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In vivo antidiabetic efficacy of Malaysian *Vernonia amygdalina* aqueous extract

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Abstract
Diabetes mellitus (DM) is a major world health concern especially DM types 2. In Malaysia, about 2.6 million adults were diagnosed with diabetes and most of them turn to herbal medicine for treatment. *Vernonia amygdalina* or locally known as bitter leaf is believed by the local people and traditional inhaler to reduce blood glucose level. This study aims to prove the antidiabetic property of Malaysian local *V. amygdalina* as claim by the local Malaysian. *V. amygdalina* aqueous extract was prepared by reflux method and was further concentrated. Twenty male Sprague Dawley rats were divided into 4 groups; non-diabetic rats as normal control (NC), diabetic induced rats as diabetic control (DC), diabetic induced rats treated with 150 mg/kg metformin and diabetic induced rats treated with 50 mg/kg *V. amygdalina* aqueous extract. The diabetic rats were induced by using 40 mg/kg of Streptozotocin (STZ). The study was conducted for 28 days. Body weights (BW) were taken at weekly interval for 4 weeks. Fasting blood glucose was measured in 3 day intervals. At the end of experiment, blood samples were collected for lipid profile test and insulin secretion assay. There were no significant differences in BW, relative organ weight (ROW) and no organs abnormalities were observed in all experimental groups. Significant decreases in triglycerides, total cholesterol and fasting blood glucose gave a prove evidence that *V. amygdalina* possessing antidiabetic property. However, the levels of insulin in diabetic induced rats treated with *V. amygdalina* aqueous extract were not significant as compared to the diabetic induced rats treated with metformin. The aqueous extract of *V. amygdalina* has antidiabetic activity. This work supports the folk use of this plant in treating diabetes. This antidiabetic property of Malaysian *V. amygdalina* may due to its phytochemical constituents as our previous study revealed the high contents of flavonoids and terpenoids. However, the aqueous extract of *V. amygdalina* did not act through regulation of insulin hormone since there was no significant change in insulin level.

Keywords: *Vernonia amygdalina*, antidiabetic, phytochemical, *in vivo*, biochemical test

Introduction
Diabetes mellitus (DM) is a chronic disease occurs due to lack of insulin production by the pancreas, known as Type 1 DM (T1DM) or the ineffectiveness of the body for insulin usage known as type 2 DM (T2DM). In definition, DM is a group of metabolic disease with characteristic of elevated blood glucose level, a condition known as hyperglycemia (Wild *et al*., 2004) [46]. DM can develop through years without showing symptoms or symptoms may be diagnosed as other conditions. Complication arises from DM includes cardiovascular disease, neuropathy, retinopathy and kidney disease, are irreversible once they develop and may result in disability for the person having the disease (International Diabetes Federation, 2012) [20]. T1DM usually occurs in children and the frequency is low relative to the T2DM, accounting for 10% or less of the total number of people with diabetes (Wild *et al.*, 2004 International Diabetes Federation, 2012; Zimmet *et al*., 2001) [46, 20]. As for T2DM, it predominantly affects middle-ages and older peoples, however, concern rises as the number of T2DM patients have increased among children and adolescents (Rosenbloom at al., 1999; Fagot-Campagna *et al*., 2001; Arslanian, 2004) [38, 12, 3].
The number of total death from DM are estimated to be elevated by more than 50% by the year 2030 with the disease predicted to be the seventh leading cause of death [1]. DM happens throughout the world, and it is most common in the developed countries, especially for T2DM. Malaysia has become top 10 countries with highest prevalence in diabetes with 11.6% in 2010 and expected to increase up to 13.8% in 2030 (Shaw *et al*., 2009) [41]. According to National Health Morbidity Survey (NHMS) of Malaysia, in 2011, the prevalence of diabetes has elevated by 31.0% in the period of 5 years, from 11.6% in 2006 to 15.2% in 2011 (NHMS,

2014) [31]. another study shows that there are about 2.6 million Malaysian adults with the age of 18 years and above are diagnosed with diabetes (Feisul and Azmi, 2013) [13].

Modern pharmaceuticals frequently employed chemicals and synthetic drugs, which believed to have adverse impact on health. Therefore, people are turning towards the use of natural as remedies of diseases or wounds. Thus, medicinal plant has become an alternative for treatment and prevention of certain diseases because it was believed to have lesser side effects. The medicinal value of plants can be found in the leaves, roots or other part of plants, arising from the rich component of phytochemicals and secondary metabolites. Plants remain to be the mainstay of the health care system in the rural area due to the limited access to modern health facilities. Despite that, the usage of the plant is depends on the ethnic beliefs, traditional usage and the availability of the plant itself.

