INVESTIGATION OF α-GLYCOSIDASE INHIBITON AND ANTIOXIDANT ACTIVITIES OF *HYDROCOTYLE ROTUNDIFOLIA* ROXB. (SAY-MYIN-KHWA)

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Abstract

This research is focused on the evaluation of α-glucosidase inhibitory effect from Myanmar Traditional Medicinal Plants *Hydrocotyle rotundifolia* Roxb. (Say-myin- khwa). The medicinal plant has been reported to possess the antidiabetic potential. According to the preliminary phytochemical tests indicated the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, steroids, terpenoids, tannins, reducing sugars, and saponins were present while starch was not detected in this sample. The α-glucosidase inhibitory effect of watery and ethanol extracts on production of glucose from sucrose was determined by using UV-Visible spectroscopy. The 50 % inhibitory concentrations (IC_{50}) of watery and ethanol extracts on α-glucosidase activity were found to be 0.73 and 0.61 µg/mL. From this experiment, it was found that ethanol extract showed higher potency than water extract. However, all of these extracts showed lower potency than that of standard drugs Voglibose (IC_{50} = 0.32 µg/mL in α-glucosidase inhibitory effect. The antioxidant activity of ethanol and watery crude extracts was investigated by DPPH free radical scavenging assay. The IC_{50} values of watery and ethanol crude extracts were12.34 µg/mL and 9.33 µg/mL. Since the lower IC_{50} value, the higher antioxidant activity of the samples occurs. Thus, the ethanol extract showed higher antioxidant activity than that water extract.

Keywords: *Hydrocotyle rotundifolia* Roxb., α-glucosidase inhibitory effect, antioxidant activity

Introduction

The systematic name of α-glucosidase glucohydrolase, (E.C 3.2.1.20), hydrolytic enzymes splitting both α-1, 4 and α-1, 6-glucosidic linkages are usually termed α-glucosidase. Mammalian α-glucosidase located in the brush-border surface membrane of intestinal cells is the key enzyme catalyzing the final step in the digestive processes of carbohydrate. Hence, α-glucosidase inhibitors can retard the liberation of D-glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose level and suppression of postprandial hyperglycemia. To control or to manage postprandial hyperglycemia, α-glucosidase inhibitors are used α-glucosidase inhibitor, a class of anti-diabetic drugs is known as "starch blocker". Taken with the first bite of a meal α-glucosidase inhibitors are especially well suited to tread postprandial hyperglycemia (a sharp rise in blood sugar after meals) a common and serious problem faced by many people with type 2-diabetes. Acarbose, miglitol, voglibose and emiglitate have been approved to use as antidiabetes drugs. Because the drug prevents the immediate breakdown of starches into monosaccharides or simple sugars, which would to absorbed into the blood stream quickly, more of the carbohydrate consumed at meal gets absorbed further "downstream" in the gastrointestinal tract, towards the end of the small intestine or the colon. Slowing the absorption of carbohydrate gives the beta-cells in the pancreases more time to secrete adequate insulin to cover the meal. α-Glucosidase enzyme are widely distributed in microorganism, plants and animals (Hong *et al*., 2008). From this point of view, many efforts have been made searching for effective and safe α-glucosidase inhibitors from neutral materials in order to develop a physiological functional food for use against antidiabetes. In this study, traditional medicinal

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plant (Figure 1) *H. rotundifolia* Roxb. (Say-myin-khwa) was chosen for the investigation of some phytochemical composition and some biological activities of its ethanol and watery extracts.

Scientific name - *H. rotundifolia* Roxb.
Family - Umbelliferae
English name - Lawn Pennyword
Myanmar name - Say-myin-khwa
Part uses - Whole plant
(Sultana & Khatun, 2010)

Figure 1 Plant of *H. rotundifolia*

Materials and Methods

Collection and Preparation of Sample

The sample of *H. rotundifolia* Roxb. (Say-myin-khwa) plant was collected from Hmawbi Township, Yangon Region in October, 2011. The plant was identified by the authorized botanist, at Botany Department, Yangon University. The collected plants samples were cleaned by washing thoroughly with water and air-dried at room temperature. The dried samples were cut into small pieces and ground into powder by a grinding machine. The dried powdered samples were stored in air-tight containers.

