Research article

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in vitro Study of Antidiabetic Effect of *Abrus precatorius* Methanol Leaves Extract against Glucose Absorption

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ABSTRACT

Diabetes mellitus is a common chronic systemic disorder characterised by hyperglycaemia as a standard feature. A traditional plant known as *Abrus precatorius* (AP) has been used for the treatment of type II diabetes mellitus in Malaysia. The potential of the 80% methanol leaves extract of *A. precatorius* has been tested for its α-glucosidase inhibition using α-glucosidase inhibitory assay and glucose diffusion activity using an *in vitro* model. It was observed that the methanol leaves extract of *A. precatorius* exhibited a high α-glucosidase inhibition at the concentrations of 25 and 50 mg/mL (65.4% and 84.6%), respectively, but low inhibition at the concentration of 6.25 to 12.5 mg/mL (25% and 28.2%) when compared to control. And it slightly affected the glucose diffusion at the concentration of 50 mg/mL (9.5%) within 24 h compared to the control group. These indicated that the methanol leaves extract of *A. precatorius* is capable of inhibiting α-glucosidase activity, besides halting glucose diffusion activity by delaying the glucose absorption in the gut.

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Introduction

Diabetes mellitus is a common chronic disease characterised by high blood glucose levels with carbohydrate, protein, and fat metabolic disorders [1, 2]. In 2017, the International Diabetes Federation (IDF) estimated that 451 million people (aged 18–99 years) worldwide had diabetes. These trends was, and it is expected to reach 693 million by 2045 [3]. Diabetes mellitus can be classified as type I diabetes, type II diabetes, gestational diabetes mellitus (GDM), and other specific types of diabetes due to other causes (e.g., drug-or chemical-induced diabetes, diseases of the exocrine pancreas, and monogenic diabetes syndromes) [4]. Among these, type II diabetes causes abnormal absorption of glucose into the blood that

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causes blood glucose level to rise and results in severe complications of various organ systems like the heart, brain, liver, kidney, and retina [5, 6].

One of the most efficient ways to control type II diabetes is to reduce glucose absorption by inhibiting carbohydrate digestion. Carbohydrate digestion occurs in the gastrointestinal tract and involves two enzymes, intestinal α-glucosidase and pancreatic α-amylase to release absorbable glucose [7]. The α-glucosidase breaks down disaccharides or make monosaccharides through the digestion of carbohydrates by α-amylase (glucose) [8, 9]. Alpha-amylase and alpha-glucosidase inhibition may help delay carbohydrate digestion in the intestine, thereby controlling type II diabetes [10].

Acarbose is the most common commercially available drug that acts as the inhibitor to α-glucosidase and α-amylase in the brush border of the small intestine, hence, ensures the delay of glucose absorption and effectively decreases postprandial blood glucose level elevation [11]. Besides acarbose, voglibose and miglitol are other drugs of the similar kind in the market [12]. However, long-term use of these compounds causes gastrointestinal side effects such as abdominal distention, diarrhoea, and abdominal pain [13]. Therefore, plants and herbs are gaining vast attention as the alternative source of α-glucosidase inhibitors, primarily due to its characteristics and cost [14]. Several dietary supplements of plant origin have been tested and exhibited potential therapeutic activities in diabetes treatment and its complications [15]. In this study, we proposed the activity of *A. precatorius* leaves methanol extract against α-glucosidase inhibition and retardation of glucose diffusion via *in vitro* study.

Materials and Methods

Chemicals and reagents

The chemicals and reagents used in the study such as methanol, sodium chloride, sodium carbonate, potassium dihydrogen phosphate, potassium hydrogen phosphate, and D-glucose were purchased from Qrec (ASIA; Bio-Diagnostic Sdn. Bhd. Petaling Jaya, Selangor, Malaysia). Other chemicals like p-nitrophenyl alpha-D-glucopyranoside and α-glucosidase, were from Sigma-Aldrich Group (Subang Jaya, Selangor, Malaysia), and glucose kit from Spinreact S.A (Sant Esteve de Bas, Girona, Spain).