Phytochemical defined as bioactive non-nutrient plant compounds that can be found in fruits, vegetables, grains and other plant foods that have been associated in reducing the risk of major chronic disease (Liu, 2004) [24]. The medicinal value of herbal plants lies in the phytochemical compounds that produce a definite physiological action on the human body (Mithraja *et al*., 2011) [30]. However, samples of medicinal plant from different plant varieties and geographical origin have different complex chemical mixture (Sim *et al*., 2004) [43].

Currently, *V. amygdalina* is popular among diabetes patients in Malaysia. They were claimed that the leaves of *V. amygdalina* have a hypoglycemic activity which able to lowering blood glucose level. The leaves were boiled with water to make a decoction and will consume two times per day. The leaves of *V. amygdalina* were also consumed as vegetables and used as condiments in a meal (Arhoghro *et al*., 2009) [2]. *V. amygdalina* is a regenerating soft wooded shrub of 2 to 10 meters in height with petiolate leaves of around 6 mm in diameter (Georgewill *et al*., 2009; Yeap *et al*., 2004) [15, 47]. In Malaysia, *V. amygdalina* also known as bitter leaf due to its bitter taste (Akah *et al*., 2009; Ong *et al*., 2011) [1, 33]. Traditional practices in other countries have been found to use *V. amygdalina* as a treatment in diabetes mellitus. In Nigeria, *V. amygdalina* was cited as the most popular antidiabetic herbal medicine. *V. amygdalina* leaves were squeezed until the juice comes out and mixed with water. The juice was also mixed with potash salt and honey to treat diabetes mellitus (Gbolade, 2009) [14]. Several studies have been reported that *V. amygdalina* exhibited the characteristics of antidiabetic (Akah *et al*., 2009; Ong *et al*., 2011; Ekpo *et al.*, 2007; Atangwho *et al*., 2010) [1, 33, 10, 4].

However, the geographical origin, locality and climate conditions could influence the biological activities of the plants (Cosentino *et al*., 1999; Hossain and Nagooru, 2011; Hossain *et al*., 2013) [8, 19, 18] as they have different complex chemical mixure (Sim *et al*., 2004) [43]. Certain metabolites or contents are only synthesized and increased under specific environments. For example, in *Rosmarinus officinalis*, the content of terpene was affected by carbon dioxide, water supply and seasonally (Yusmazura *et al*., 2016) [48]. Furthermore, previous studies have demonstrated that in different environments of medicinal plants growth, the contents of secondary metabolites produce were different. Therefore, the medicinal qualities produce were varied (Penuelas and Llusia, 1997). A study on *Sinopodophyllum hexandrum*, an herbaceous perennial plant showed that in the different locations throughout China, the active ingredients of the roots and rhizomes significantly affected, indicating that ecological and geographical differences influence the content of plants [26]. A preliminary study of the essential oil from *Xylopia aethiopica* has also demonstrated that the composition of essential oil was quantitatively difference according to the climate conditions, soil, geographical origin and genetic factors (Liu *et al*., 2015; Elhassan and Ayoub, 2014) [25, 11].

Therefore, the phytochemical contents of Malaysian *V. amygdalina* have been identified in our previous study (Yusmazura *et al*., 2016) [48]. Thus, this present study aims to investigate the antidiabetic property of Malaysia *V. amygdalina* as claimed by local people and to prove that the respective biological effect is due to its phytochemical contents. The STZ-diabetic rats were used as an animal model. The use of STZ induced hyperglycemia rat has been described as a useful experimental animal model in diabetic studies (Rerup, 1970). This procedure has been used in over 7600 PubMed citations making this the most used animal model to diabetic study of human (Tom *et al*., 1992). Many studies were revealed that the rats administered with STZ in dose range of 40 - 65 mg/kg, have been developed moderate and stable non-fasting hyperglycemia without any significant changes in plasma insulin levels. STZ causes only minor damage to pancreatic beta cell mass, producing T2DM. Hence, this model is found to be an advantageous tool for investigation of antidiabetic agents in the treatment of T2DM.