Phytochemical Investigation

The dried powdered samples were used for the chemical tests on the phytochemicals by using standard procedure (Harborne, 1984; M-Tin Wa, 1972; Marini-Bettolo *et al*., 1981; Robinson, 1983; Shriner *et al*., 1980; Trease and Evans, 1980; Vogel, 1966).

Isolation and Identification of α-Glucosidase Enzyme from Flint Corn Seeds

Ingeminated seeds of flint corn (100 g) were powdered by using a blender. The powder obtained was then suspended with 140 mL of 0.1 M acetate buffer (pH 5). After the suspended had been stirred with magnetic stirrer for 5 h at room temperature, it was filtered by using thin cloth and 817 mL of pale yellow crude extract was obtained. About 204.3 g of solid ammonium sulphate were added to the crude extract under stirring. The resulting precipitate was removed by centrifuging at 10,000 rpm for 20 min. The supernatant was obtained as first filtrate. Subsequently, 188 g of solid ammonium sulphate were slowly added to the supernatant. The resulting precipitate was collected by the centrifuge at 12,000 rpm for 15 min and dried at room temperature. The crude enzyme precipitate was obtained. The supernatant is called second filtrate which was discarded. The extracted α-glucosidase enzyme was identified as follows. 1 mL of starch and 1 mL of distilled water were added into the first test tube and allowed to stand for 30 min. Then 1 mL of iodine was added and deep blue colour was observed. In the second test tube, 1 mL of starch and 1 mL of enzyme were mixed and allowed to stand for 30 min. Then 1 mL of iodine was added. No colour was observed (Aung Myint, 1997).

Screening of α-Glucosidase Inhibitory Effect of Plant Extracts

The enzyme inhibition assay is based on the breakdown of substrate to produce a colour product, followed by measuring the absorbance over a period of time. In this experiment, the α-glucosidase inhibition activity of 95 % ethanol and watery extracts from selected plant was

studied by determining the α-glucosidase inhibitory effect on the production of glucose from sucrose at 505 nm wavelength. This experiment was done in triplicate for each sample solution. Absorbance values obtained were used to calculate % inhibition and 50 % inhibitory concentrations (Astumi *et al.*, 1990; Cannel *et al.*, 1987; Kurihara *et al.*, 1994; Xiao and Rongli, 2005).

Preparation of test sample solution

2 mg of each extract or each isolated compound and 10 mL of distilled water were thoroughly mixed by vortex mixer. The mixture solution was filtered and the stock solution was obtained.

Procedure

Firstly, the control solution was prepared by mixing 1 mL of sucrose, 1 mL of enzyme and 1 mL of DMSO with vortex mixer and incubated for 30 min at 37 ºC followed by addition of glucose oxidase reagent (0.5 mL). After the incubation of the above mixture at 37 ºC for 30 min, the reaction was stopped by immersing the test tube into a boiling water bath for 10 min and allowed to cool to room temperature. Secondly, the background solution was prepared by mixing 1 mL of sucrose 1 mL of 6 % DMSO with vortex mixer according to the above procedure. Finally, the test solution was prepared by mixing 1 mL of sucrose, 1 mL of sample solution and 1 mL of 6 % DMSO with vortex mixer and incubated for 30 min at 37 ºC followed by addition of 1 ml of enzyme. After the incubation of the above mixture at 37 ºC for 30 min, glucose oxidase reagent (0.5 mL) was added. After the incubation of the above mixture at 37 ºC for 30 min, the reaction was stopped by immersing the test tube into a boiling water bath for 10 min and allowed to cool to room temperature. The different concentrations (0.125, 0.25, 0.5, 1.0, 2.0 µg/ mL) of the sample solution were used. Absorbance of all solutions was measured by using a UV-7504 spectrophotometer at 505 nm. Voglibose is an α-glucosidase inhibitor used for lowering postprandial blood glucose levels in people with diabetes mellitus was used as a reference.

Absorbance measurements were done in triplicate for each of the sample solutions. From the mean absorbance values, percent inhibition of the sample on α-glucosidase enzyme activity and average percent inhibition on α-glucosidase enzyme activity were calculated by using following equations (Yuhao, 2004):

$$\%\ \text{inhibition} = \frac{A_c - A - A_b}{A_c} \times 100$$

where,

% Inhibition = percent inhibition of test sample on α-glucosidase enzyme activity
A_c = absorbance of control solution
A_b = absorbance of background solution
A = absorbance of test sample solution

The IC_{50}, 50 % inhibitory concentration of the sample on α-glucosidase enzyme activity was calculated by Linear Regressive Excel Program.

Determination of Antioxidant Activity by DPPH Radical Scavenging Assay

The antioxidant activity of ethanol and water extracts of plant material was assayed according to a published method with slight modification (Marinova and Batchvarov, 2011). The commercially available DPPH (2, 2-diphenyl picrylhydrazyl) is a stable free radical, which is purple in colour. The antioxidant molecule presents in the test extracts, when incubated react with DPPH and convert it into di-phenyl hydrazine, which is yellow in colour. For the preparation of

the coloured reaction, the sample extract with the concentration of 1000 ug/mL was prepared by dissolving 20 mg of the extract in ethanol or water and the final volume was made up to 20 mL (the preparation was used as a stock solution). Then different concentrations like 5 μg/mL, 10 μg/mL, 20 μg/mL, 40 μg/mL and 80 μg/mL were prepared by dilution with ethanol or water from the stock solution. The control solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of ethanol in the brown bottle. The sample solution was prepared by mixing 1.5 mL of 0.002 % DPPH solution and 1.5 mL of test sample solution. These bottles were incubated at room temperature and were shaken on a shaker for 30 min.

The absorbance for the degree of discoloration of purple to yellow was measured at 517 nm against ethanol as blank. Ascorbic acid was used as a standard. Each experiment was done triplicate. The DPPH radical scavenging activity of the plant extracts were calculated by the following formula:

$$\% \text{ RSA} = [\ \{(A_{DPPH} - A_{sample}) - A_{blank}\ \} / A_{DPPH}] \times 100$$

where, % RSA = %radical scavenging activity of test sample

A_{DPPH} = absorbance of DPPH in EtOH solution

A_{Sample} = absorbance of sample+ DPPH solution

A_{blank} = absorbance of sample + EtOH solution

IC_{50} is defined as the concentration of substrate that causes 50 % loss of DPPH activity (colour).

Results and Discussion

Phytochemical Constituents Present in *H. rotundifolia*

According to the experiments, alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, steroids, tannins, terpenoids, reducing sugars, saponins and organic acids were found to be present while starch was not detected. In addition, alkaloids, α-amino acids, carbohydrats, flavonoids, glycosides, steroids, terpenoids, reducing sugar and organic acids were found to be present in larger amount in this sample.

In Vitro α-Glucosidase Inhibitory Effect of Crude Extracts of *H. rotundifolia*

In vitro α-glucosidase inhibitory effects of watery and 95 % ethanol extracts from *H. rotundifolia* plants were determined by using α-glucosidase inhibition assay. In this method, α-glucosidase enzyme can produce the glucose and fructose from sucrose by enzymatic hydrolysis. Therefore, the presence or absence of α-glucosidase enzyme inhibition effect of a sample can be demonstrated by the enzyme inhibition effect of a sample can be demonstrated by the enzymatic production of glucose from the substrate sucrose.

If glucose is not produced from sucrose by α-glucosidase in the presence of the herbal extracts, it can be inferred that the sample has the α-glucosidase inhibitory effect, i.e., it is an enzyme inhibitor. If the glucose is still formed from the sucrose by α-glucosidase enzyme in the presence of the herbal extracts, the herbal may not possess the α-glucosidase inhibitory effect. The formation of glucose can be quantitatively determined by using UV-Visible spectrophotometric technique. If the glucose amount increases, the absorbance of the red pigment will be increased. Hence, the lower the absorbance value, the lower the glucose content. The absorbance of the red pigment formed from the glucose that produced from sucrose by α-glucosidase enzymatic hydrolysis, was found to be higher than that for the glucose produced from sucrose by α-glucosidase enzymatic hydrolysis in the presence of plant extracts. This observation showed that the extracts inhibited the α-glucosidase enzyme activity. From the mean

absorbance values, the percent inhibition of the crude extracts and reference drug (Voglibose) in various concentration: 0.125, 0.25, 0.5, 1.0, 2.0 µg/mL on α-glucosidase enzyme activity were calculated and it was found that the % inhibition of the samples on α-glucosidase enzyme activity increased with increasing the concentrations. From the % inhibition, the respective IC_{50} values of the plants extracts were calculated and the results are respectively tabulated in Table 1.

According to the results shown in Table 1, it can be seen that the 50 % inhibition concentration (IC_{50}) values for the ethanol extract (0.61 µg/mL) and watery extract (0.73 µg/mL) from Say-myin-khwa plant. Since the lower the IC_{50} values indicate the higher the α-glucosidase inhibitory effect of the samples. Alpha-glucosidase inhibitory effect of ethanol extract showed higher potency than that of watery extract. But it was observed that the watery and ethanol extracts of plant samples showed lower potency than standard drug Voglibose (IC_{50} = 0.32 µg/mL) in α-glucosidase inhibitory effect. These observations are depicted with a bar graph in Figure 2.

Table 1 % Inhibition of Various Concentrations and IC_{50} Values of Different Crude Extracts from *H. rotundifolia* and Standard Voglibose on α-Glucosidase Enzyme Activity

Tested Sample	% inhibition of different concentrations (µg/mL)					IC_{50} (µg/mL)
	0.125	0.25	0.5	1.0	2.0	
Water extract	41.77 ± 0.02	45.68 ± 0.11	47.04 ± 0.03	53.37 ± 0.21	61.71 ± 0.01	0.73
Ethanol extract	39.87 ± 0.35	47.46 ± 0.12	49.57 ± 0.03	51.47 ± 0.01	65.66 ± 0.05	0.61
Standard Voglibose	31.06 ± 0.01	48.22 ± 0.02	55.41 ± 0.01	58.33 ± 0.03	64.39 ± 0.02	0.32

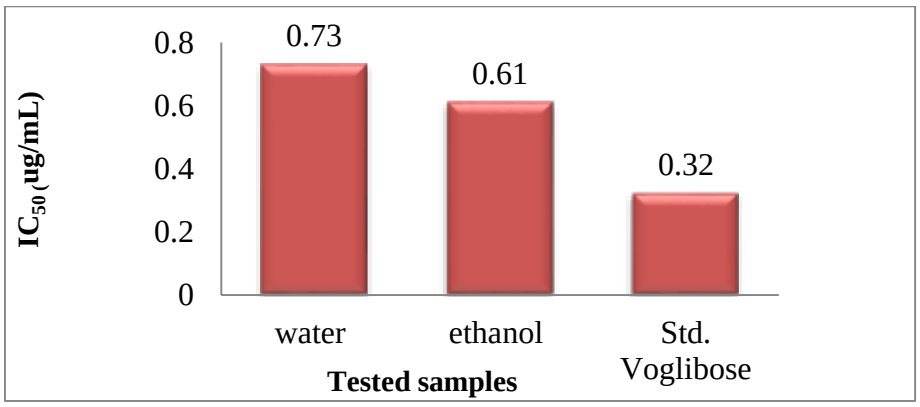

Figure 2 IC_{50} values of water and ethanol extracts from compared with standard voglibose on α-glucosidase enzyme activity

Antioxidant Activity of Crude Extracts of *H. rotundifolia* by DPPH Free Radical Scavenging Assay

The antioxidant activity was studied on the watery and 95 % ethanol extracts from two selected plant samples by DPPH free radical scavenging assay method. DPPH (2, 2 – diphenyl -1- picryl hydrazyl) method is most widely reported method for screening of antioxidant activity of many plant drugs. This method is based on the reduction of ethanolic solution of

coloured free radical DPPH by free radical scavenger. Determination of radical scavenging activity by DPPH method bases on the change in absorbance of sample solution in various concentrations. The activity was expressed as 50 % inhibitory concentration (IC_{50}).

The present study was carried out to investigate the radical scavenging activity of two crude extract such as ethanol, water from plant of *H. rotundifolia* by using DPPH according to the spectrophotometric method. In this experiment, five kind of different concentrations for each crude extract were prepared with ethanol solvent. The percent inhibition values and IC_{50} values of crude extracts were measured at different concentrations and the results were summarized in Table 2. And also the IC_{50} values was shown in Figure 3. From these experimental results, for all extracts, it was found that as the concentrations increased, the absorbance values decreased, i.e., increase in concentration, increase in radical scavenging activity of crude extracts usually expressed in term of % inhibition. Form the average values of % inhibition, IC_{50} (50 % inhibition concentration) values in µg/mL were calculated by linear regressive excel program.

From these results, it can be clearly seen that IC_{50} values were 12.34 µg/mL for water extract and 9.33 µg/mL for ethanol extract. Among these extracts, radical scavenging activity of ethanol extract was found to be the higher than water extract and it was also found to be lower than that of standard ascorbic acid (IC_{50} = 5.94 µg/mL).

Table 3 Radical Scavenging Activity (% RSA) and IC_{50} Values of Crude extracts of from *H. rotundifolia* and Standard Ascorbic acid

Tested Sample	% inhibition of different concentrations (µg/mL)					IC_{50} (µg/mL)
	5	10	20	40	80	
Water extract	39.77 ± 0.52	48.68 ± 0.31	54.32 ± 0.23	62.32 ± 0.12	68.35 ± 0.21	12.34
Ethanol extract	42.06 ± 0.11	51.22 ± 0.32	67.35 ± 0.30	72.43 ± 0.21	78.34 ± 0.11	9.33
Std. Ascorbic acid	48.32 ± 0.25	57.23 ± 0.02	55.41 ± 0.51	58.33 ± 0.43	62.78 ± 0.12	5.94

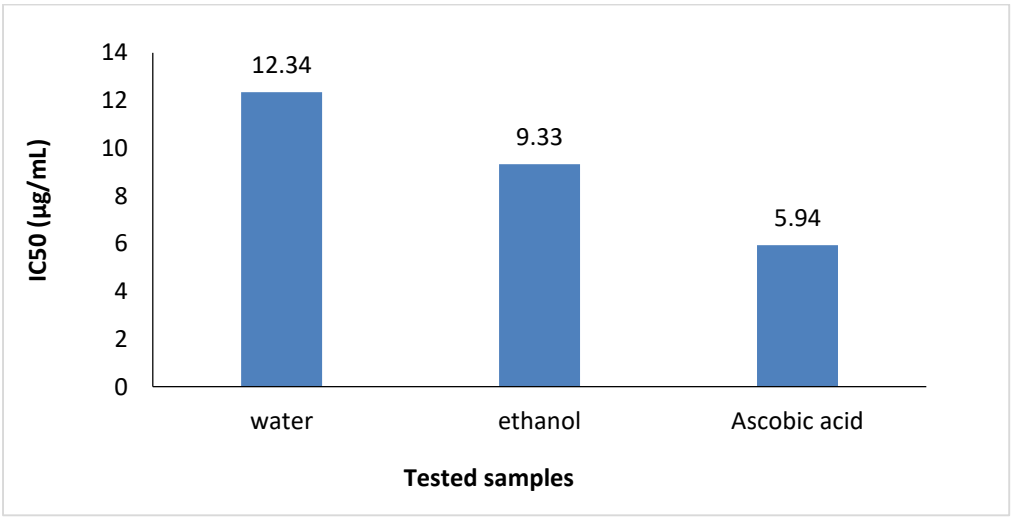

Figure 3 IC_{50} values of water and ethanol extracts compared with standard ascorbic acid in antioxidant activity

Conclusion

From these observations it can be suggested that selected medicinal plants was generally found to possess α-glucosidase inhibitory effect. Therefore, *H. rotundifolia* plant may be used as α-glucosidase inhibitor in control or managements of the postprandial hyperglycemia, Type 2 diabetes. The findings from the present work will contribute to the scientific development of Myanmar traditional medicine, specifically in the areas concerned with diabetes mellitus, α-glucosidase inhibitory effect, the diseases related to oxidative stress.

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