Plant materials

The *A. precatorius* (*Akar Saga*) plant was obtained from Nursery Pak Ali (Skudai, Johor Bahru, Malaysia). Green, mature, and intact leaves were harvested from the plant and washed with tap water and dried in a shady place with enough ventilation for 3–5 days. The dried leaves were ground by a standard blender to get a fine powder. The leaves powder was kept in a sealed container in a dry and cool place to be used throughout the study period [16].

Preparation of *A. precatorius* leaves extract

About 25 g of the leaves powder was soaked in 80% methanol for three days. Then, the mixture was filtered by filter paper No 1 (Advantec, Medigene Sdn Bhd, Selangor, Malaysia). The filtered methanol extract was evaporated using a rotary evaporator (EYELA-Tokyo Rikakikai Company, Tokyo, Japan) and dried using a freeze dryer (Martin Christ Company, Osterode am Harz, Germany) to obtain dark crude *A. precatorius* extract [16, 17]. The percentage yield of the crude methanol extract obtained was 1.86 grams, and kept at 4 °C for further use.

Alpha-glucosidase inhibition assay

The activity of α-glucosidase was determined using kinetic endpoint assay in a 96-well plate (NEST, Jiangsu, China). The absorbance was measured using an Epoch Microplate Spectrophotometer (BioTek Instruments Inc, Winooski Vermont, USA). Approximately 20 µL different concentrations of *A. precatorius* extract were mixed with 50 µL of 50 mM phosphate buffer, followed by 10 µL of α-glucosidase from *S. cerevisiae* (1 U/mL). These samples were further incubated for 5 min at 37 °C [16]. A 20 µl of p-nitrophenyl alpha-D-glucopyranoside (PNP-G) was then added to the mixture as the substrate. The inhibitory activity of α-glucosidase was assessed using p-nitrophenyl alpha-D-glucopyranoside (PNPG) as the substrate based on the formation of the yellow p-nitrophenol colour [18]. The reaction was incubated in an incubator (Panasonic-Biomedical Company, Japan) for 30 min at 37 °C. Subsequently, 50 µL of a sodium carbonate solution was added to stop the reaction. Acarbose, a synthetic α-glucosidase inhibitor (used for type II diabetes patient), was employed as a positive control. This drug is a synthetic drug used by type II diabetic patients to inhibit α-glucosidase within the small intestine [16, 19]. The absorbance was recorded at

405 nm, and the percentage inhibition was calculated according to the following formula [20]:

% inhibition = [(*absorbance* of *control* – *absorbance* of *extract*)/*absorbance* of *control*] ×100

Effects of *A. precatorius* methanol leaves extract on glucose movement

In this experiment, a simple *in vitro* dialysis tube model was used to assess the potential of the *A. precatorius* methanol leaves extract to retard the diffusion of glucose in the small intestinal tract [21]. The movement in this system is not by true diffusion, but it is assisted by the convective activity of intestinal concentrations [22, 23]. This model requires the use of a dialysis tube (150 mm × 25 mm) soaked in sodium chloride (NaCl) before use [24]. Then, one end of the tube was tightly tied with a rubber band. A volume of 1 mL *A. precatorius* leaves extract was added to a dialysis tube and mixed with 1 mL of 0.15 M NaCl containing 0.22 M D-glucose. The other end of the dialysis tube was also tied and put in a 50 mL centrifuge tube containing 0.15 M NaCl placed in a shaker incubator (Hotech Instruments Corp, New Taipei City, Taiwan) at room temperature [25]. The effect of methanol leaves extract *A. precatorius* leaves on glucose diffusion was investigated at varying concentrations (6.25, 12.5, 25, and 50 mg/mL) and at different time intervals (0, 4, 8, 12, 16, 20, and 24 h), with distilled water as the control. The percentage of glucose that moved into the external solution was analysed by the glucose oxidase kit [26, 27].

Statistical analysis

The results of three replicate experiments were collected and expressed as mean ± standard deviation (SD), and the diagrams were obtained using the Origin70 program.