Materials and Methods

Plant material

The fresh leaves of *V. amygdalina* were collected from Bachok, Kota Bharu, Kelantan, Malaysia. The identification of the plant was performed by Natural Medicinal Products Centre, Kulliyah of Pharmacy, and International Islamic University Malaysia (IIUM) with voucher specimen number PIIUM 0233. The name of plant also been checked with www.theplantlist.org.

Preparation of *V. amygdalina* aqueous extract

The leaves were cleaned with towel to remove any debris and dust. After that, they were dried in an oven at 55°C ± 1°C for 72 hours prior to crush into coarse powder. The 25 g of *V. amygdalina* coarse powder was extracted in 250 ml of distilled water at 60°C ± 1°C for 72 hours. The extract was filtered and further dried in an oven incubator at 37°C. The solid residue was referred as aqueous extract and the percentage of yield was calculated.

Animal study

Twenty male Sprague Dawley (SD) rats were obtained from Animal Research and Service Center (ARASC), Universiti Sains Malaysia (USM), Kubang Kerian, Kelantan. The rats weighing from 250-300 g and 8-12 weeks old were chosen as an experimental model. The rats were kept in individual cages. Water and standard pellet food are available at all times (ad libitum). The rats were allowed to acclimatize for one week prior to the experiment. The Principles of Laboratory Animal Care (NIH, 1985) were followed during the experimentation period. The experimental protocol was approved by the Animal Ethics Committee, USM (AECUSM) under USM/Animal Ethics Approval/2012/(82)(418). The ARRIVE Guidelines for reporting animal research was apply throughout the study (Kilkenny *et al*., 2010) [23].

Induction of diabetes with streptozotocin (STZ)

The rats (n=15) were fasted for overnight prior to single intra peritoneal injection of 40 mg/kg of body weight (BW) of STZ ((Nacalai Tesque, Japan). STZ was freshly dissolved in 1 ml of citrate buffer (pH4.5) prior to injection. After 7 days of injection, fasting blood glucose (FBG) level was measured with glucometer (Accu-Chek Performa). Rats with FBG of 12 mmol/L and higher were considered diabetic and were used for the study.

Animal and treatment

The diabetic rats induced- STZ were divided randomly into 3 groups consisting of 5 rats per group. Group 1 has contained of diabetic rats given 1 ml phosphate buffer (diabetic control group). Group 2 are the diabetic rats treated with 150 mg/kg metformin (CCM Pharmaceutical, Malaysia) and group 3 are the diabetic rats which received 50 mg/kg *V. amygdalina* aqueous extract. Metformin and *V. amygdalina* aqueous extracts were freshly prepared according to the BW of the rats and were dissolved in 1 ml of phosphate buffer saline (Nacalai Tesque, Japan) and 1 ml of distilled water, respectively. Treatments were given twice daily, at 9 a.m. and 4 p.m. for 28 days. One group of non-diabetic rats (n=5) or clean rats was used as normal control (NC). Body weight was recorded weekly.

Blood collection and biochemical analysis

After 28 days, all the rats were fasted overnight before euthanization by intra peritonal injection of sodium pentobarbital (Alfasan Woerden, Holland). Approximately 5 ml of blood was collected through cardiac puncture and transferred into a blood plain vacuum tube. The blood samples were centrifuged at 4500 rpm for 10 min for serum separation. The serum obtained was evaluated for lipid profile test and FBG level.

Insulin secretion assay

The insulin secretion assay was done by using Insulin ELISA kit (ab10057, Abcam). This kit employs an antibody specific for insulin coated on a 96-well plate. Serum samples were pipetted into the well plate and procedures were followed as to manual. The intensity of colour formation is proportional to the amount of insulin bound, which was measured at 450 nm by microplate reader (Model 680, Biorad).

Statistical analysis

Data were expressed as Mean ± SEM. The statistical analysis were analyzed using Kruskal Wallis test to evaluate the differences in the lipid profile test, FBG, mean insulin absorbance between each group. Results with (^{a}P value < 0.05) were considered statistically significant.

3. Results and Discussion

Body weight (BW) and relative organ weight (ROW)

V. amygdalina traditionally claim has a potential in lowering blood glucose among diabetes patients. In order to confirm the effectiveness of *V. amygdalina* and to prove the claim by traditional practice, aqueous extract of local Malaysian *V. amygdalina* leaves were used in this study in order to mimic as close as possible. Most of the traditional healers used water to make herbal decoctions juices. In addition, the aqueous extract was chosen as water is the universal solvent, non-toxic and does not interfere with the end product of extraction.

