Anti-Diabetic Activities of Aqueous Extract from *Actinidia kolomikta* Root Against α-glucosidase

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**ABSTRACT**

The present study was to evaluate the anti-diabetic activity of the aqueous extract of *Actinidia kolomikta* root against baker's yeast α-glucosidase and rat intestinal α-glucosidase. As a result, the aqueous extract of *Actinidia kolomikta* root exhibited excellent baker's yeast α-glucosidase inhibitory activity which was better than acarbose. It also showed rat intestinal α-glucosidase inhibitory activity not only in maltase, but also in sucrase in a dose dependent manner. With these results, it can be concluded that *Actinidia kolomikta* root existed the anti-diabetic activity depend on their inhibitory activity on glucose absorption in the small intestine. The results also give scientific support to the utilization of *Actinidia kolomikta* root as folklore medicine for the treatment of diabetes.

**Keywords:** *Actinidia kolomikta*, Anti-diabetic activity, α-Glucosidase

1. Introduction

Diabetes mellitus is a global health problem and the diabetic was constantly increasing. The number of diabetic people is expected to rise to 366 million in 2030 [1], and most of them were type 2 diabetes. As we all know that the hyperglycemia can damage many of the body's organs and systems, and then lead to renal failure, blindness, cerebrovascular disease and so on. One of the therapeutic approaches is to decrease the postprandial hyperglycemia [2]. The postprandial hyperglycemia can be decreased through inhibiting the activity of α-glucosidase enzyme in the brush border of the small intestine, and then delay the digestion and absorption of carbohydrates, reduce the rate of glucose absorption and consequently blunting the postprandial plasma glucose rise [3]. Therefore, α-glucosidase inhibitors is one of the choice for diabetic, and now many α-glucosidase inhibitors such as acarbose, miglitol, voglibose as oral anti-diabetic drugs are widely used in clinical trials [4]. However, a lot of clinical and laboratory researches have reported that the α-glucosidase inhibitors has serious side effects such as hepatotoxicity, abdominal pain, flatulence, diarrhea, and hypoglycemia [5, 6, 7, 8]. So it is necessary to search for effective components with low cost, no side effects as a substitute. On the other hand, traditional plant was wildly used for treatment of diabetes throughout the world as it was effective, non-toxic, with less or no side effects [9]. More than 1200 plants have been used to treat diabetes in folk medicine [10], and there are 136 plants clearly showed the anti-diabetic effects [11]. More researchers are trying to search for more effective inhibitors of anti-diabetic compounds from natural plants. *Actinidia kolomikta* (Rupr. & Maxim.), which is named Tonh Khidum (Laotian) in Latin, is a species of deciduous dioecious woody vines in the genus Actinidia. In our lab, various biological activities of *Actinidia kolomikta* root aqueous extract such as antitumor activities [12], antiproliferative activities [13], Immunomodulatory activities [14], anti-oxidant activities [15] have been evaluated. Although the root extract of *Actinidia kolomikta* has been used as a folk remedy for the treatment of diabetes for many years in Laos, there is no scientific evidence to support the anti-diabetic effect of *Actinidia kolomikta*. In the present study, the anti-diabetic effects of *Actinidia kolomikta* root was investigated in vitro by using α-glucosidase inhibitory methods. The bioactive compound was also identified.
2. Material and Methods

2.1 Chemicals
Rat intestinal acetone powder, 4-Nitrophenyl-a-D-glucopyranoside (pNPG), yeast α-glucosidase (E.C.3.2.1.20) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA), Glucose C II kit and Acarbose were purchased from Wako Pure Chemical Industries (Osaka, Japan). Other solvents and chemicals were obtained from Wako Pure Chemical Industries.

2.2 Plant Material and Preparation of Extract
The roots of *Actinidia kolomikta* were collected from Laos. Firstly, the roots of *Actinidia kolomikta* were washed thoroughly with distilled water and dried in the shade, shade dried roots were finely powdered. The powder (100 g) was boiled in 1 L distilled water three times and 2 hours for each time. Each extract was then filtered through a Whatman No. 47 paper filter, the filtrate was combined, concentrated by rotary evaporation under reduced pressure, then lyophilized to get powder (yield:10%, w/w). The root aqueous extract was stored at -20°C until further use.