Result and Discussion

***In vitro* α-glucosidase inhibition study**

In the present study, the acarbose showed the lowest and highest inhibitory effects of 77.9% and 95.7% at the concentrations of 6.25 and 50 mg/mL, respectively (Figure 1). On the other hand, the methanol leaves extract of *A. precatorius* displayed the lowest and highest inhibitory activity of 25% and 84.6% at the concentration of 6.25 and 50 mg/mL, respectively. The results show that the inhibitory activity of *A. precatorius* leaves extract and acarbose are increased proportionately to the increase in their concentrations, (Figure 1).

Acarbose exhibited better inhibitory effects against glucose diffusion compared to *A. precatorius* methanol leaves extract. The control of postprandial hyperglycaemia is critical in the management of diabetes to reduce its complications [28]. Postprandial hyperglycaemia strongly depends on the amount of monosaccharide absorbed by the small intestine [29]. The control of postprandial hyperglycaemia can be achieved by retarding the absorption of glucose through the inhibition of α-glycosidase, which cleaves oligosaccharide to monosaccharide in the small intestine [30]. However, acarbose and other synthetic drugs have undesirable toxic side-effects compared to traditional medicines, leading to an increased interest in the use of traditional medicines [31, 32]. Two different studies indicated that long-term use of acarbose cause side effects like flatulence, stomach distention, and diarrhoea [33, 34].

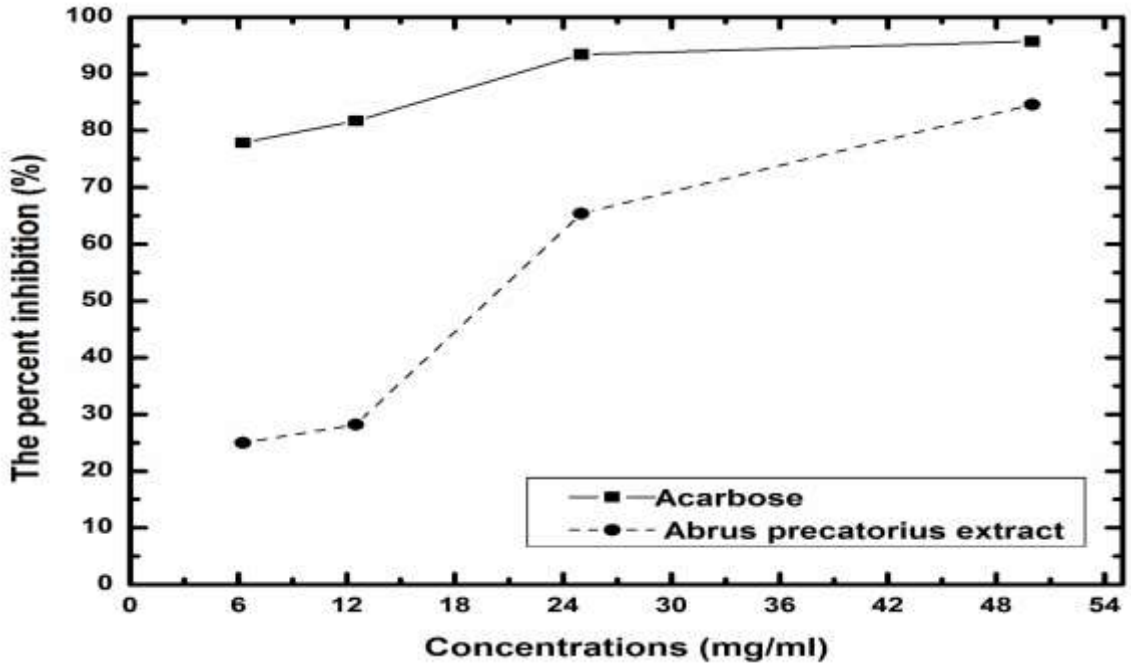

Fig 1 The percent inhibition of α-glucosidase enzyme by methanol leaves extract of *A. precatorius* and acarbose at various concentrations. Each value is the mean± SD of three replicate analyses