The group contains of clean rats or normal control (NC) showed a slightly gain in body weight throughout the experiment. However, diabetic induced groups have shown a pattern of decrease in BW throughout the 28 days of experimental period. Diabetic induced rats treated with *V. amygdalina* aqueous extract showed increased of weight in Day 15. Despite that, there were no significant differences in BW and ROW between each group (Table 1 and Table 2). There were no organs abnormalities observed in all the rats.

Table 1: The body weight recorded for each group in one week of interval for 28 days

Body weight (g)	GROUP			
	NC	DC	Metformin	VA
Week 0	403.3 ± 9.1	385.8±12.1	388.4 ± 8.5	386.4 ± 23.2
Week 1	403.0 ± 11.4	357.0±10.3	364.4 ± 10.3	366.2 ± 28.7
Week 2	414.8 ± 11.1	360.0±10.0	364.0 ± 11.9	371.0 ± 32.2
Week 3	419.0 ± 9.1	358.3 ± 9.1	363.8 ± 12.9	369.2 ± 33.4
Week 4	408.3 ± 11.1	327.5±11.8	339.8 ± 17.5	343.0 ± 37.9

Value were expressed as mean ± SEM, n=5, **P*<0.05, Kruskal Wallis test not significant. NC = normal control, DC = diabetic control, Metformin = 150 mg/kg/BW metformin-treated diabetic, VA = 50 mg/kg/BW V.amgdalina aqueous extract-treated diabetic.

Table 2: The relative organ weight (ROW) recorded for each group at day 28

Relative organ weight (g)	GROUP			
	NC	DC	Metformin	VA
Liver	2.7 ±0.1	3.3±0.2	3.4 ± 0.2	3.4±0.2
Heart	0.4±0.02	0.4±0.03	0.4 ± 0.02	0.4±0.02
Kidney	0.6±0.04	0.8 ± 0.1	0.8 ± 0.04	0.8 ± 0.1

Value were expressed as mean ± SEM, n=5, **P*<0.05, Kruskal Wallis test not significant. NC = normal control, DC = diabetic control, Metformin = 150 mg/kg/BW metformin-treated diabetic, VA = 50 mg/kg/BW V. amgdalina aqueous extract-treated diabetic.

Lipid profile test

In this present study, *V. amygdalina* aqueous extract possessed antidiabetic characteristic which the ability to reduce blood glucose level. There were no significant differences in the level of total cholesterol and LDL cholesterol between each group. *V. amygdalina* aqueous extract-treated group has significant increase in total cholesterol/HDL ratio compared to others group. NC group has significant lower level in triglycerides, HDL cholesterol and FBG level among each group. *V. amygdalina* aqueous extract-treated group has significant reduced in triglycerides, HDL cholesterol and FBG level compared to metformin treated rats and diabetic control group (Table 3). Metformin treated rats have shown a slight increase in FBG as compared to diabetic control rats.

Table 3: Lipid profile test and fasting blood glucose level after 28 days of treatment

GROUP	NC	DC	Metformin	VA
Total cholesterol (mmol/L)[a]	1.50±0.04	1.80±0.11	1.88 ± 0.15	1.68 ± 0.07
Triglycerides (mmol/L)[a]	0.42±0.06*	0.96±0.29	0.90 ± 0.17	0.45±0.03**
HDL cholesterol (mmol/L)[a]	0.31±0.01*	0.49±0.05	0.36 ± 0.03	0.32±0.02**
LDL cholesterol (mmol/L)[a]	1.00 ± 0.02	0.87±0.11	1.11 ± 0.10	1.15 ± 0.08
Total cholesterol/HDL ratio	4.88 ± 0.15	3.73 ± 0.16	5.30 ± 0.33	5.32 ± 0.42*
Fasting blood glucose (mmol/L)[a]	6.78±0.68*	10.90±1.49	14.70±2.38	8.80±1.06**

[a]Values were expressed as mean ± SEM. (n=5). P value < 0.05. * Significantly different from other groups, ** significantly different among diabetic induced groups. NC = normal control, DC = diabetic control, Metformin = metformin-treated diabetic, VA = V. amgdalina aqueous extract-treated diabetic.

Diabetic rats treated with *V. amygdalina* aqueous extract showed a significant reduced in triglycerides, HDL cholesterol and FBG level. These findings were correlates to the previous findings that describe *V. amygdalina* ethanol extract significantly reduced glucose level and decreased triglycerides level, and *V. amygdalina* aqueous extract significantly decreased triglycerides level and normalized cholesterol concentrations (Ekpo *et al*., 2007; Atangwho *et al*., 2010; Trease and Evans, 1989) [10, 4, 44]. A related study from Ghana also showed that the leaf of *Vernonia amygdalina* either young leaf or old leaf has possess antidiabetic property. Both young leaf and old leaf significantly reduce the levels of blood glucose (Asante *et al*., 2016) [5]. The decreasing of body weight in diabetic control group was observed throughout the experiment period but not significantly different (p>0.05). Severel studies have shown the relationship between hyperglycemia and decreased body weight of diabetic animals. STZ by producing diabetes or hyperglycemia and hypoinsulinemia causes reduction in the body weight of diabetic animals (Nwanjo, 2005) [32]. The obligatory renal water loss along with hyperosmolarity in diabetes triggered the osmoreceptor of the thirst centre of the brain and polydipsia which resulted in water intake. Hence, this catabolic effect resulted in weight loss (Akah *et al*., 2009) [1]. The treatment with *V. amygdalina* aqueous extract showed the increased of body weight. The rats gained the body weight within 15 days. However, the data was not statistically significant. Previous finding was reported that the body weight of diabetic induced rats treated with *V. amygdalina* extract slightly increase after a few days of treatment (Zafar and Hassan, 2010) [50]. ROW did not show any significant differences between each group and there were no organs abnormalities, gross lesions or enlargement of organs observed in all the rats. The observations were similar to one of the previous studies, which were reported that there were no significant differences in wet organ weight and no significant abnormalities in the vital organs in diabetic treated *V. amygdalina* extract (Akah *et al*., 2009) [1].

Insulin secretion assay

The insulin secretion assay showed that there were no significant changes in the level of insulin in the diabetic rats treated with *V. amygdalina* extract as compared to metformin treated group. Metformin- treated group has the highest insulin level (Table 4).

Table 4: The mean absorbance for insulin level after 28 days of experiment

GROUP	NC	DC	Metformin	VA
Mean absorbance (nm)[a]	0.17±0.003*	0.14±0.002	0.17±0.009**	0.14±0.008

[a]Values were expressed as mean ± SEM. (n=5). P value < 0.05. * Significantly different from other groups, **significantly different among diabetic induced groups. NC = normal control, DC = diabetic control, Metformin = metformin-treated diabetic, VA = V. amgdalina aqueous extract-treated diabetic.

The result is in line with the previous study which reported that metformin decreased the plasma glucose level by enhanced the insulin release (Yoshida *et al*., 2009) [49]. Based on the result, it was showed that Malaysian *V. amygdalina* act as glucose lowering agent without regulate the insulin action. There was a slight increase in fasting blood glucose of metformin-treated group as compared with diabetic control. Metformin is currently the drug of first choice for the treatment of T2DM and being prescribed to at least 120 million people worldwide. Despite that, the exact molecular mechanism of action of metformin has not been fully elucidated. Metformin is regarded as an anti-hyperglycemic agent because it lowers blood glucose concentrations in T2DM, reduces insulin resistance and significantly lowering plasma fasting glucose level. Metformin could be activated insulin receptor expression and tyrosine kinase activity, thus improve insulin sensitivity (Gunton *et al*., 2003) [16]. In addition, the action of metformin to inhibit complex I of the respiratory chain, mitochondrial respiration and ATP synthesis, has been proposed (Rutter *et al*., 2003) [37]. Although metformin is generally considered to have no direct effect on the pancreatic islet β-cell, an early report demonstrated a dose-related inhibition of glucose-stimulated insulin secretion and insulin biosynthesis by metformin (Schatz *et al*., 1972) [40]. An observation usually attributed to the increase in peripheral insulin sensitivity (Rutter *et al*., 2003) [37]. Clinical studies also show that metformin may reduce plasma dipeptidyl peptidase-4 activity and increase circulating levels of glucagon-like peptide 1 (GLP-1) (Maida *et al*., 2011) [28]. Another research study shows that metformin activates AMPK activity in MIN6 cells and human islets of Langerhans and inhibits insulin release (Isabelle *et al*., 2004) [21], therefore, might reduce the sensitivity of insulin and increases glucose level. This kind of action might have occurred in our present study, thus the FBG levels of metformin-treated diabetic rats slightly increase as compared to diabetic control group. The effects of metformin on T2DM been somewhat contradictory, with some reports demonstrating that metformin improve insulin secretion, increase insulin gene expression and insulin content, and reduce islet apoptosis in human islets exposed to high glucose (Marchetti *et al*., 2004) [29]. In contrast, metformin was also reported to inhibit insulin secretion (Isabelle *et al*., 2004) [21], impairs glucose-responsivity and enhances susceptibility to apoptosis (Kefas *et al*., 2004) [22]. Therefore, these discrepancies possibly reflect variation in experimental models, species-specific differences, doses of metformin and duration of metformin exposure, which need further study.

