

## Antidiabetic Activity of Aqueous Extract of *Leptospermum flavescens* in Alloxan Induced Diabetic Rats

(Aktiviti Antidiabetik Ekstrak Akues *Leptospermum flavescens* dalam Tikus Diinduksi Aloksan)

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### ABSTRACT

*Leptospermum flavescens*, commonly known as 'Gelam bukit' has been used by the Malays as traditional plants in Malaysia for antidiabetic treatment. However, at this moment there is no scientific evidence and data available to validate such claim. In the present study, the aqueous extract of leaves and stems were studied for its antidiabetic activity. The total phenols and flavonoids were determined and correlated with antidiabetic activity. The detection of aqueous leaves extract with LCMS/MS showed the presence of flavonoids aromadendrin glucoside, kaempferol rhamnoside, quercetin rhamnoside and vindoline. The extract has significantly inhibited glycogen phosphorylase at 85% with  $IC_{50} = 0.18$  mg/mL. In the alloxan induced diabetic rats showed that extract at 500 mg/kg decreased significantly fasting plasma glucose level by 61.9% ( $p < 0.001$ ) on the 20<sup>th</sup> day as compared to diabetic control. The treatment with *Leptospermum flavescens* at 500 mg/kg showed that it decreased the total cholesterol and triglycerides but restored the HDL level. The high antidiabetic activity was correlated with high total phenol at  $1.57 \pm 0.01$  GAE/g and total flavonoids at  $1.41 \pm 0.01$  mg QE/g. Thus, the high antidiabetic activity of the aqueous leaves extract attributed due to the presence of aromadendron glucoside, kaempferol rhamnoside, quercetin rhamnoside and vindoline in aqueous extract of *Leptospermum flavescens*.

**Keywords:** Antidiabetics; flavonoids; glycogen phosphorylase; *Leptospermum flavescens*; phenols

### ABSTRAK

*Leptospermum flavescens* yang lazimnya dikenali sebagai Gelam bukit telah digunakan oleh orang Melayu dalam perubatan tradisi untuk rawatan diabetes. Walau bagaimanapun, pada masa ini tiada bukti saintifik dan data tersedia untuk mengesahkan dakwaan tersebut. Dalam kajian ini, ekstrak akues daun dan batang dikaji untuk aktiviti antidiabetiknya. Jumlah fenol dan flavonoid ditentukan dan berkorelasi dengan aktiviti antidiabetik. Pengesanan ekstrak akues daun dengan LCMS/MS menunjukkan kehadiran flavonoid glikosida aromadendrin, kaempferol rhamnoside, quercetin rhamnoside dan vindoline. Ekstrak mempunyai rencatan signifikan glikogen fosforilase pada 85% dengan  $IC_{50} = 0.18$  mg/mL. Di dalam tikus induksi diabetik aloksan menunjukkan ekstrak pada 500 mg/kg signifikan menurunkan paras glukosa plasma puasa sebanyak 61.9% ( $p < 0.001$ ) pada hari ke 20 berbanding dengan kumpulan kawalan. Perlakuan dengan *Leptospermum flavescens* pada 500 mg/kg menunjukkan penurunan jumlah kolesterol dan trigliserida tetapi mengekalkan paras HDL. Aktiviti antidiabetik yang tinggi berkorelasi dengan jumlah fenol pada  $1.57 \pm 0.01$  GAE/g dan jumlah flavonoid pada  $1.41 \pm 0.01$  mg QE/g. Maka, aktiviti antidiabetik yang tinggi ekstrak akues daun adalah disebabkan kehadiran glikosida aromadendron, kaempferol rhamnoside, quercetin rhamnoside dan vindoline di dalam ekstrak akues *Leptospermum flavescens*.

**Kata kunci:** Antidiabetik; fenol; flavonoids; glikogen fosforilase; *Leptospermum flavescens*

### INTRODUCTION

Diabetes is a type of hormonal deficiency disease and almost all of diabetes mellitus has been diagnosis as type 2 or non-insulin dependent diabetes mellitus (Oikonomakus 2002). It is caused by the elevation of blood glucose level due to either resistance to the biological activity of insulin or by abnormal insulin secretion rate (Ross et al. 2004). There are several drugs available for the treatment of type 2 diabetes such as biguarides (phenformin and metaformin) (Bailey 1992), thiazolidinediones (TZDs) (Barnett et al. 1977),  $\alpha$ -glucoside inhibitors (Acarbose) (Asano 2003; Buse et al. 1998) and sulfonylureas (glipizide and gliimeperide) (Yun et al. 2006). However, these drugs have side effect

such as weight loss, bloating, metallic taste, abdominal pains, hepatic toxicity, weight gain, gastrointestinal discomfort and skin rashes. Thus, a new medical treatment for diabetes mellitus needs to be developed to control blood glucose homeostasis without inherent side effects. Glycogen phosphorylase (GP) has been recognized as a therapeutic target in searching phytochemical compounds of GP inhibitors from medicinal plants for the diabetic treatment. In the glycogen metabolism, the liver GP is directly responsible for the regulation of hepatic glucose output and maintained the blood sugar level. Therefore, the inhibition of GP has become accepted treatment for type 2 diabetes (Moller 2001; Zimmet et al. 2001) to reduce the

hepatic glucose production. Secondary plant metabolites flavanoids (Prakash et al. 2011), terpenoids (Min-Jia et al. 2008) and alkaloids (Jiyin et al. 2009) has been shown to possess hypoglycemic effect *in vitro* and *in vivo* in induced diabetic rats. Furthermore, the uses medicinal traditional plants for diabetes treatments are much more save, effective, non-toxic with less or no side effect. The search for safer and affordable antidiabetic drugs has led to the testing of many plants for such activity.