Although present study showed that acarbose represents better result than the *A. precatorius* methanol leaves extract, this *A. precatorius* showed a promising effect by exhibiting an increasing pattern of glucose diffusion inhibition closely resembling those characterised for acarbose. *A. precatorius* methanolic leaves extract showed good inhibitory activity of 65.4%

and 84.6% at the concentration of 25 and 50 mg/mL, respectively, when compared to the positive control (Figure 1). This could be a promising treatment in the management of postprandial hyperglycaemia to avoid undesirable toxic side-effects of synthetic drugs. In another study using aqueous extract the inhibitory activities of aqueous extract of twelve medicinal plants for α-glucosidase enzyme were investigated; and out of the twelve medicinal plants, the aqueous extract of *A. precatorius* leaves showed potent inhibition of 76.8% [35]. Thus, the present study established that the *A. precatorius* methanol leaves extract is more potent than the aqueous leaves extract of *A. precatorius*. Therefore, further studies are required to identify the potential bioactive compounds that act as α-glucosidase enzyme inhibitors.

The effects of *A. precatorius* methanol leaves extract on glucose diffusion via *in vitro* study

The present study shows that the effect of methanol leaves extract of *A. precatorius* at various concentrations was very close to the control reading (Figure 2). This study indicates that methanol leaves extract of *A. precatorius* did not show any effect on glucose diffusion. Nonetheless, a slight inhibition effect on glucose diffusion was observed at the concentrations of 12.5, 25, and 50 mg/mL after 16 h when compared to the control. Besides, the methanol leaves extract exhibits a higher effect (9.5%) of the 50 mg/mL concentration within 24 h compared to other concentrations. Thus, concentrations higher than 50 mg/mL may show a greater inhibitory effect on glucose diffusion. Generally, the study demonstrated that methanol leaves extract of *A. precatorius* did not show effect on glucose diffusion in these concentrations. However, previous studies showed that there is a relationship between the viscosity of the soluble polysaccharide constituents of plants and their ability to inhibit glucose absorption [21, 24, 26]. Further study is needed to investigate the role of the viscosity of suspensions of *A. precatorius* methanol leaves extract on glucose diffusion or absorption. Similar results were reported by another study that used the same method to assess the potential of the ethanolic and aqueous extracts of *Teucrium polium* to retard the movement and diffusion of glucose from a sealed dialysis tube into the external solution [23]. The results showed that *T. polium* did not show any entrapment ability in decreasing glucose movement into the external solution [23]. It is noteworthy that the methanol leaves extract of *A.*

precatorius might show inhibitory effect on glucose diffusion at the concentration of 50 mg/mL, while *T. polium* did not show any inhibitory effect on glucose diffusion. This observation suggested that the antidiabetic action of *T. polium* is not be related to glucose diffusion and may depend on other mechanisms of inhibition of carbohydrate hydrolysing enzymes, increased insulin production from pancreatic β-cells, or decreased insulin resistance. Therefore, the antidiabetic activity of the methanol leaves extract of *A. precatorius* might be due to α-glucosidase inhibition, which appeared to be more potent than retarding the diffusion of glucose in the small intestinal tract or might also involve different mechanism as an antidiabetic agent.

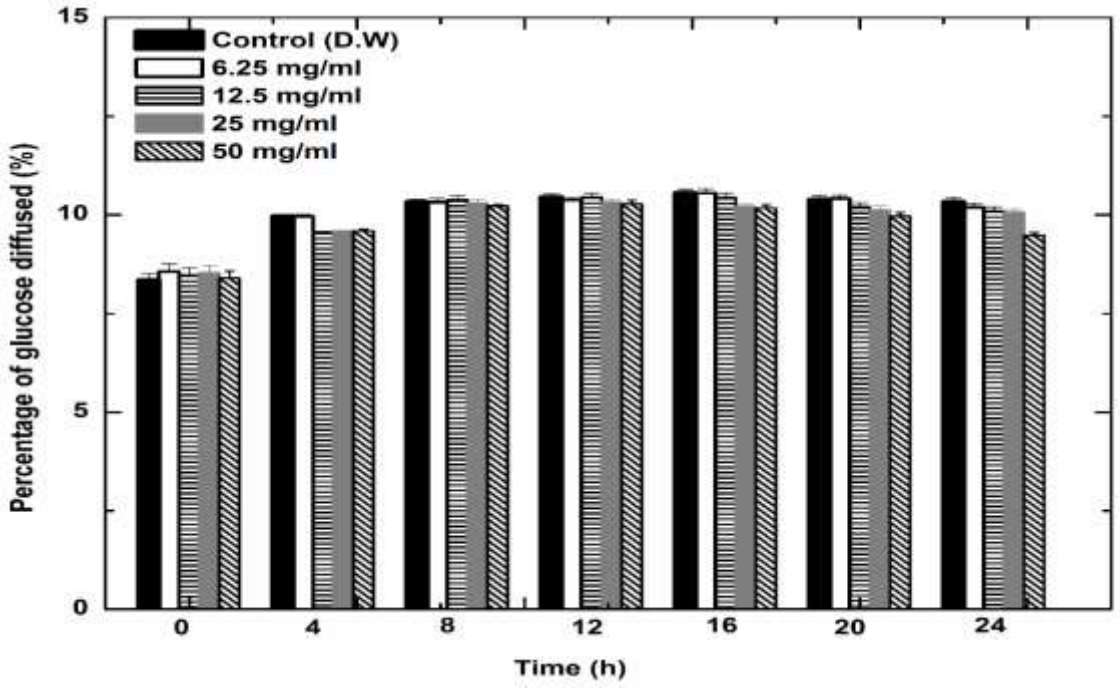

Fig 2 The effect of methanol leaves extract of *A. precatorius* at varying concentrations on the diffusion of glucose out of dialysis tube through 24 h incubation period. Each value is the mean ± standard deviation of four replicate analyses.

In another study, an aqueous extract of the whole plant for ten plants (*Agaricus campestris*, *Agrimony eupatoria*, *Coriandrum sativum*, *Eucalyptus globulus*, *Juniperus communis*, *Medicago sativa*, *Persea americana*, *Sambucus nigra*, *Urtica diocia*, and *Viscum album*) with antihyperglycemic properties have been tested to evaluate their potential effects on gastrointestinal glucose diffusion [21]. *P.americana* and *A.eupatoria* decreased glucose

diffusion in vitro by more than 50%. Aqueous extracts of *V.album*, *J.communis*, *A.campestris*, *E.globulus*, *M.sativa*, and *C.sativum* decreased the activity of glucose diffusion significantly but were less effective than *P.americana* and *A.eupatoria*. Meanwhile, *S.nigra* and *U.diocia* extracts did not significantly decrease glucose diffusion [21]. The study indicated that the plants have antihyperglycemic properties, and some of them can inhibit glucose diffusion. Whereas, other plants did not show any ability to inhibit glucose diffusion. It is noteworthy that the antidiabetic plants have different mechanisms, which assist in treating diabetes and its complications. These include glycosidase (glucosidase) inhibitor mechanism, α-amylase inhibitor mechanism, inhibition of hepatic glucose metabolising enzyme mechanism, antioxidant mechanism, inhibition of glycosylation of haemoglobin mechanism and modulation of glucose absorption from the gut [36].

Conclusion

Based on the results, *A. precatorius* methanol leaves extract showed good inhibitory activity for the α-glucosidase enzyme. On the other hand, it did not show any effect on glucose diffusion, except a slight effect at the highest concentration of 50 mg/mL within 24 h. In our opinion, the ability of *A. precatorius* plant to inhibit the activity of the intestinal α-glucosidase enzyme is one of the reasons that can explain the traditional healer's use of *A. precatorius* leaves for the treatment of type II diabetes. The 80% methanol leaves extract of *A. precatorius* showed excellent inhibitory activity for the α-glucosidase enzyme. Therefore, further studies are recommended to isolate and test the potential compounds responsible for inhibitory activity of the α-glucosidase enzyme.

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