfat *et al.,* 2018). In this recent study, ethanolic extract of the unripe fruit seed was found to be more effective in antioxidant, α-glucosidase and α-amylase inhibitory activities than that of its pulp (Mirfat *et al.,* 2020).

To the best of our knowledge, the *in vivo* evaluation of the antidiabetic effect of *L. fruticosa* fruits is still lacking. Therefore, the current work was undertaken to investigate the antidiabetic and antioxidative potentials of *L. fruticosa* ethanolic fruit seed extract in a T2DM animal model. In addition, phytochemical identification of the fruit extract using LC-MS/MS was also described for the first time.

2. Materials and methods

2.1 Preparation of L. fruticosa seed extract (LFSE)

The fruits were manually harvested at their unripe stage from Malaysian Agricultural Research and Development Institute (MARDI) fruit germplasm, Serdang, Selangor, Malaysia. The seeds were separated from the pulps and kept at -80°C. Then, they were lyophilised at -85°C, pressure 250 mtorr for 3 days using a bench-top freeze dryer (Virtis, USA). The lyophilised seed samples were successively extracted with increasing polarity of organic solvents; hexane, chloroform, ethyl acetate and ethanol. The extraction started with hexane by soaking the samples in the respective solvent (1:10) with continuous stirring on an orbital shaker (Protech, Malaysia) set at 100 rpm, for approximately 24 hrs. Extraction was repeated three times under identical conditions. The filtrates were combined and brought to complete dryness using a rotary evaporator (Buchi, Switzerland) to obtain hexane crude extract. The same extraction procedure was applied to the consecutive solvents until ethanolic seed extract (LFSE) was obtained. The extract was kept at −80°C overnight prior to lyophilisation. The LFSE was prepared at two different dosages; 300 and 600 mg/kg body weight using 0.03% (w/v) carboxy methyl cellulose (CMC), which represented as low dosage treatment (LT) and high dosage treatment (HT), respectively.

2.2 Experimental animals

The ethical approval was obtained from the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia (UPM/IACUC/AUP-R091/2018). Thirty healthy male *Sprague Dawley* (SD) rats (N=30), 4 weeks of age and weighing approximately between 100 and 150 g were purchased from Sapphire Enterprise, Selangor, Malaysia. The rats were housed in

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an individual ventilated cage and maintained on a controlled environmental condition (12 hrs light and 12 hrs dark cycle, 25±30°C, 35–60% humidity). They were given free access to a standard rat diet (Specialty Feeds, Australia) and plain drinking water during a 1-week acclimatisation period. Following acclimatisation, the rats were randomly divided into five groups with six rats per group (n=6).

2.3 Induction of obesity

Obesity was induced using a commercial saturated high-fat pelleted diet (MP Biomedicals, USA). Four groups (excluding the normal control group), were given the high-fat diet (HFD) of 25 g/rat daily for 8 weeks of obesity induction. The purpose of obesity development prior to diabetes induction was to mimic the human T2DM. The HFD contained a total caloric value of 439 kcal/100 g. The normal control (NC) group was given a standard rat diet (Specialty Feeds, Australia) of 25 g/rat daily which contained 368 kcal/100 g. Bodyweight (BW) was measured weekly to ensure the development of obesity.

2.4 Induction of diabetes

At week 9, the overnight fasted HFD-fed rats were injected intraperitoneally (i.p) with a fresh solution of low-dose streptozotocin (STZ) (35 mg/kg BW) (Sigma Aldrich, USA) prepared in 0.05 M cold citrate buffer (pH 4.5) (Mokiran *et al.,* 2014). Following STZ injection, the rats were provided with 10% of glucose solution in their drinking bottles for 24 h to avoid the occurrence of hypoglycaemia. After 1 week, the fasting blood glucose (FBG) level was measured by tail pricking method using a strip glucometer (Accu-Chek, Roche Diagnostics, USA) to confirm the development of diabetes. As recommended by WHO, rats with FBG levels of more than 11.1 mmol/L (equivalent to 200 mg/dL) were considered diabetic (Kumar *et al.,* 2012) and therefore, were further used in the study.

2.5 Experimental design

Experimental animals were randomly divided into five groups of six rats (n=6) each and treated as follows:

Group I (Normal Control): Normal rats administered 0.03% (w/v) CMC (10 mL/kg BW)

Group II (Diabetic Control): Diabetic rats administered 0.03% (w/v) CMC (10 mL/kg BW)

Group III (Positive Control): Diabetic rats administered 10 mg/kg BW metformin (10 mg/kg BW)

Group IV (Low Treatment): Diabetic rats administered 300 mg/kg BW LFSE

Group V (High Treatment): Diabetic rats administered 600 mg/kg BW LFSE.

One week after experimental diabetes induction, week 10, was commenced as the first week of the treatment in the group of rats. The LFSE treatment doses were selected based on a previous study by Mokiran *et al.* (2014). All other treatments were administered once daily for 4 weeks by gastric intubation using a force-feeding needle. Fasting blood glucose levels and body weight was measured weekly. Water and food intake were monitored daily.

2.6 Oral glucose tolerance test

On the 13th week, 24 hrs before sacrifice day, all experimental animals underwent an oral glucose tolerance test (OGTT). The OGTT determines the short-term effect of the treatments on glucose metabolism. The rats fasted for 18 hrs with free access to water before OGTT was performed. Treatments were given to the rats 30 mins before administering 10% glucose (2 g/kg BW) by gastric intubation. Blood samples were acquired by tail pricking method to obtain baseline blood glucose levels (0 min, just before glucose load). Subsequently, blood was collected at 15, 30, 60, 90 and 120 mins after glucose loading. Blood glucose levels were estimated by a glucometer and the results were expressed as mmol/L. Comparisons were made on the basis of the glucose response curve (0 – 120 mins) and the area under the curve (AUC) between each experimental group.

2.7 Biochemical analysis

All rats fasted overnight before collection of blood at week 9 (baseline) and after 4 weeks of treatment, week 13 (final). The blood was collected with or without anticoagulant for plasma and serum separation, respectively, and centrifuged using a refrigerated centrifuge (Hettich Zentrifugen, Germany). Plasma antioxidant enzyme superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were assayed using a commercially available standard kit by Cayman Chemical (USA), while plasma catalase (CAT) activity and serum insulin level were measured according to Elabscience (USA) kits. Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARS) using a commercial kit from Cayman Chemical (USA). Serum lipid profile which includes total cholesterol (TC), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-c) and high-density lipoprotein-cholesterol (HDL-c) was evaluated by an automated chemistry analyser (Dimension Xpand Plus, Siemens Medical Solutions Inc., USA) using CHOL, TGL, ALDL and AHDL Flex reagent cartridges following standard procedures of the manufacturer (Dimension, Siemens Medical Solutions Inc., USA).

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2.8 Identification of phytochemical compounds using LC -MS/MS

Ultra-high pressure liquid chromatography (UHPLC) was performed on the ACQUITY UPLC I-Class system from Waters (Milford, USA), consisting of a binary pump, a vacuum degasser, and an auto-sampler and a column oven. Compounds were chromatographically separated using a column ACQUITY UPLC HSS T3 (100 mm × 2.1 mm × 1.8 µm), maintained at 40°C. A linear binary gradient of water (0.1% formic acid) and acetonitrile was used as mobile phases A and B, respectively. The mobile phase composition was changed during the run as follows: 0 min, 1% B; 0.5 min, 1% B; 16.00 mins, 35% B; 18.00 mins, 100% B; 20.00 mins, 1% B. The flow rate was set at 0.6 mL/min and the injection volume was 1 µL.

For MS/MS characterisation, the UHPLC system was coupled to a Vion IMS quadrupole time-of-flight (QTOF) hybrid mass spectrometer (Waters) operated at 50,000 FWHM and equipped with a Lock Spray ion source. The ion source was operated in negative electrospray ionisation (ESI) mode under the following specific conditions: capillary voltage, 1.50 kV; reference capillary voltage, 3.00 kV; source temperature, 120°C; desolvation gas temperature, 550°C; desolvation gas flow, 800 L/h, and cone gas flow, 50 L/h. Nitrogen (>99.5%) was employed as desolvation and cone gas. Data were acquired in high-definition MSE (HDMSE) mode in the range of m/z 50 - 1500 at 0.1 s/scan. Thus, two independent scans with different collision energies (CE) were alternatively acquired during the run: a low-energy scan at a fixed CE of 4 eV, and a high-energy scan where the CE was ramped from 10 to 40 eV. Argon (99.999%) was used as collision-induced-dissociation gas. The MS data were processed and analysed with UNIFI Software (Waters). All compounds were putatively identified by comparing full scan mass spectra, interpreting their parent ions $[M-H]^-$, MS/MS fragmentation pattern and by matching the above data with that in internal and web databases such as PubChem and ChemSpider.

2.9 Statistical analysis

All experiments were carried out in triplicates and presented as means ± standard error (SE). Data were statistically analysed by two-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) using Statistical Analysis System (SAS) 9.4 for Windows. The significance level was set at $P<0.05$.

3. Results and discussion

3.1 Changes of body weight in HFD/STZ-induced diabetic rats

The combination of the HFD and STZ model is a valid experimentally-induced animal model of T2DM to mimic human diabetes syndrome. As previously described, prolonged feeding with HFD may lead to the development of obesity which is due to hyperinsulinemia, insulin resistance and/or glucose intolerance (Skovsø, 2014). The animals become mildly hyperglycaemic or pre-diabetic which refers only to the early stage of T2DM pathology (Barrière *et al.,* 2018). Therefore, the incorporation of STZ is important to reduce the pancreatic β-cells capacity and develop significant hyperglycaemia (Furman, 2016). The body weight changes of an experimental group of rats throughout the study period are displayed in Figure 1. All groups showed significant differences ($P<0.05$) in body weights between week 0 (before HFD feeding) and week 8 (after HFD feeding). Rats in the HFD groups (DC, PC, LT and HT) put on more than 70% of their original body weights (week 0), whereas the NC group gained approximately 60%. After 8 weeks of obesity induction, the body weights of HFD groups were significantly ($P<0.05$) higher (24-27%) than NC, ranging from 427.99-446.99 g. Based on the significantly higher body weight gain relative to the negative control, it can be inferred that HFD-induced obesity was successfully achieved in the rats. According to Thibault (2013), moderate obesity in rats has been defined as having 10–25% higher body weight than chow-fed control, whereas an increment greater than 40% indicates severe obesity.

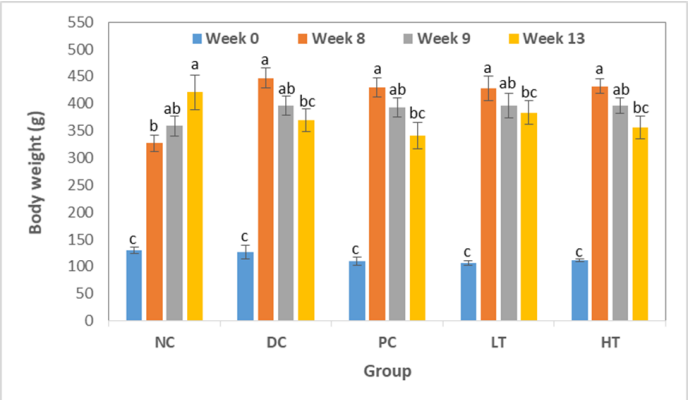

Figure 1. Body weight changes of HFD/STZ-induced diabetic rats. Week 0 (initial body weight before HFD feeding), week 8 (after HFD feeding except NC group), week 9 (after STZ injection to HFD groups, before treatment) and week 13 (after treatment). Values are expressed as the mean ± standard errors. Bars with different alphabet notations are significantly different between groups at the level of $P<0.05$. NC: normal control (normal diet + vehicle), DC: diabetic control (HFD + STZ + vehicle), PC: positive control (HFD + STZ + metformin), LT: low dose treatment (HFD + STZ + 300 mg/kg BW LFSE), HT: high dose treatment (HFD + STZ + 600 mg/kg BW LFSE).

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However, at week 9 (after the injection of STZ), a gradual loss of body weight was observed in the HFD groups, ranging from 392.26-395.98 g. After a 4-week treatment period (week 13), there were further reductions in the body weights of the HFD/STZ-induced rats (340.84-382.84 g), in contrast to NC (420.45 g). Treatment with LFSE at two different dosages, LT (300 mg/kg BW) and HT (600 mg/kg BW) showed no significant recovery in the body weights of the rats. This is in accordance with Abiola *et al.* (2018) that diabetes induced by STZ is characterised by a severe loss in bodyweight which might be the result of increased muscle wasting and excessive degradation of structural proteins. Other studies indicated that owing to the destruction of β-cells, insulin action was impaired thus compromising the conversion of glucose into glycogen, catabolism of fats and inhibition of lipolysis (Gheibi *et al.,* 2017).

3.2 Effects of Lepisanthes fruictosa fruit seed extract on fasting blood glucose level

The first line of therapeutic target for DM is to keep blood glucose levels as normal as possible and maintain glucose homeostasis (Jeszka-Skowron *et al.,* 2014). The effect of LFSE in regulating blood glucose levels is shown in Table 1. It was observed that STZ injection significantly ($P<0.05$) caused a marked elevation in the FBG levels in diabetic groups (21.9-22.3 mmol/L), as compared to the NC group (5.2 mmol/L). High dosage LFSE (600 mg/kg BW), HT progressively lowered the FBG level with a significant reduction evident after 4 weeks of the treatment period. Interestingly, the glucose-lowering effect of HT was also significantly ($P<0.05$) comparable to PC (metformin) with the final FBG levels of 14.0 mmol/L and 13.8 mmol/L, respectively. The possible mechanisms by which HT mediated its anti-hyperglycemic actions may result from the insulin-like effect such as enhancing glucose uptake metabolism by inhibiting hepatic gluconeogenesis, promoting glycolytic and glycogenic processes, reducing glucose absorption in the intestine by inhibiting α-glucosidase activity, and stimulating the pancreatic secretion of insulin through the regeneration process and revitalisation of the remaining β-cells (Kaur *et al.,* 2016; Mohammed *et al.,* 2016).

3.3 Effects of Lepisanthes fruictosa fruit seed extract on oral glucose tolerance test

The OGTT measures the ability to respond to an acute glucose challenge, where diabetic rats usually have difficulties regulating their blood glucose levels after glucose loading compared to normal rats (Mokiran *et al.,* 2014). The test is also useful to evaluate apparent insulin release and insulin resistance due to insulin response to glucose administration reflecting the capability of pancreatic β-cells to secrete insulin (Gheibi *et al.,* 2017). As presented in Figure 2, all diabetic groups showed a high spike in the FBG levels, 30 mins after oral glucose loading (2 g/kg BW). The FBG levels and AUC values were greater in the diabetic groups as compared to NC. The highest AUC was observed in LT (2418.36 mmol/L)

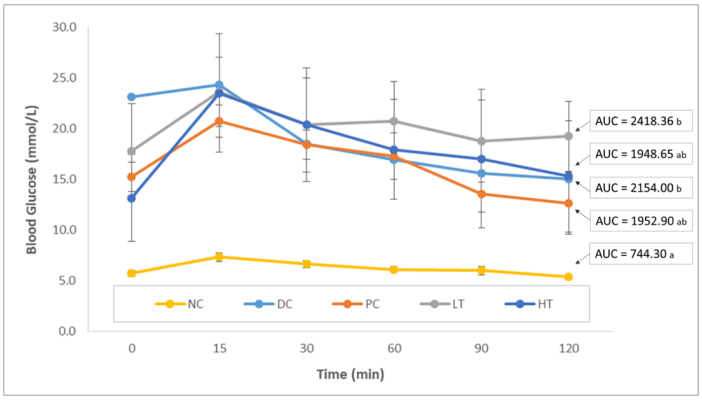

Figure 2. Oral glucose tolerance test and area under the curve of experimental group of rats. Values are expressed as the mean ± standard errors. Values with different letters are significantly different between groups at the level of $P<0.05$. NC: normal control (normal diet + vehicle), DC: diabetic control (HFD + STZ + vehicle), PC: positive control (HFD + STZ + metformin), LT: low dose treatment (HFD + STZ + 300 mg/kg BW LFSE), HT: high dose treatment (HFD + STZ + 600 mg/kg BW LFSE).

Table 1. Fasting blood glucose and insulin levels of the experimental group of rats

	Blood glucose (mmol/L)		Serum insulin (pmol/L)	
Group	Baseline	Final	Baseline	Final
NC	5.2±0.2^{b}	6.0±0.4^{b}	413.75±12.97^{a}	392.27±30.08^{a}
DC	21.9±1.0^{a}	21.7±5.3^{a}	165.56±21.37^{b}	138.70±30.41^{b}
PC	22.3±1.6^{a}	13.8±0.3^{ab*}	174.98±6.93^{b}	249.97±49.93^{b}
LT	22.1±2.3^{a}	19.2±3.3^{a}	168.42±24.39^{b}	186.96±20.44^{b}
HT	22.2±1.8^{a}	14.0±3.0^{ab*}	150.83±24.82^{b}	204.47±27.49^{b}

Values are expressed as the mean ± standard errors. Values with different letters in the same column (between groups) are significantly different at the level of $P<0.05$. Asterisk (*) in the same row indicates that the values are significantly different at the level of $P<0.05$ as compared to baseline. Baseline and final represent week 9 (after STZ injection, before treatment) and week 13 (after treatment), respectively. NC: normal control (normal diet + vehicle), DC: diabetic control (HFD + STZ + vehicle), PC: positive control (HFD + STZ + metformin), LT: low dose treatment (HFD + STZ + 300 mg/kg BW LFSE), HT: high dose treatment (HFD + STZ + 600 mg/kg BW LFSE).

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indicating its weak glucose tolerance. However, treatment with HT slightly improved the impaired glucose tolerance in the diabetic rats. The OGTT result of HT was also consistent with the reduction in the AUC (1948.65 mmol/L). Based on the findings, it can be postulated that high dosage LFSE (600 mg/kg BW) is more effective in regulating blood glucose as indicated by the acute and sub-chronic anti-hyperglycemic activities.

3.4 Effects of Lepisanthes fruictosa fruit seed extract on insulin level

Insulin is an anabolic hormone that plays a major role in glucose homeostasis. In response to elevated blood glucose levels, insulin is released by the pancreatic β-cells to regulate the amount of glucose in the blood and the rate at which glucose is absorbed into insulin-responsive cells (Coman *et al.,* 2012). In the present study, induction of diabetes by STZ caused a significant ($P<0.05$) decrease in serum insulin levels in all diabetic groups, as compared to NC (Table 1). Treatment with different dosages of LFSE for 4 weeks showed an increased trend in the levels of insulin by approximately 40% as compared to DC, suggesting an insulin secretagogue activity of the extract. Even though the exact mechanism is unclear, the effect could be due to the presence of active phytochemicals in the extract which enhances insulin secretion from the remaining or regenerated β-cells and protects them from further deterioration (Cherbal *et al.,* 2017). Interestingly, the improvement shown by HT was also consistent with the decrease of blood glucose level as discussed earlier.

3.5 Effects of Lepisanthes fruictosa fruit seed extract on antioxidant enzyme activities

The protective effect of LFSE in diabetic animals was evaluated as a function of SOD, CAT and GPx enzymes which represent the front line of the body's endogenous antioxidant defence system (Irondi *et al.,* 2015). Superoxide dismutase (SOD) catalyses the dismutation of superoxide radicals ($\cdot O_2^-$), the most toxic reactive oxygen species (ROS), into less toxic hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) (Dontha, 2016; Gargouri *et al.,* 2016). From Table 2, both LFSE-treated diabetic groups showed a significant ($P<0.05$) recovery in the SOD activities, with approximately 20% improvement after 4 weeks of study. Interestingly, the final activities were also significantly ($P<0.05$) comparable to PC, indicating their strong free radical scavenging abilities. On the contrary, no significant improvement in the CAT activities was observed in LFSE-treated diabetic groups after the treatment period. However, the final activity of CAT was significantly ($P<0.05$) greater in HT-treated rats (43%) than untreated DC. The results indicated that high dosage LFSE (600 mg/kg BW) may have a protective ability against the deleterious effects of H_2O_2 radicals. Similar to CAT, GPx is also responsible for the detoxification of H_2O_2, hence protecting the cell from oxidative damage (Irondi *et al.,* 2015). Although there were no significant differences observed among the diabetic groups, the GPx activities of LFSE increased by 19% and 26% as compared to PC and DC, respectively. The presence of various antioxidants phytochemicals could largely contribute to the antioxidant capacity of LFSE. This agrees with Abiola *et al.* (2018) that free radical scavenging activities of date palm *Phoenix dactylifera* seed may be attributed to synergistic actions of various phytochemicals e.g flavonoids, phenols, steroids and saponins.

3.6 Effects of Lepisanthes fruictosa fruit seed extract on lipid peroxidation level

Free radicals generated along with hyperglycaemia have been shown to not only reduce antioxidant capacity but also induce lipid peroxidation (Gargouri *et al.,* 2016). Lipids, especially polyunsaturated fatty acids (PUFA) in the cell membrane are prone to oxidative damage by free radicals through sequential peroxidation processes,

Table 2. Antioxidant enzymes and lipid peroxidation levels of experimental group of rats

Group	Plasma SOD (U/mL)		Plasma CAT (U/mL)		Plasma GPx (U/mL)		Serum MDA (μM)	
	Baseline	Final	Baseline	Final	Baseline	Final	Baseline	Final
NC	17.18±1.08^{a}	21.20±1.05^{a*}	139.81±6.58^{a}	139.72±4.73^{a}	381.50±26.64^{a}	403.77±4.73^{a}	40.22±7.64^{a}	41.33±4.49^{c}
DC	18.19±0.50^{a}	17.93±0.79^{b}	114.48±6.03^{b}	65.13±12.38^{c*}	167.49±21.74^{b}	148.19±4.89^{b}	52.11±5.74^{a}	69.91±4.82^{a}
PC	17.39±0.62^{a}	19.19±0.65^{ab}	108.16±2.88^{b}	86.24±3.81^{bc*}	151.92±51.59^{b}	157.27±25.71^{b}	59.49±8.15^{a}	57.93±1.41^{ab}
LT	17.66±0.75^{a}	22.17±1.07^{a*}	101.16±4.61^{b}	90.90±10.59^{bc}	179.69±24.32^{b}	186.82±20.39^{b}	51.75±11.84^{a}	55.15±2.56^{abc}
HT	18.04±0.62^{a}	21.97±0.92^{a*}	116.89±4.86^{b}	113.70±10.22^{ab}	174.61±34.01^{b}	187.79±18.72^{b}	49.67±6.66^{a}	50.22±4.97^{bc}

Values are expressed as the mean ± standard errors. Values with different letters in the same column (between groups) are significantly different at the level of $P<0.05$. Asterisk (*) in the same row indicates that the values are significantly different at the level of $P<0.05$ as compared to baseline. Baseline and final represent week 9 (after STZ injection, before treatment) and week 13 (after treatment), respectively. NC: normal control (normal diet + vehicle), DC: diabetic control (HFD + STZ + vehicle), PC: positive control (HFD + STZ + metformin), LT: low dose treatment (HFD + STZ + 300 mg/kg BW LFSE), HT: high dose treatment (HFD + STZ + 600 mg/kg BW LFSE).

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forming lipid hydroperoxides and subsequently malondialdehyde (MDA), a well-established biomarker for oxidative stress (Kong *et al.,* 2016). In addition to improving endogenous antioxidants, LFSE-treated diabetic group, particularly HT prevented membrane damage by decreasing lipid peroxidation reported as MDA level. At the end of the study, HT exhibited a significantly ($P<0.05$) reduced MDA level by 28% as compared to DC (Table 2). Based on the findings, it can be suggested that this activity could be related to the strong antioxidant ability of the extract. Shakirin *et al.* (2012) described that antioxidant phenolics play a major role in scavenging free radicals and attenuating oxidative stress-related lipid peroxidation.

3.7 Effects of Lepisanthes fruictosa fruit seed extract on lipid profile

Dyslipidaemia or abnormalities in lipid metabolism is one of the most common complications of diabetes (Irondi *et al.,* 2015). The condition could be attributed to the excess mobilisation of free fatty acids from the adipose tissues due to under-utilisation of glucose or inhibition of the hormone-sensitive lipase by insulin (Okoduwa *et al.,* 2017). Results of serum lipid profile comprising TC, TG, LDL-c and HDL-c are presented in Table 3. All experimental groups showed no significant improvements in most of the parameters tested. The most affected group was the untreated diabetic control, DC. After 4 weeks of study, the levels of TC and TG in DC were significantly ($P<0.05$) increased by 22% and 51%, respectively. This is in agreement with the previous finding that increased TC and TG are commonly associated with chronic diabetic situations (Tang *et al.,* 2017). The increased level of serum lipids occurs when lipolytic hormone fails to function properly on the fat stores due to the deficiency of insulin (Ali *et al.,* 2017). This is supported by Hahm *et al.* (2011) that resistance to the insulin action on lipoprotein lipase in peripheral tissues may result in elevated TG and LDL-c levels. Despite having weak lipid-lowering abilities, LFSE exhibited a significant promotion in HDL-c level. The HDL-c is the advantageous lipoprotein that protects the arterial walls from cholesterol deposition and thereby preventing atherosclerosis and other cardiovascular diseases (Irondi *et al.,* 2015). Interestingly, the lower dosage (300 mg/kg BW), LT was more effective than HT as indicated by its significantly ($P<0.05$) improved level of HDL-c (39%). Although the exact mechanism is unclear, the presence of flavonoid genistein could possibly contribute to the increase by enhancing apolipoprotein A-1, the main protein component of HDL -c (Mokiran *et al.*, 2014).

3.8 Phytochemical compounds in Lepisanthes fruictosa fruit seed extract

On the basis of its potential antidiabetic and antioxidant effects, LFSE was further analysed using LC -MS/MS to identify the putative phytochemical compounds. Figure 3 depicts the total ion chromatogram (TIC) obtained from LC-MS/MS analysis of LFSE under the ESI-negative ion mode. A total of 21 compounds were proposed from LFSE including alkaloid, benzoid, carbohydrate, coumarin, fatty acid, flavonoid, sphingolipid, tannin and terpene (data not shown), which were previously reported to have antidiabetic and antioxidant properties. Among these, α-kojibiose, genistein-7,4'-di-O-β-D-glucoside and soyacerebroside II were the most predominant ones.

Peak 1, eluting at 0.57 min (m/z 341.109) was predicted as α-kojibiose (Figure 4). This compound showed good intensity in the TIC, being one of the most predominant peaks in the chromatogram. Furthermore, MS/MS analysis yielded fragment ions at m/z 221.069, 179.056, 161.046, 89.024 and 71.013. The neutral loss of 221.069 (120 amu) was associated with the cross-ring cleavages of sugars. The successive losses of 179.056 (162 amu) and 161.046 (180 amu) corresponded to the presence of a hexose moiety and additional glucose (or galactose) unit, respectively. Meanwhile, fragments at m/

Table 3. Serum lipid profile of experimental group of rats

Group	TC (mmol/L)		TG (mmol/L)		LDL-c (mmol/L)		HDL-c (mmol/L)	
	Baseline	Final	Baseline	Final	Baseline	Final	Baseline	Final
NC	1.75±0.05^{a}	1.73±0.03^{a}	1.12±0.26^{a}	0.57±0.12^{a}	0.41±0.04^{b}	0.53±0.02^{a}	1.41±0.04^{a}	1.29±0.04^{a}
DC	1.60±0.05^{a}	2.06±0.08^{a*}	0.74±0.12^{b}	1.51±0.32^{a*}	0.49±0.03^{ab}	0.53±0.03^{a}	1.15±0.07^{ab}	1.61±0.22^{a}
PC	1.65±0.06^{a}	1.98±0.14^{a}	0.57±0.07^{b}	1.27±0.45^{a}	0.49±0.05^{ab}	0.55±0.07^{a}	1.15±0.10^{ab}	1.70±0.28^{a}
LT	1.65±0.15^{a}	2.10±0.21^{a}	0.94±0.24^{ab}	1.45±0.16^{a}	0.57±0.04^{a}	0.56±0.01^{a}	1.00±0.08^{b}	1.64±0.02^{a*}
HT	1.60±0.06^{a}	1.93±0.12^{a}	0.70±0.16^{b}	1.12±0.21^{a}	0.44±0.03^{ab}	0.66±0.09^{a}	1.28±0.04^{ab}	1.29±0.19^{ab}

Values are expressed as the mean ± standard errors. Values with different letters in the same column (between groups) are significantly different at the level of $P<0.05$. Asterisk (*) in the same row indicates that the values are significantly different at the level of $P<0.05$ as compared to baseline. Baseline and final represent week 9 (after STZ injection, before treatment) and week 13 (after treatment), respectively. NC: normal control (normal diet + vehicle), DC: diabetic control (HFD + STZ + vehicle), PC: positive control (HFD + STZ + metformin), LT: low dose treatment (HFD + STZ + 300 mg/kg BW LFSE), HT: high dose treatment (HFD + STZ + 600 mg/kg BW LFSE).

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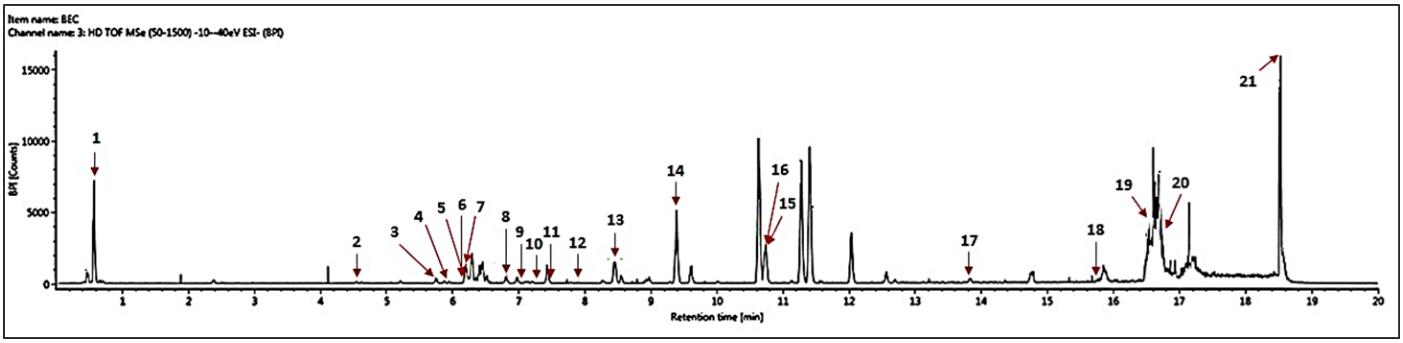

Figure 3. Total ion chromatogram of the main compounds of LFSE under negative ion mode.

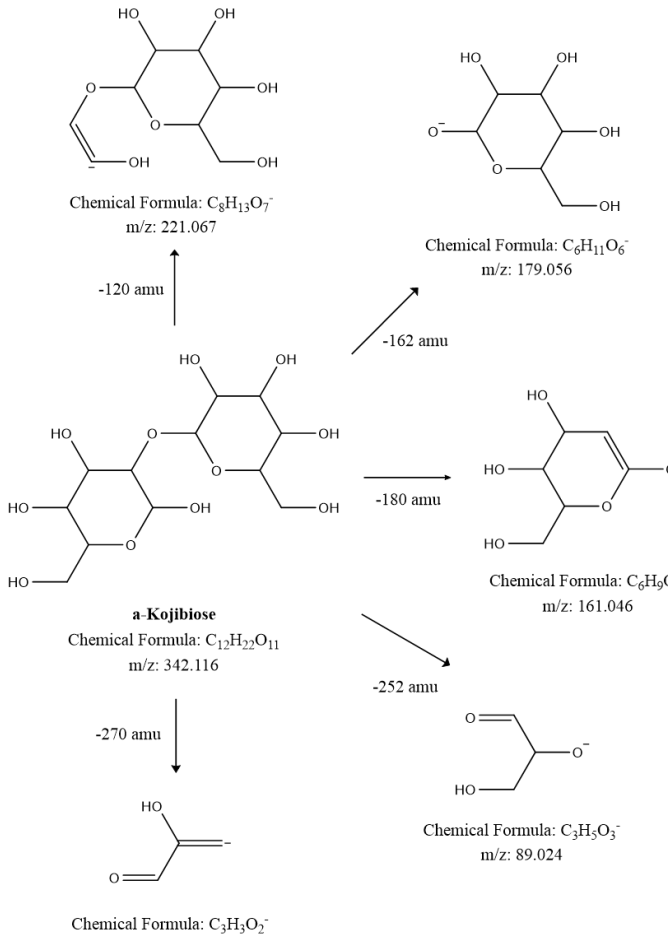

Figure 4. Possible fragmentation pattern of peak 1, tentatively identified as α-kojibiose.

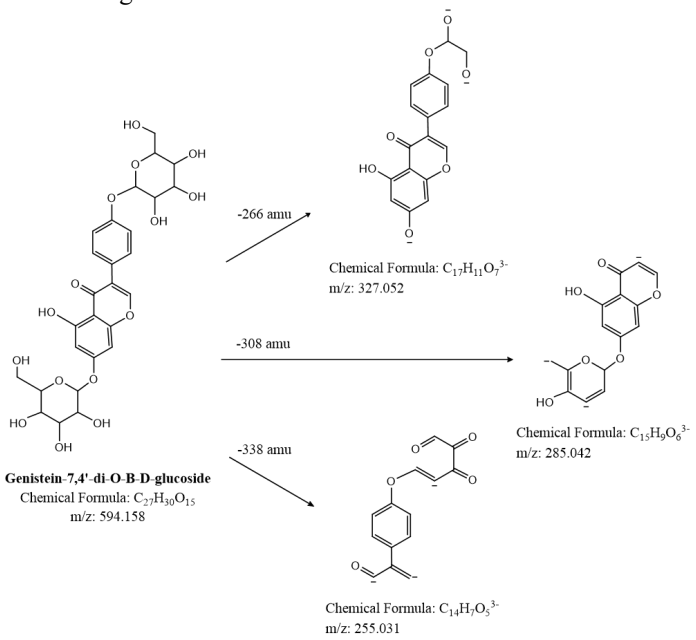

Figure 5. Possible fragmentation pattern of peak 14, tentatively identified as genistein-7,4'-Di-O-B-D-glucoside.

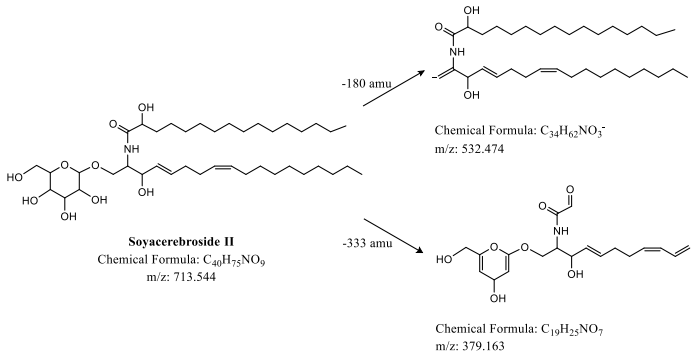

Figure 6. Possible fragmentation pattern of peak 21, tentatively identified as soyacerebroside II.

z 89 and 71 indicated the losses of 252 and 270 amu, respectively. Kojibiose is a non-digestible isomaltooligosaccharide that inhibits α-glucosidase I (Canneyt, 2015), the key enzyme that plays an important role in carbohydrate metabolism (Lee *et al.,* 2014). It promotes the proliferation of microbiota and improves intestinal balance, which is one of the main therapies recommended to diabetic patients (Bharti *et al.*, 2015). This compound also potentially enhances glucose tolerance, as well as reduces fat and cholesterol absorption (Bharti *et al.,* 2015; Canneyt, 2015).

Another clearly visible peak was observed at peak 14 (RT 9.39 min) which was putatively assigned to genistein-7,4'-di-O-β-D-glucoside (Figure 5). This flavonoid glycoside yielded a deprotonated molecular ion at m/z 593.152. Its major MS/MS fragments at m/z 327.051, 285.039 and 255.029 were formed through the consecutive losses of 266, 308 and 338 amu, respectively. The neutral loss of 308 amu could be attributed to the loss of rutinose disaccharide side-group. Genistein-7,4'-di-O-β-D-glucoside was previously reported to show glucose lowering effect in *Morus alba* (mulberry) (Bae *et al.,* 2018). Genistein was described to exert its antidiabetic activities through enhancing β-cell proliferation, glucose-stimulated insulin secretion and protection against apoptosis (Gilbert and Liu, 2013). The naturally-occurring isoflavone mainly exists in the form of glycosides. The glycosides were also reported to have similar antioxidant potency to their aglycone (Popović *et*

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al., 2013).

Peak 21 (RT 18.52 min) was the most abundant in the TIC, suggesting that the putatively identified compound, soyacerebroside II (Figure 6), may largely contribute to antioxidant and antidiabetic activities of LFSE. It yielded a deprotonated molecular $[M–H]^-$ ion at m/z 712.540. The MS/MS analysis of the parent ion resulted in the fragment ions at m/z 532.473 and 379.156, which the former was attributed to the loss of glucose moiety (180 amu). Soyacerebroside II was a sphingolipid detected in the seed extract of longan fruit, *Dimocarpus longan* that possessed antioxidative and hypoglycaemic effects (Sharajabian *et al.,* 2019). Sphingolipids are important metabolic mediators that regulate intracellular signalling related to insulin, as well as many other processes, which makes them useful targets to prevent and mitigate metabolic anomalies (Fernández-Ochoa *et al.,* 2020).

4. Conclusion

The present study has revealed, for the first time, the antidiabetic potential of *L. fruticosa* ethanolic fruit seed extract which might be attributed to its anti-hyperglycaemic effect and enhanced antioxidant status in HFD/STZ-induced diabetic rats. Although the underlying mechanisms are beyond the scope of this study, the antidiabetic and antioxidant properties of various putative phytochemicals present in the extract could synergistically contribute to the amelioration of the biochemical anomalies. The findings may suggest that *L. fruticosa* fruit species may be viewed as a potential candidate for a new and safe alternative remedy for the prevention and treatment of T2DM. However, further work is warranted to isolate and characterise the active principle(s) responsible for the anti-diabetic effect and to elucidate the precise mechanisms of actions.

Conflict of interest

The authors declare no conflict of interest.

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