Thus, this would give traditional medicines plants to play an important role in the control of diabetes mellitus (Ahmad et al. 2004; Karunanayake & Tennekoon 1993). There are many traditional medicinal plants possesses antidiabetic activity has been reported (Day 1998; Mankil et al. 2006) such as *Tinospora cordifolia* (Patel et al. 2009) and *Jatropoda cucu* (Obatomi et al. 1994). *Leptospermum flavescens* belong to the family of Myrtaceae which commonly known as 'Gelum bukit' by the Malays in Malaysia. It has been used in Malay folks to stimulate appetite, relieve stomach disorder, menstrual discomfort and diabetes (Riley 1994). The present work was undertaken to explore the antidiabetic potential of a plant *Leptospermum flavescens* for type 2 diabetic treatments. No reports are available on the hypoglycemic activity of *Leptospermum flavescens* in streptozotocin induced diabetic rats.

## MATERIALS AND METHODS

### COLLECTION OF PLANTS SAMPLE

The *Leptospermum flavescens* was collected from the forest at Genting Highland, Pahang, Malaysia. The authentic of the plant was identified by plant taxonomist Professor Dr. Ong Hean Choi from Institute of Biological Science, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia. The leaves, stems and roots were separated and air-dried at room temperature for four days. It was then grinded into fine powder and kept in sample bottle until further used.

### PREPARATION OF AQUEOUS EXTRACTS

The powdered sample of leaves, stems and roots were extracted with 100 mL of water. Then the mixture was incubated in water bath at 40°C for 2 h. After the incubation, the mixture was filtered and the water extract was dried in the freeze drier.

### SEPARATION AND DETECTION OF CHEMICAL COMPOUNDS

The chemical compounds of the aqueous extract were separated using Thin Layer Chromatography (TLC). The extract was loaded as a band onto TLC plate size 20 cm × 20 cm with capillary tube. The TLC separation was developed in chromatography tank in 10% methanol/chloroform solvent system. The presence of phenols, terpenoids and alkaloids were detected with phenol, vanillin H<sub>2</sub>SO<sub>4</sub>

and Dragendorff reagent, respectively. The chemical constituents of the extract were determined using LCMS/MS with known standard references using ionisation mode: Positive and Negative; Column: Phenomenex Aqua C18 – 50 mm × 2.0 mm × 5 µM; Buffer: Water and Acetonitrile with 0.1% formic acid and 5 mM ammonium formate. 1.0 mL of sample extracts were diluted five times with methanol and filtered with 0.2 µM nylon filter prior to analyses. The mass fragmentations were based on using standards samples.

### DETERMINATION OF TOTAL PHENOLS

The total phenolic contents of aqueous extracts were determined with the Folin-Ciocalteu reagent using method from Spanos and Wrosltd (1990). First, the Folin-Ciocalteu reagent was prepared by dissolving Folin-Ciocalteu reagent in distilled water in 1:10 ratio. 500 µL of the the crude extracts was mixed with 5 mL of Folin-Ciocalteu reagent and 4 mL of 1M of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>). The reaction mixture was incubated in water bath at 45°C for 15 min. The absorbance of the sample was measured at 765 nm with spectrophotometer. The total phenol content were determined using standard curve prepared from 0, 50, 100, 150, 200, 250 and 500 mg/L of gallic acid in methanol solution (50:50/v:v). The results were expressed as milligram of gallic acid equivalent per gram of dry sample. All of the aqueous extracts were tested in duplicate and mean value were calculated.

### DETERMINATION OF TOTAL FLAVONOIDS

The total flavonoid contents of extracts were determined using Aluminium chloride colorimetric method with slightly modification as described by Liu et al. (2007). 10 mg/mL of standard concentration samples in methanol (1:1) were mixed with 0.3 mL 5% Sodium nitrate and incubated for 5 min at 37°C. 0.3 mL 10% aluminium chloride was added and incubated for 6 min at 37°C. 2 mL of Sodium hydroxide and 10 mL distilled water was added. Absorbance was measured at 510 nm using spectrophotometer. The total flavonoids content was determined using a standard curve prepared from 0, 50, 100, 150, 200, 250 and 500 mg/L of quercetin. The results from two replicates were expressed as mg of quercetin equivalent per gram of dry sample.

### ENZYMATIC GLYCOGEN PHOSPHORYLASE BIOASSAY

The inhibitory activity of the test compounds against rabbit muscle glycogen phosphorylase (GP<sub>a</sub>) was monitored using microplate reader (BIO-RAD) following the method as described by Martin et al. (1998). In brief, GP<sub>a</sub> activity was measured in the direction of glycogen synthesis by the release of phosphate from glucose-1-phosphate. Each test compound was dissolved in DMSO and diluted at different concentrations for IC<sub>50</sub> determination. The enzyme was added into 100 mL of buffer containing 50 mM Hepes (pH 7.2), 100 mM KCl, 2.5 mM MgCl<sub>2</sub>, 0.5 mM glucose-1-

phosphate, 1 mg/mL glycogen and the test compound in 96-well microplates (Costar). After the addition of 150 mL of 1 M HCl containing 10 mg/mL ammonium molybdate and 0.38 mg/mL malachite green, reactions were run at 22°C for 25 min and then the phosphate absorbance was measured at 655 nm. The caffeine was used as a positive control standard at 0 – 1 mg/mL. The IC<sub>50</sub> values were estimated by fitting the inhibition data to a dose-dependent curve using a logistic derivative equation.

#### ANIMALS

The male Sprad Dawley rats aged 2 months and weighing 120 – 150 g were used for the study. The animals were kept in rat cages of six rats per cage and maintained under standard conditions at 12 h light and 12 h dark cycle, room temperature 30°C, 70–80% relative humidity. They were fed with standard rat pellet diet and the animal study was carried out under Animal Ethics Committee of Animals House, Faculty of Medicine, University of Malaya with animal ethic No: ISB/03/03/2010/JM(R).

#### ACUTE TOXICITY STUDY

The Sprad Dawley rats in a group of six rats were starved overnight. They were fed with the leaves and stems aqueous extracts at doses of 200 mg/kg and 500 mg/kg body weight, respectively. The acute toxicity study was carried out according to OECD guidelines - 425. The toxic effect on alertness, restlessness, irritability, fearless, spontaneous activity, defecation and urination was observed continuously for 14 days to see any lethality or death.

#### ORAL GLUCOSE TOLERANCE TEST (OGTT)

The oral glucose tolerance test was carried out according to Shirwaikar et al. (2006). The normal rats were fasted overnight. The rats were divided into six group consisting of six rats in each group and administered with glucose (3 g/kg), glipizide (2 mg/kg), leaves aqueous extract 200 mg/kg, 500 mg/kg, stem aqueous extract 200 mg/kg, 500 mg/kg respectively. The glucose (3 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the retro orbital sinus under ether inhalation at 0, 30, 60 and 120 min of glucose administration. The glucose level was estimated using glucometer Accu check (USA) with glucose-oxidase-peroxidase reactive strips.

#### INDUCTION OF NON-INSULIN-DEPENDENT DIABETES MELLITUS (NIDDM)

The rats weighing 120 – 150 g were induced with 150 mg/kg body weight of alloxan by single intraperitoneal injection. Hyperglycemia was confirmed by the evaluated glucose level determined at 72 h. Animals with blood glucose level more than 12 mmol/L were considered as diabetics. Rats found with permanent NIDDM were used for the antidiabetic study. Glipizide at 2 mg/kg was used as the standard drug.

#### ANTIDIABETIC STUDY

The animals were divided into seven groups, each consisting of six rats. The extracts were administered for 20 days. Group I: Normal control rats administered saline (0.9%, w/v); Group II: Diabetic control rats administered saline (0.9%, w/v); Group III: Diabetic rats administered glipizide (2 mg/kg) daily for 20 days; Group IV: Diabetic rats administered leaves aqueous extract (200 mg/kg); Group V: Diabetic rats administered leaves aqueous extract (500 mg/kg); Group VI: Diabetic rats administered stem aqueous extract (200 mg/kg); Group VII: Diabetic rats administered stem aqueous extract (500 mg/kg). The effects of administration of *Leptospermum flavescens* extracts in normal and diabetic rats were observed by measuring fasting blood glucose, serum lipid profile and changes in body weight. Fasting blood glucose was estimated on days 0, 5, 10, 15 and 20 of extracts administration. Serum lipid profiles were measured by an auto analyzer (Star 21).

#### STATISTICAL ANALYSIS

Data were statistically evaluated using one-way ANOVA, followed by Dunnett test using STAT software. The values were considered significant when  $p < 0.05$ .

#### RESULTS AND DISCUSSION

The present study reported for the first time the potential of *Leptospermum flavescens* as antidiabetic agent. In this study the assessment of the antidiabetic activity of aqueous extract from leaves and stem of *Leptospermum flavescens* was investigated. The extract showed a dose-dependent fall in FBG (fasting blood glucose) in alloxan induced diabetic rats. Alloxan induces diabetes by pancreatic cell damage mediated through generation of cytotoxic oxygen free radicals. The primary target of these radicals is the DNA of pancreatic cells causing DNA fragmentation (Shankar et al. 2007). The phytochemical screening of leaves and stem extract with thin layer chromatography showed the presence of phenolics and terpenoids compounds. Table 1 shows that aqueous extract of leaves contained highest amount of phenols and flavonoids at 1.57±0.01 mg and 1.41±0.07 mg per g of dry weight, respectively. The leaves aqueous extracts also showed high value of LC<sub>50</sub> with BSLA (609.14 ug/mL) compared with others extracts which indicated that the leaves aqueous extract is not toxic and safe to be used as decoction for traditional treatment of diabetes. The phenolic compounds have been found to be beneficial in controlling diabetes. Several workers have shown that the flavonoids, triterpenoids, alkaloids and phenolics possessed antidiabetic activity (Atta-Ur-Rhemann & Khurshid Zaman 1989; Ivorra et al. 1989; Kameswara Roa et al. 1997; Oliver-Bener 1986). Chakravarthy et al. (1980) have reported that flavonoids are able to regenerate the damaged beta cells in the alloxan diabetic rats. Similarly, phenolic compounds are found to possess antihyperglycemic properties (Manickam et al. 1997). Lu et al. (2009) have shown that total

TABLE 1. Total of phenol, flavonoid contents and LC<sub>50</sub> BSLA of crude extract of *Leptospermum flavescens*

Sample	Total Phenol (mg of GAE/g)	Total Flavonoid (mg of QE/g)	LC <sub>50</sub> BSLA (µg/mL)
Leaves Hexane	0.30 ± 0.01	1.10 ± 0.01	124.23
Stems Hexane	0.17 ± 0.01	1.12 ± 0.01	116.33
Leaves Chloroform	0.41 ± 0.01	1.02 ± 0.01	21.54
Stems Chloroform	0.19 ± 0.01	0.94 ± 0.01	124.57
Leaves Aqueous	1.57 ± 0.07	1.41 ± 0.01	609.14
Stems Aqueous	1.30 ± 0.02	1.16 ± 0.01	295.65