2.3 Assay for yeast α-glucosidase Inhibitory Activity
The assay was according to a report [16, 17] using p-nitrophenyl-a-D-glucopyranoside as substrate. Briefly, a mixture of 50 μl of different concentrations of the extracts and 100μl of 0.1 M phosphate buffer (pH 6.9) containing yeast α-glucosidase solution (1.0 U/ml) was incubated in 96-well plates at 25°C for 10 min. After pre-incubation, 50 μl of 5 mM p-nitrophenyl-a-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to each well at timed intervals. The reaction mixtures were incubated at 25°C for 5 min. Absorbance was recorded at 405 nm before and after incubation with a micro-plate reader (BIO-RAD, USA) and was made into a pellet by using a hydraulic press. The scans were made in a wave number range from 4000 and 400 cm⁻¹. All determinations were performed in triplicate.

%Inhibition= (ΔAc − ΔAs)/(ACB−AC) ×100

Where ΔAc represent the changes in absorbance for the sample, ΔAs represent the changes in absorbance for the control, respectively.

2.4 Assay for Rat Intestinal Maltaise Inhibitory Activity
Rat intestinal maltase inhibitory activity was determined using a literature method [18] with a slight modification. The assay was carried out in the same manner as the assay for rat intestinal maltase inhibitory activity, except for the supernatant was diluted with 0.1 M phosphate buffer by 2 times and sucrose substrate solution (2% w/v in phosphate buffer).

2.5 Assay for rat Intestinal Sucrase Inhibitory Activity
Rat intestinal sucrase inhibitory activity was determined using a literature method [19] with a slight modification. The assay was carried out in the same manner as the assay for rat intestinal maltase inhibitory activity, except for the supernatant was diluted with 0.1 M phosphate buffer by 2 times and sucrose substrate solution (2% w/v in phosphate buffer).

2.6 Fourier Transform Infrared Spectroscopy (FTIR) Analysis
FT-IR spectrum was recorded on a FTIR 300 spectrometer (Jasco, Japan). The root extract was mixed with potassium bromide (KBr) and was made into a pellet by using a hydraulic press. The scans were made in a wave number range from 4000 and 400 cm⁻¹. All determinations were performed in triplicate.

2.7. UV–vis spectra analysis
The UV–Vis spectrophotometer UV–1800 (shimadzu, Japan) was used to measure UV–Vis absorbance spectra of *Actinidia kolomikta* root aqueous extract. The absorbance measurements were made in 200–400 nm range with a 1 nm step.

3. Result and Discussion

3.1 Assay for yeast a-glucosidase inhibitory activity

![Graph showing yeast a-glucosidase inhibitory activity of *Actinidia kolomikta* root aqueous extract at different concentration](image)

The optical density (OD) of the wells was measured at 490 nm with a micro-plate reader (Cambridge, UK) and the inhibitory activity was calculated using following formula:

%Inhibition= (ACB − Ac)/(ACB − Ac) ×100

where ACB was the absorbance with α-glucosidase and without the sample, Ac was the absorbance without α-glucosidase and the sample, AAB was the absorbance with α-glucosidase and the sample, and As was the absorbance with the sample but without a-glucosidase.

The yeast α-glucosidase inhibition of *Actinidia kolomikta* root extract at final concentrations of 0.078, 0.156, 0.313, 0.625 and 1.25 mg/ml were shown in Fig. 1. The results suggest that *Actinidia kolomikta* root aqueous extract showed a dose dependent inhibition of yeast α-glucosidase activity. *Actinidia kolomikta* root aqueous extract showed superior α-glucosidase inhibitory activity compared to acarbose, which is used as anti-diabetic drug. According to the results, *Actinidia kolomikta* root aqueous extract exhibited remarkable potential anti-diabetic properties. The results also give scientific support to the utilization of *Actinidia kolomikta* root as folklore medicine for the treatment of diabetes.
3.2 Assay for rat Intestinal Maltase Inhibitory Activity