The antidiabetic property of the *V. amygdalina* might have an association with its phytochemical contents. Our previous study on phytochemicals analysis by standard method revealed that the aqueous extract of Malaysian *V. amygdalina* leaves were contained of flavonoids, tannin, saponin and terpenoids. ATR-FTIR spectrum also demonstrated the present of phenol and a high content of flavonoids and terpenoids (Yusmazura *et al*., 2016) [48]. Previous research on other localities of *V. amygdalina* have found polyphenols, glycosides, steroids, carbohydrates, alkaloids, saponin, tannin, flavonoids and glycosides in the aqueous extract of *V. amygdalina* leaves (Akah *et al*., 2009; Siddiqui *et al*., 2009) [1, 42]. Thus, the main difference between Malaysian *V. amygdalina* and any other reported *V. amygdalina* is in the content of terpenoids. Terpenoids is one of the plant antioxidants substances besides ascorbic acid and tocopherols which perform an important function in human and plant. During the assessing of plant antioxidant activity, an important point to consider is the interaction with other antioxidants. Flavonoids also act as an antioxidant (Arhoghro *et al*., 2009) [2]. The combinations of hydrophilic and lipophilic antioxidants may exert synergistic effects which could significantly increase the potential of the antidiabetic property. One of the major triggers in inducing hyperglycemia in diabetic complications has been proposed was an increase of oxidative stress. Hyperglycemia is the main symptom of

diabetes, which generates reactive oxygen species (ROS) and eventually causes lipid peroxidation and membrane damage (Pate *et al*., 2010) [34]. Lipid peroxidation also plays a role in the long term complications of diabetes (Logani and Davis, 1979) [26]. Thus, antioxidants play an important role to protect the human body against damage by reactive oxygen species (Lollinger, 1981) [27].

Therefore, the effectiveness of Malaysian local *V. amygdalina* may be due to the high content of flavonoids. Previous studies reported that flavonoids act as an insulin secretagogues or insulin mimetics (Collier *et al*., 1990) [7]. This flavonoids probably influencing the pleiotropic mechanisms to attenuate diabetic complications with protecting β-cells against ROS-mediated damage, leading to cellular antioxidant defenses and minimizing hyperglycemia in STZ-induced diabetes (Collier *et al*., 1990) [7]. Thus, the Malaysian *V. amygdalina* extract might have insulin like activity. The anti-hyperglycemic effect of the extract may be due to an increase in peripheral glucose consumption as well as protection against oxidative damage in diabetic rats (Halliwell and Gutteridge, 1985; Logani and Davis, 1979; Saghizadeh *et al*., 1996) [17, 26, 39]. There is also a possibility that the Malaysian *V. amygdalina* extract reduces the effect of inflammatory cytokine release during diabetes which may be one of the causative agents for the tissue distraction and insulin resistance (Saghizadeh *et al*., 1996) [39]. However the exact mechanism of *V. amygdalin*a extract in lowering blood glucose need to be further confirmed.

Conclusion

Malaysian *V. amygdalina* possessed antidiabetic activity. Based on the findings, together with our previous study, the effect of Malaysian *V. amygdalina* leaves as an antidiabetic agent may be due to the high content of flavonoids and terpenoids. As compared to other locality of *V. amygdalina*, the present of terpenoids and flavonoids in Malaysian *V. amygdalina* leaves become a good combination in order to act as an antidiabetic agent. The synergistic effects of both compounds make the plant more effective not only in lowering blood glucose but also as prevention against ROS in the body. Flavonoids mimic the insulin in order to reduce blood glucose while terpenoids act as an antioxidant and protect from organ damage. As a final conclusion, the findings in this present study clearly indicate an exciting promising of alternative potent antidiabetic drug from Malaysian *V. amygdalina*.

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