Values indicate mean ± SEM (n = 2)

flavonoids from leaves of *Eriobotrya japonica* possessed hypoglycemic activity in streptozotocin-induced diabetic mice. The LCMS/MS analysis of leaves aqueous extract of *Leptospermum flavescens* showed the presence of flavonoid aromadendrin glucoside (Figure 1), kaempferol rhamnoside (Figure 2), quercetin rhamnoside (Figure 3) and vindoline (Figure 4). Chen et al. (2010) have determined aromadendrin in *Euonymus alatus* whereas Wei Yun et al. (2011) have reported that aromadendrin isolated from *Gleditsia sinensis* stimulated the glucose uptake and improve the insulin resistance in mouse predipocytes cell culture. The kaempferol rhamnoside and quercetin rhamnoside extracted from *Bauhinia megalandra* leaves have also been shown by Rodriquez et al. (2010) that it inhibited glucose intestinal absorption. Similarly, quercetin rhamnoside found in *Agrimonia eupatoria* have been reported by Zhang and Cheng (2009) are able to reduce blood glucose level in agrimony plants. Several researches have reported that Vindoline isolated from *Catharanthus roseus* possess antidiabetic activity (Chattopadhyay et al. 1991, 1992; Chattopadhyay 1999; Ghosh & Gupta

1980). Thus, the presence of these compounds provides explanation and evidence for the antidiabetic effect of *Leptospermum flavescens*. Figure 5 shows that the aqueous leaves crude extract possessed high inhibition of glycogen phosphorylase at 85% inhibition with IC<sub>50</sub> at 0.18 mg/mL. The positive control standard drug caffeine at 1 gm/mL gave 95% inhibition and its IC<sub>50</sub> was 0.12 mg/mL. The high inhibition of glycogen phosphorylase of aqueous leaves crude extract was attributed to the presence of aromadendrin glucoside, kaempferol rhamnoside, quercetin rhamnoside and vandoline which has been detected by LCMS/MS. Thus, these results supported the claim made by traditional practitioners that aqueous extract of leaves *Leptospermum flavescens* are effective for diabetic treatment. In the present studies during the acute toxicity test, there are no toxic sign such as restless, response to touch, fearfulness, urination or death was found in male rats at any of the doses given to each rats until the end of experimental works. Figure 6 depicts the hyperglycemic effect of single oral administration of the leaves and stem aqueous extract at 200 mg/kg and 500

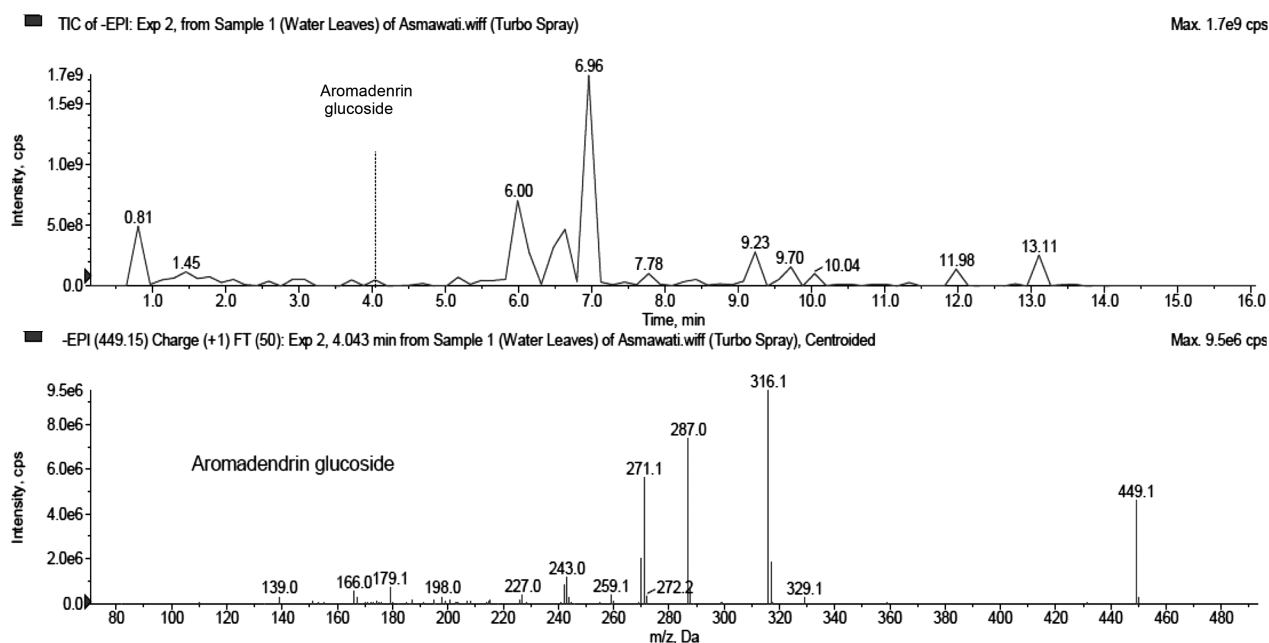


FIGURE 1. LCMS/MS profile of Aromadendrin glucoside from leaves aqueous extract of *Leptospermum flavescens*

mg/kg on OGTT of normal rats, respectively. It showed that the glucose administration to normal rats fasted for 16 h increased plasma glucose level from 5 to 8 at 30 min and returned to normal glucose level after 2 h. The administration of leaves aqueous extract of *Leptospermum flavescens* at the dose of 500 mg/kg bodyweight showed significant decrease in plasma glucose level at 60 min than the dose of 200 mg/kg body weight as compared to the normal group. This indicated that aqueous leaves extract of *Leptospermum flavescens* at 500 mg/kg are able to improve glucose tolerance in normal rats. The antihyperglycemic activity of the leaves and stem aqueous extract was further evaluated in alloxan induced diabetic rats. The results in Table 2 shows that fasting blood glucose levels were

significantly increased in alloxan treated group compared with the normal control group ( $p < 0.05$ ; diabetic control vs. normal control). This is due to the lack of insulin in alloxan induced rats by destructing the  $\beta$  cells which leads to hyperglycemia. The treatment of diabetic rats with *Leptospermum flavescens* leaves aqueous extract (500 mg/kg) decreased fasting plasma glucose levels significantly ( $p < 0.05$ ) on the 20<sup>th</sup> day by 61.9% compared to the 0<sup>th</sup> day. Another group treated with leaves aqueous at the dose of 200 mg/kg body weight did not show significant ( $p > 0.05$ ) glucose lowering effect at the end of experiment compared to the 0<sup>th</sup> day, but it decreased glucose level on the 20<sup>th</sup> day by 12.4%. Glucose lowering activity of the extract (200 mg/kg) was remained statistically significant compared

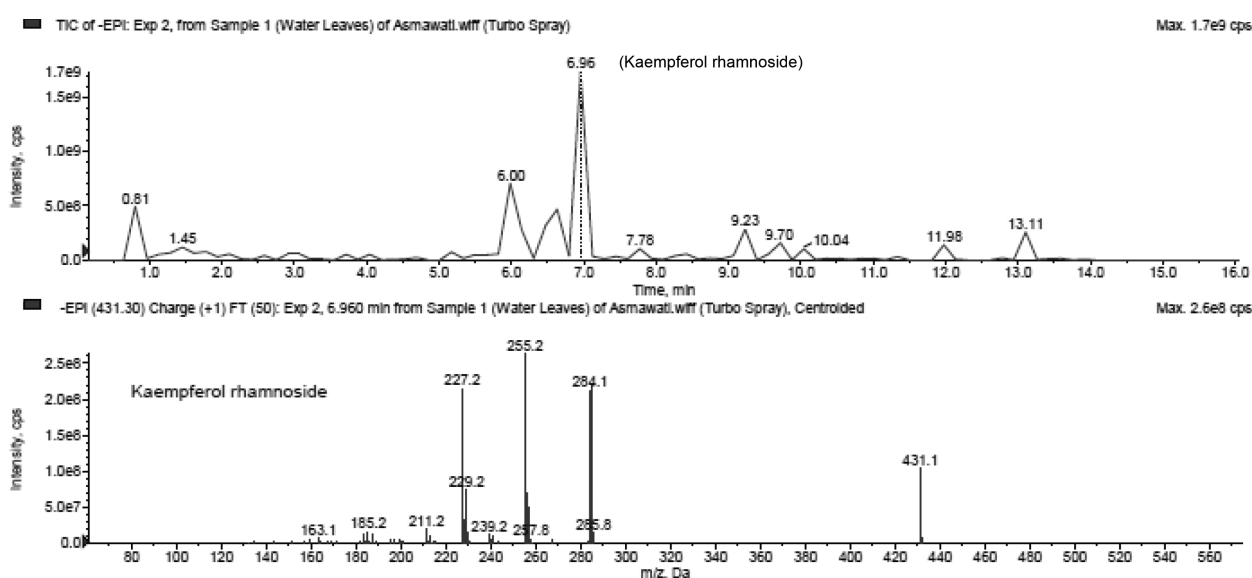


FIGURE 2. LCMS/MS profile of Kaempferol rhamnoside from leaves aqueous extract of *Leptospermum flavescens*

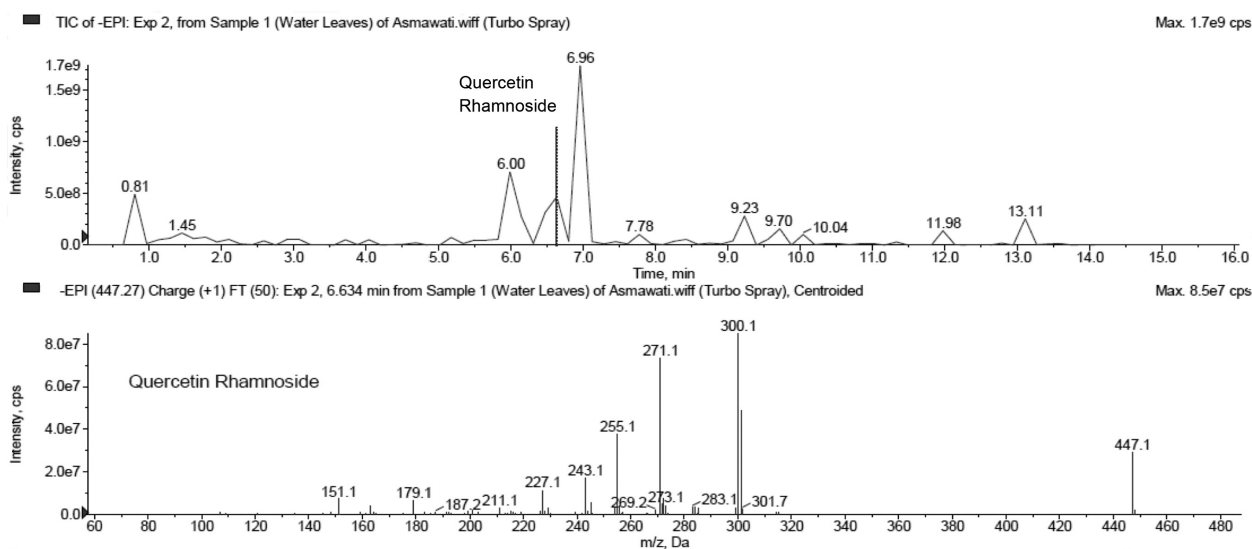


FIGURE 3. LCMS/MS profile of Quercetin rhamnoside from leaves aqueous extract of *Leptospermum flavescens*