Recent years, yeast α-glucosidase is widely used to search α-glucosidase inhibitors, but the results do not always agree with those obtained in mammals [20]. Therefore, the rat intestinal acetone powder as a source of α-glucosidase was used to evaluate the α-glucosidase inhibitory activity of *Actinidia kolomikta* root aqueous extract. The rat intestinal maltase inhibitory activity of *Actinidia kolomikta* root aqueous extract was presented in Fig. 2. The result showed that the extract inhibited rat intestinal maltase activity dose-dependently (0.125–2 mg/mL), and the rat intestinal maltase inhibition was 57.9% at the concentration of 2 mg/ml. Acarbose as a positive control showed strong rat intestinal maltase inhibitory activity, even at the concentration of 0.125 mg/ml, the rat intestinal maltase inhibition reached to 96.2%. However, as shown in Fig.1, acarbose showed a weaker yeast α-glucosidase inhibitory activity than *Actinidia kolomikta* root aqueous extract. This result was consistent with the past reports [21]. The high rat intestinal maltase activity of *Actinidia kolomikta* root aqueous extract maybe is bound up with its high content of phenolic compounds [13].

3.3 Assay for rat Intestinal Sucrase Inhibitory Activity

The rat intestinal sucrase inhibition of *Actinidia kolomikta* root aqueous extract at final concentrations of 2, 1, 0.5, 0.25, 0.125 mg/ml was showed in Fig. 3. *Actinidia kolomikta* root aqueous extract elicited a dose dependent inhibition of rat intestinal sucrase activity. The extract at 2 mg/ml had a percentage inhibition of 53.3% while acarbose at the same concentration inhibited 98.5% of the enzyme activity. Many studies confirmed that phenolic compounds had anti-diabetic activity [22, 23, 24]. According to previous research [23], the high content of phenolic compounds was found in *Actinidia kolomikta* root aqueous extract, maybe it can explain its high rat intestinal sucrase activity.

3.4 Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR spectra and the functional group of compounds present in the root aqueous extract of *Actinidia kolomikta* was shown in Fig. 4. The FTIR spectrum shows a Strong and broad absorption peak at 3318.89 cm\(^{-1}\) corresponding to the overlapping of –OH peaks [25]. Another peak located at 1608–1580 cm\(^{-1}\) was assigned to C–C ring vibration [26]. The peak at 1284.36 cm\(^{-1}\) represents the C–O stretching, the peak at 1060.66 cm\(^{-1}\) represents the O–H stretching [27]. It has been reported that phenolic compounds exhibited an absorption peak at 1650–1450 cm\(^{-1}\) and 1420–1330 cm\(^{-1}\) [28]. Therefore, the FTIR spectrum of *Actinidia kolomikta* root aqueous extract was similar to phenolic compounds. It has been reported that phenolic compounds play a major role in anti-diabetic activity.

3.5 UV–vis Spectra Analysis

*Actinidia kolomikta* root aqueous extract were examined by UV-VIS spectrophotometry, and the results are shown in Fig. 5. As we all know that phenolic compounds including phenolic acids, phenols, phenyl propanols, flavonoids, minor flavonoids, quinines and tannins. Phenolic acid derivatives showed a single peak at 270-290 nm [29]; flavonoids showed double maximum absorption peaks at 240-280nm and 330-400nm [30]. The spectrum of *Actinidia
Actinidia kolomikta root aqueous extract showed a single peak at 278 nm, which suggests to be related to the presence of phenolic acids. It has been reported that phenolic acids such as Chlorogenic Acid [31], Caffeic acid [32], 3,5-dicaffeoylquinic acid methyl ester [33] exhibited anti-diabetic activity.

4. Conclusion
In conclusion, anti-diabetic activities of Actinidia kolomikta root aqueous extract were firstly established through α-glucosidase inhibitory activity. The results give scientific support to the use of Actinidia kolomikta root in folklore medicine for the treatment of diabetes. Further studies were needed to isolate the active compounds and make clear the mechanism of anti-diabetic activities.

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6. Reference:


