**Journal of Medicinal Plants and By-products (2018) 1: 67-73**

## Original Article

# *Vitis Elegan* as a Promising Antidiabetic Herb: Phytochemical and Pharmacological Assessment

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Article History: Received: 01 August 2017 /Accepted in revised form: 06 January 2018


## Abstract

In this research, we investigated the antidiabetic activity of *Vitis elegans* rhizome, which is used as traditional treatment for diabetes mellitus. The methanol, chloroform, petroleum ether, and hexane extracts of the plant root were obtained through serial exhaustive extraction and were analyzed by Thin Layer Chromatography (TLC). Glycogen phosphorylase (GP) assay was done to determine the inhibitory effects of respective extracts on GP enzyme. Total phenol content was measured using the Folin-Ciocalteu method and brine shrimp test was done to evaluate the toxicity of the extracts. Evaluation of TLC plates alone and after spraying with different reagents indicated that terpenoid was the major component of the sample followed by alkaloid and phenol. Chloroform extract applied more inhibitory effects on GP enzyme activity with percentages of 39.65 in concentration of 2.5 mg/ml. This suppression effect was higher than glucose, as a standard inhibitory agent in the body. The highest amount of phenol was found in the methanol extract, equal to 49 mg GAE g-1. Petroleum ether, chloroform and methanol extracts were considered as non-toxic solvents with LC50 values of 8.9, 3.5 and 3.7 mg/ml respectively. While hexane with 0.089 mg/ml LC50 value was classified as toxic extract. Based on the results of this study, we concluded that *Vitis elegans* rhizome, has the potential to be further studied for anti-hyperglycemic properties.

**Keywords:** Anti-diabetic, Enzyme Inhibition, Glycogen phosphorylase, Phytochemical screening, *Vitis elegans*

## Introduction

Diabetes mellitus (DM) is a complex metabolic disorder, caused by abnormal high level of blood glucose (hyperglycemia) [1]. In the past decade an explosive increase in the number of people diagnosed with DM worldwide has been accrued, from 170 million persons in 2005 [2] to 422 million in 2014 [3]. Type 2 diabetes mellitus (T2DM) is the most common type of DM with prevalence of affecting 9% of the world population [3]. Therefore, research for exploring innovative therapeutic targets with fewer side effects is still warranted.

Several classes of molecules are available to control T2DM. Oral hypoglycemic drugs (Glucophage, incretin mimetics, sulfonylureas, thiazolidinediones) [4-6] and α-glucosidase inhibitors (acarbose, miglitol, voglibose) [7,8] are widely used. However, there are several adverse

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side effects such as danger of causing hyperinsulinaemia or sometimes hypoglycemia [9]. Inhibition of hepatic glycogen phosphorylase (GP) is a new therapeutic potential for decreasing high glucose level [10-12] through suppressing hepatic glucose output in T2DM. Recent studies reported low hepatic glucose secretion in animal models of diabetes after administration of synthetic GP inhibitors [13-15]. Liver is the predominant source of blood glucose. This organ utilize GP enzyme to release glucose into the blood stream through process of glycogenolysis. Moreover, liver is capable to synthesis glucose from 3-carbon precursor in process called gluconeogenesis. Therefore, inhibition of hepatic GP could suppress glucose production from pathways glycogenolysis and gluconeogenesis and could alleviate the diseases-related complications [16].

Beside, some studies have shown the inhibitory effects of herbal-remedies on GP enzyme [17,18]. Herbs are cost effective, easy available and have less or no side effects [19]. Based on current NAPRALERT database more than 1300 remedies have been determined to possess anti-hyperglycemic activity [20]. However, the active components in herbs are not well defined. It is necessary to specify the active components and their metabolism effects for evaluating their therapeutic efficiency.

The rhizome of *Ampelopsis glandulosa* (Wall.) Momiy, commonly known as *Vitis elegans*, belongs to *Vitaceae* family. It has been used for treatment of T2DM in Asia. The anti-hyperglycemic activity of different species of this family has been studied before [27-30]. The presence of different components including phenols, tannin and flavonoid were considered as the natural source of antioxidant and anti-hyperglycemic in this family [28,31]. To the best of our knowledge no scientific study has been conducted on the *Vitis elegans* to evaluate its anti-diabetic activity. Therefore, we aim to investigate the phytochemical screening, total phenol content and GP inhibitory activity of this species.

## Material and Methods

### Plant Material

The rhizome sample of *Vitis elegans* was purchased from Biology Institute, Faculty of Science, University of Malaya in spring 2011, and then dried under shade and then milled to a fine powder before extraction.

### Animal

Total 6 healthy adult Wistar rat prepared from ISB farm, University Malaya. The rats were housed in polypropylene cages for one week under standard conditions (12 h light and 12 h dark cycle, 25±3 °C); they were fed with standard diet (Sai Foods, Bangalore) and water ad libitum. Rats were scarified by stretching the necks. Since chloroform could have adverse effect on enzymes. This study full considered the ethical principles approved by international animal care.

### Extraction

Serial exhaustive extraction was performed using four different solvents, according to ascending order of their polarity. This method involves successive extraction with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. We used hexane, as a non-polar solvent, to extract the low polar compounds such as flavonoid, terpenoid [21] and fatty compounds. Chloroform, a semi-polar organic solvent, was chosen to extract the semi-polar compounds like alkaloids [22], polyphenol [23] and terpenoid. Petroleum ether also detects bioactive compound with intermediate polarity. Methanol, as a most common solvent, extracts polar compounds such as saponin, tannin [24], starches, terpenoids and polypeptides. In serial exhaustive extraction 20.0 gr of plant powder extracted successively with hexane (200 ml), chloroform (200 ml), petroleum ether (200 ml) and methanol (200 ml) respectively. All samples incubated for 3 hours in a water bath at 40 °C of temperature. The extracts were filtered through a buchner funnel and concentrated using a rotary evaporator with medium speed under a vacuum at 65_C [25].

### Phytochemistry Screening Using thin Layer Chromatography (TLC)

TLC was performed as reported elsewhere [26].Thin layer chromatography was done for all extracts in two sets. Once chloroform was used as mobile phase and once the combination of chloroform: methanol (8:2). We identified and labeled the exposed band on chromatography papers when the papers were dried. We also checked TLC plates under UV light to detect any

further bands. We recorded the color of each bands and calculated retardation factor (RF).

Determination of Bioactive Compounds Using Reagent Test

We sprayed TLC plate with Dragendorff reagent. Orange appeared bands are marker of nitrogen compounds like alkaloid. A Vanillin-H2SO4 reagent was used to detect terpenoid. After heating, blue and purple exposed bands show the presence of terpenoid and red bands indicate the existence of phenol. Anisaldehyde reagent could detect different types of terpenes through giving red or pink color bands. The blue bands are representative of phenolic bioactive compound in the extracts after spraying with Folin-ciocalteu reagent. We checked color changes under UV light and calculated the RF of all the labeled compounds [27].

Preparation of Crude Extracts

An empty sample bottles were weight before serial dilution being carried out. 500 µl of hexan, chloroform, petriulem ether and methanol extracts were added into sample bottles respectively. The extracts were left to dry and the dry weigh of the bottles was calculated. The dried sample was dissolved in a 100 µl of DMSO (dimethyl sulphoxide) and diluted to prepare 5 different concentrations: 5.0000, 2.5000, 1.2500, 0.6250 and 0.3125 mg/ml [28].

Preparation of Isolated Compounds

We performed the TLC using big silica plate and used combination of chloroform: methanol (8:2) solvent as mobile phase. The reagent test wasn't carried out in this step. The labeled compounds on big TLC plate were scarped and placed in micro centrifuge tube. 1ml of distilled water was added to each tube and centrifuged at 5000 rpm for 10 minutes and the supernatant was collected for GP inhibitory assay.

Glycogen Phosphorylase Inhibitory Assay

We performed this test with both crude extracts and isolated compounds. The inhibition effect of the extracts against GP activity was monitored, by measuring the total carbohydrate after the reaction, using phenol-sulfuric acid method [29]. We prepared GP enzyme from the rats' livers after centrifuging at 4000 RPMI for 15 minutes. 200 µl of GP enzyme (in phosphate buffer) was added into micro-plate containing 170 µl of 10mM glycogen and 670 µl of plant extract with different concentrations. The mixture was incubated at 38 °C for thirty minutes. After half an hour 170 µl of 3 M sulfuric acid was added and the mixture centrifuged at 3,000 RPM for 5 minutes. After the centrifuged process 330 µl of supernatant was taken out into test tube and 330 µl of 25% phenol solution was added followed by 1.7ml of concentrated sulphuric acid. We used distilled water as a positive control and glucose as a standard or reference antidiabetic agent. We measured the optical density (OD) of the wells two times at 490 nm against blank. The inhibitory activity was calculated using the following equation.

Glycogen phosphorylase inhibitory activity (%):

$=[(\text{OD control}_{490nm}-\text{OD sample}_{490nm})/\text{OD control}_{490nm}]\times 100$

Measurement of Total Phenolic Compounds

We determined the phenolic compounds using the Folin-Ciocalteu method. The Folin-Ciocalteu reagent was diluted 10 times. We added 50 ml of the sample extractions to 5ml of Folin-Ciocalteu and 4ml of 1M sodium carbonate solution (Na2CO3). The reaction mixture was incubated in water bath at 45 °C for 15 minutes and the absorbance of the sample was measured at 765 nm with spectrophotometer [30]. We computed the data by comparison of standard curve of 0, 50, 100, 150,200, 250 and 500mg/L Gallic solution in methanol water (50:50). The data were expressed as mg of Gallic acid / g dry matter.

Brine Shrimp Lethality Assay

We prepared the crude extracts with three different concentrations (1000, 100 and 10 mg/ml) of sample. We added 100 µl of the crude extracts in micro plate and 4900 µl of filtered sea salt water, containing the nauplii in each vial. In the control group the samples were replaced with distilled water. Live brine shrimp were maintained under illumination and being left at room temperature for observation. After 2 days, the number of dead nauplii was calculated. The percentage of mortality at each concentration and control group was recorded and LC50 value was calculated using Finney computer program [31].

## Results

Phytochemical Test

The preliminary phytochemical screening of hexane extract of *Vitis elegans* rhizome revealed no visible color due to nonexistence of pigment. Four compounds appeared as light blue bands under UV light. They contained aromatic rings. Phenol, alkaloid and trepans were absent because no band was presented after spraying with folin, dragendorff and anisaldehyde respectively. Four types of terpenoid bioactive compounds were detected after spraying with vanillin-H2SO4 followed by heating. The number of exposed bands in chloroform and petroleum ether extracts was almost the same as hexane. The best phytochemical screening obtained from methanol extract. The yellow exposed band indicated the presence of carotenoid pigment. UV light exposed 13 blue bands, which indicated 13 compounds with aromatic ring exist in respective plant. The exposed brown color band after spraying with dragendorff, indicated the presence of alkaloid bioactive compounds. In addition, four types of terpenoid compounds were detected after spraying with vanillin.

Glycogen Phosphorylase Inhibitory Aassay

The chloroform-extracted isolated compounds had the highest level of GP inhibition activity (35.1%) followed by, methanol (24.7%), petroleum ether (21.3%) and hexane (20.5%) solvents respectively (Fig. 1). The crude extracts, which were prepared from chloroform solvent, suppressed the GP activity more than glucose, in all concentrations (Fig. 2). Glucose was considered as a standard inhibitor.

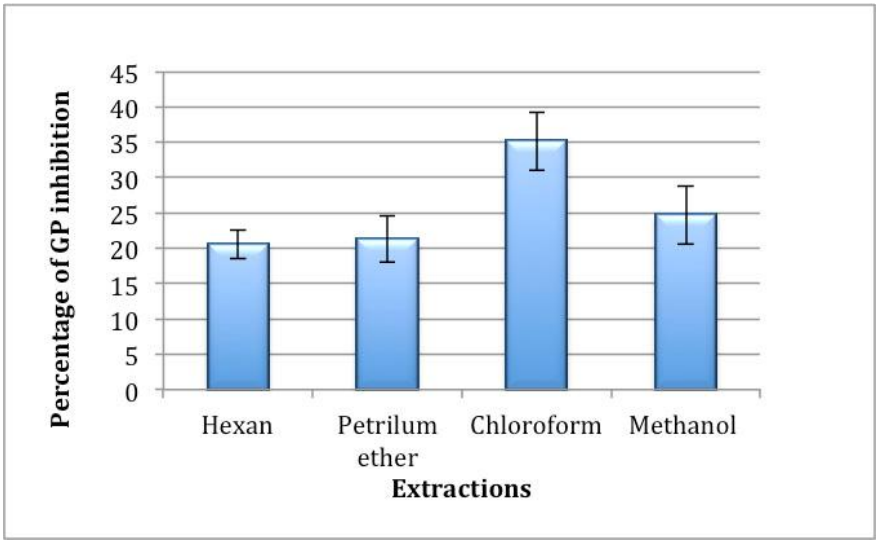


**Fig. 1** Inhibition rate of glycogen phosphorylase by isolated compounds of four extracts of *Vitis elegans* Kurz rhizome

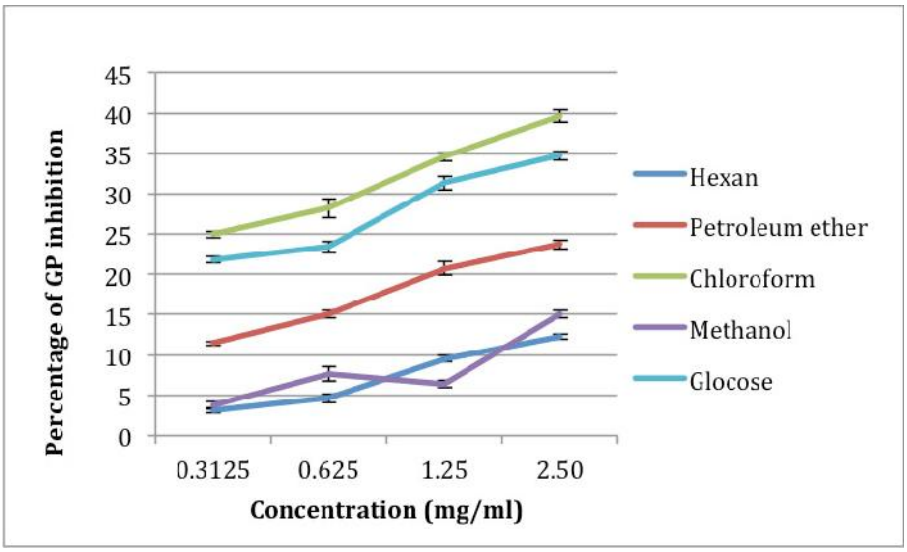

**Fig. 2** Comparison of glycogen phosphorylase inhibition rate of crude extracts of *Vitis elegans* Kurz rhizome with glucose (standard)

Measurement of Total Phenolic Compounds

We found the highest amount of phenol in methanol extract, which was equal to 49 mg GAE g-1. Then hexane, chloroform and petroleum ether extracts contained more level of phenol with amounts of 32, 16 and 9 mg GAE g-1 respectively.

Brine Shrimp Lethality Assay

Petroleum ether, chloroform and methanol extracts were considered as non-toxic solvents with LC50 values of 8.9, 3.5 and 3.7 mg/ml respectively. While hexane with 0.089 mg/ml LC50 value was classified as the toxic extract.

## Discussion

This study found the anti-hyperglycemic potential of *Vitis elegans*, a plant that is found in tropical area of Asia. We showed this plant contain terpenoid, alkaloids, carotenoid, aromatic compounds and phenol. The phenolic content ranged from 9 mg GAE g-1 for petroleum ether extract to 49 mg GAE g-1 for methanol extract. The result of the glycogen phosphorylase assay demonstrated that chloroform extract had the highest rate of GP activity suppression by percentage of 35.2 in isolated compounds and by percentage of 39.65 in concentration of 2.5 mg/ml in crude extracts.

The results of the phytochemical screening carried out on the different extracts of *Vitis elegans* rhizome showed the presence of terpenoid, alkaloids, carotenoid and aromatic compounds. Methanol extract contained a great proportion. Studies reported that these phytochemicals contribute the anti-hyperglycemic activity in Vitaceae family [32-34] and the others [35-37]. However, their effects on GP enzyme have not been cleared yet. Moreover, we isolated 13 different aromatic compounds from methanol extract. G. Soman reported skeletal muscle GP enzyme could be inhibited by a wide variety of soluble aromatic compounds. They hypothesized that soluble aromatic compound will also limit hepatic GP enzyme [38].

In this study chloroform extract applied more inhibitory effects on GP activity comparing to glucose. Glucose creates a negative feedback mechanism, stop the glycogenolysis process and inhibit the GP enzyme from further release of glucose from glycogen. Therefore, if the plant extract could reach at almost the same percentage of GP inhibiting activity, it has full potential to be further studied as anti-hyperglycemic agent. It was indicated previously that Vitis vinifera, the other species of Vitaceae family, has strong inhibitory potency against GP [39]. However, no scientific study has been conducted on *Vitis elegans* before, and this is the first study that investigated the anti-hyperglycemic potential of this plant.

In contrast to limited studies on plant-derivate inhibitors, many synthetic GP inhibitors have been studied as a potential therapy for decreasing the blood glucose in T2DM. Lilla Nagy, et.al demonstrated N-(3,5-Dimethyl-Benzoyl)-N'-(β-D-Glucopyranosyl)Urea , as a GP inhibitor, could improve glucose tolerance and rearrange hepatic metabolism under diabetic condition [40]. Privilege of GP inhibitors comparing to other types of hypoglycemic agents is that, GP inhibitors prevent further hyperinsulinaemia in body. In a murine model of diabetes, administration of GP inhibitors reduced hyperglycemia without causing further hypoglycemia [14,41]. In other study, Docsa, Tibor et.al indicated insulin sensitivity was modified by a GP inhibitor in streptozotocin-induced diabetic rats [42].

Glycogegn phosphorylase has different isozymes that mainly found in muscle and liver. It seems that skeletal muscle GP inhibitors could inhibit hepatic GP enzyme as well, since there is significant homology between hepatic and skeletal muscle [38]. Importantly, the effect of hepatic GP inhibitors on muscle functions should be considered. Although, David J. Baker et.al showed that hepatic GP inhibition is unlikely to negatively impact muscle metabolic and functional capacity [43], further invivo studies should investigate the effect of this plant on muscle function.

We found that total phenolic content ranged from 9 mg GAE g-1 for the petroleum ether extract to 49 mg GAE g-1 for methanol extract. There are significant linear correlations between the total phenol concentration and antioxidant activity

values [44,45]. Antioxidants prevent destruction of β-cells and reactive oxygen species formation, which could help in insulin production and prevent complications such as cataract, neuropathy and nephropathy [46]. Gallic acid and hydroxycinnamic acid are the phenolic acids that are found commonly in Vitaceae family [47].

In conclusion our results indicated that *Vitis elegans* extracts have good potential for the management of hyperglycemia and diabetes. The inhibitory of glycogen phosphorylase has been associated with their anti-diabetic properties. *Vitis elegans* also possess antioxidant potential that may be beneficial for related condition of oxidative stress. However, the plant should be further investigated in vivo and the inhibitory effect of hepatic GP on muscle should be examined.

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