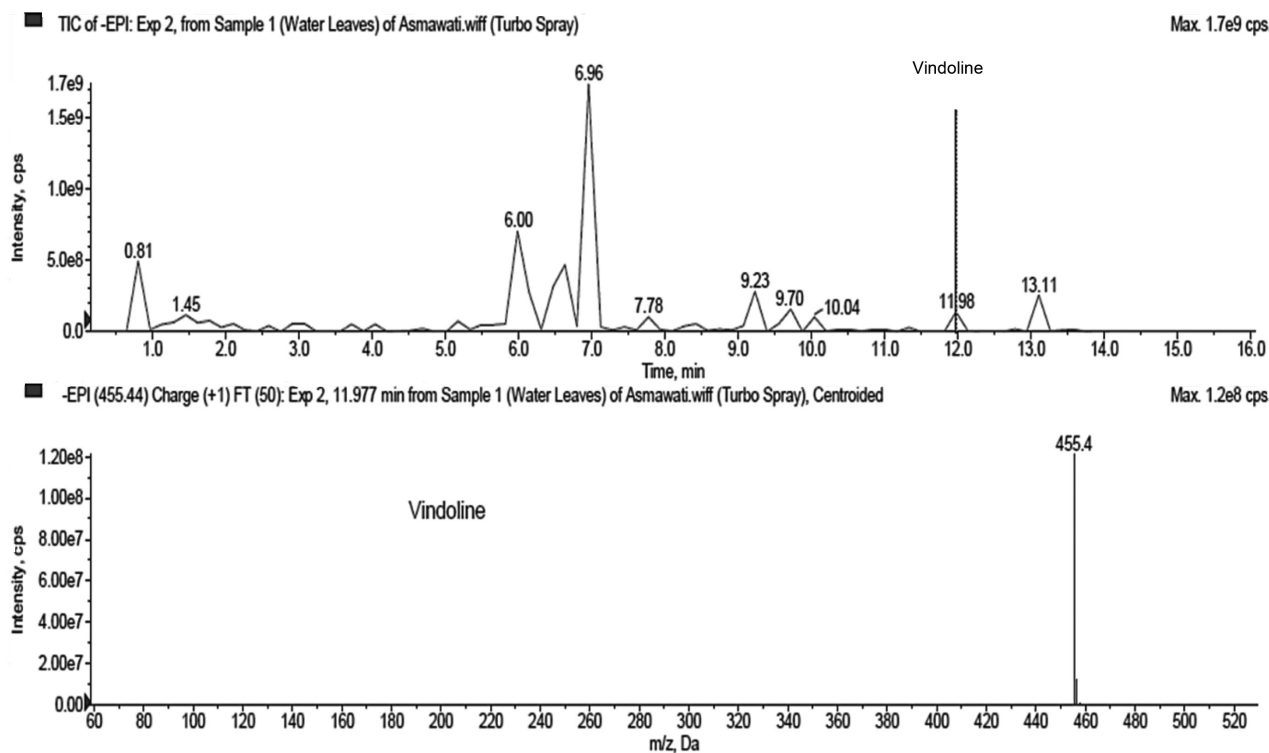


FIGURE 4. LCMS/MS profile of Vindoline from leaves aqueous extract of *Leptospermum flavescens*

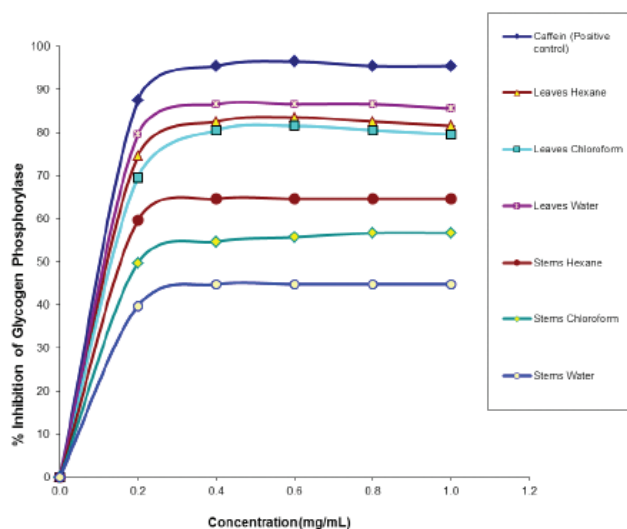
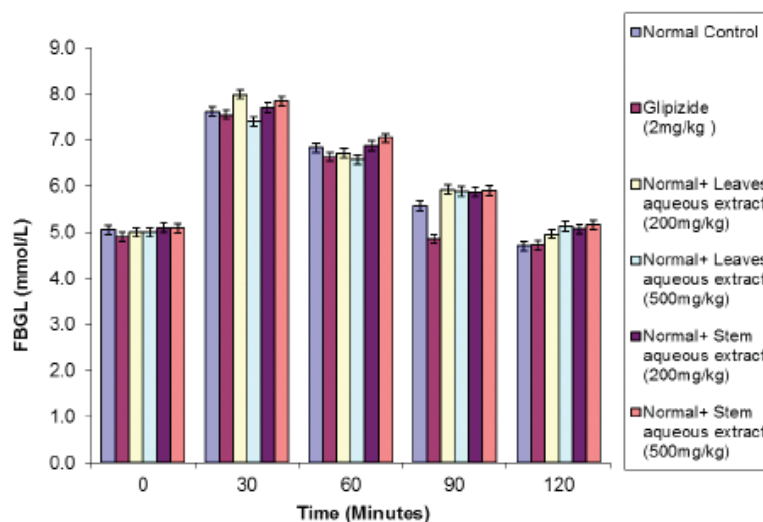


FIGURE 5. Percentage of inhibition of glycogen phosphorylase of crude extract from *Leptospermum flavescens*

to the diabetic control. From this result it indicates that *Leptospermum flavescens* leaves extract can decrease fasting plasma glucose level in a dose-dependent manner in alloxan induced diabetic rats. The diabetic rats treated with standard drug glipizide showed significant reduction in fasting plasma glucose levels compared to diabetic control group ( $p < 0.05$ ; diabetic control vs. group III) and the same effect was observed in *Leptospermum flavescens* (500

mg/kg) treated group. It is well known that sulfonylurea causes hypoglycemia by increasing insulin secretion from pancreas (Del Prato & Pulizzi 2006). The results in Table 2 shows that *Leptospermum flavescens* aqueous leaves extract in both of the doses 200 mg/kg and 500 mg/kg produced hypoglycemia in diabetic rats. This indicated that there is impaired in insulin sensitivity through insulin receptor of mechanism of enzyme involved in glucose



Each values are expressed as Mean  $\pm$  SEM ( $n = 6$ )

FIGURE 6. Hyperglycemic effect of aqueous extract of *Leptospermum flavescens* on fasting blood glucose level (FBGL) of normal rats during OGTT

TABLE 2. Effects of aqueous crude extracts of *Leptospermum flavescens* on fasting blood glucose level in diabetics rats

Group ( $n=6$ )	Treatment	Fasting plasma glucose concentration (mmol/L)				
		0th day	5th day	10th day	15th day	20th day
I	Normal Control	4.300 $\pm$ 0.292	4.800 $\pm$ 0.357	4.700 $\pm$ 0.478	4.000 $\pm$ 0.510	4.4 $\pm$ 0.747
II	Diabetic Control	14.100 $\pm$ 0.402	15.1 $\pm$ 0.727	16.200 $\pm$ 0.804	18.100 $\pm$ 1.425	20.7 $\pm$ 0.597*
III	Glipizide (2 mg/kg)	14.900 $\pm$ 1.931	13.5 $\pm$ 1.924	12.900 $\pm$ 1.682	10.600 $\pm$ 0.835	9.50 $\pm$ 0.257*
IV	Diabetic + Leaves aqueous extract (200 mg/kg)	15.200 $\pm$ 2.342	14.32 $\pm$ 3.013	14.12 $\pm$ 1.21	13.400 $\pm$ 1.940	13.31 $\pm$ 0.053*
V	Diabetic + Leaves aqueous extract (500 mg/kg)	14.500 $\pm$ 2.247	10.40 $\pm$ 1.206	8.50 $\pm$ 1.34	6.71 $\pm$ 1.52	5.52 $\pm$ 1.07*
VI	Diabetic + Stem aqueous extract (200 mg/kg)	15.000 $\pm$ 1.76	15.22 $\pm$ 1.22	15.83 $\pm$ 1.71	15.82 $\pm$ 1.87	15.45 $\pm$ 1.09*
VII	Diabetic + Stem aqueous extract (500 mg/kg)	15.700 $\pm$ 2.01	15.74 $\pm$ 2.11	15.44 $\pm$ 2.21	15.55 $\pm$ 2.54	15.15 $\pm$ 3.32*

Each value represents Mean  $\pm$  SEM ( $n = 6$ ). \* $p < 0.05$  compared with diabetic control

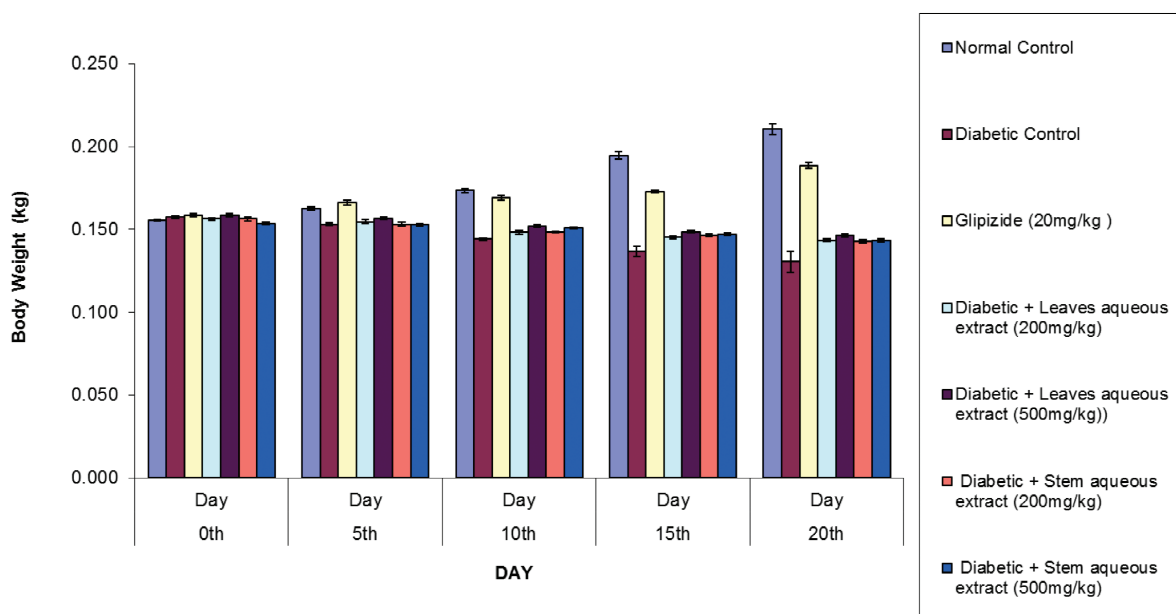
phosphorylation (Benhahhord et al. 2001; ElHilaly & Lyoussi 2002). This strongly suggests that the possible mechanism by which the antihyperglycemic activity is enhanced insulin secretion effect from pancreatic  $\beta$  cells due to the presence of aromadendrin glucoside, kaempferol rhamnoside, quercetin rhamnoside and vindoline in aqueous extract of leaves from *Leptospermum flavescens* which have been detected by LCMS/MS.

Weight loss is one of the major complications in diabetes and it arises due to the impairment in insulin action caused by alloxan toxicity (Sancheti et al. 2010). Due to this there is a decrease in the body weight of alloxan induced diabetic animals. As shown in Figure 7, treatment with alloxan caused a significant ( $p < 0.05$ ) weight loss on the 20<sup>th</sup> day compared with normal control. This may be

due to the reduction of insulin release from pancreatic  $\beta$  cells which leads to hyperglycemia, as a result of increase muscle wasting and loss of tissue protein in diabetic rats (Salahuddin & Jalalpure 2010). In contrast, rats in normal control group continued to gain weight (42%) during the 4-week of experimental period. Treatment with standard drug glipizide increased body weight by 13% in diabetic rats. Whereas, diabetic rats treated with *Leptospermum flavescens* leaves aqueous extract (200 mg/kg and 500 mg/kg) are not significantly reduced the body weight after 20 days. Thus, it can maintained the lost of the body weight compared with their initial level. The levels of serum lipids are usually altered in diabetic mellitus (Shirwaikar et al. 2006). In this study it was observed that in diabetic rats the serum triglycerides and total cholesterol levels were

significantly ( $p < 0.05$ ) elevated and serum HDL cholesterol levels were decreased 44.9% in comparison with normal control (Table 3). Treatment with *Leptospermum flavescens* leaves extracts at the dose of 500 mg/kg for 20 days to the diabetic rats significantly ( $p < 0.05$ ) decreased serum triglycerides and total cholesterol levels compared with the diabetic control. Treatment with standard drug glipizide significantly decreased triglyceride, but there was no significant decrease in serum total cholesterol levels

compared with diabetic control. The serum HDL levels in both of the *Leptospermum flavescens* and glipizide treated groups were restored to the control level. This is due to the effect of aqueous extract increased the utilization of glucose and depressing the metabolism of fat. These data are consistent with previous report which stated that administration of high-fat diet with powdered fruit of *Sophora species* significantly decreased body weight in diabetic mice, exhibited lowering triglyceride and



Each values are expressed as Mean  $\pm$  SEM ( $n = 6$ )

FIGURE 7. The effect of aqueous extract of *Leptospermum flavescens* on body weight of diabetic rats

TABLE 3. Effect of aqueous extracts of *Leptospermum flavescens* on serum lipid profile in diabetic rats

Group ( $n=6$ )	Treatment	Serum lipid profile (mmol/L)			
		TG	TC	HDL	LDL
I	Normal control	0.619 $\pm$ 0.051	1.647 $\pm$ 0.053	0.631 $\pm$ 0.037	0.736 $\pm$ 0.064
II	Diabetic control	1.838 $\pm$ 0.100*	3.200 $\pm$ 0.068*	0.348 $\pm$ 0.031*	1.509 $\pm$ 0.072*
III	Glipizide (2 mg/kg)	0.949 $\pm$ 0.108*	1.686 $\pm$ 0.073*	0.697 $\pm$ 0.107*	0.631 $\pm$ 0.111*
IV	Diabetic + Leaves aqueous extract (200 mg/kg)	1.523 $\pm$ 0.047*	3.075 $\pm$ 0.083	0.788 $\pm$ 0.065*	0.685 $\pm$ 0.005*
V	Diabetic + Leaves aqueous extract (500 mgg/kg)	0.859 $\pm$ 0.076*	1.456 $\pm$ 0.058*	0.702 $\pm$ 0.066*	0.803 $\pm$ 0.047*
VI	Diabetic + Stem aqueous extract (200 mg/kg)	0.800 $\pm$ 0.000*	1.150 $\pm$ 0.018*	0.670 $\pm$ 0.080*	0.623 $\pm$ 0.092*
VII	Diabetic + Stem aqueous extract (500 mg/kg)	0.710 $\pm$ 0.073*	1.750 $\pm$ 0.018*	0.645 $\pm$ 0.064*	0.750 $\pm$ 0.011*

Each value represents Mean  $\pm$  SEM ( $n = 6$ ). \* $p < 0.05$  compared with diabetic control



cholesterol effects while at the same time increasing HDL cholesterol in hyperlipidemic and cholesterol-fed rats (Hyun et al. 2008; Park et al. 2009).

#### CONCLUSION

The leaves aqueous extract of *Leptospermum flavescens* has been shown to contain aromadendrin glucoside, kaempferol rhamnoside, quercetin rhamnoside and vandoline with LCMS/MS. It showed no toxic effect of *Leptospermum flavescens* on male rats. Furthermore, the leaves aqueous extract showed high glycogen phosphorylase activity and reduced glucose significantly at concentration 500 mg/kg in diabetic rats. In addition, the leaves aqueous extract decreased the serum triglycerides and total cholesterol level in diabetic rats while the level of HDL are maintained compared with the control rats. Thus, the results obtained from this study provide novel evidence in supporting the traditional uses of *Leptospermum flavescens* as an antidiabetic remedy and its complications.

#### ACKNOWLEDGEMENTS

The authors wish to thank University of Malaya for the financial support through University Malaya Research Grant (UMRG) RG025/09BIO.

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Received: 28 December 2012

Accepted: 28 January 2014