IN VITRO ANTIDIABETIC POTENTIALS OF SIDA ACUTA, ABUTILON INDICUM AND MALVASTRUM COROMANDELIANUM

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ABSTRACT

Objective: Starch metabolizing enzyme inhibitors are able to retard postprandial glucose absorption. This study aimed to investigate the in vitro inhibitory activities of alpha-glucosidase and alpha-amylase of three Malvaceous weeds. The inhibition of alpha-glucosidases as well as alpha-amylase is one of the powerful interventions to decrease glucose absorption.

Methods: The stems, roots and leaves of S. acuta, A. indicum and M. coromandelianum were sequentially extracted in dichloromethane and methanol, respectively. All fractions were tested for the inhibitory activities on yeast alpha-glucosidase, rat intestinal alpha-glucosidase and porcine alpha-amylase. p-Nitrophenyl-alpha-D-glucopyranoside and 2-chloro-4-nitrophenol-alpha-D-maltotrioside were used as the substrate for glucosidase and amylase respectively.

Results: The dichloromethane fraction of the roots and stems from A. indicum and dichloromethane as well as methanolic fractions of the stems of M. coromandelianum could inhibit yeast alpha-glucosidase compared to 1-deoxynojirimycin with the IC₅₀ of 0.36, 0.45, 0.48, 0.48 and 0.58 mg/ml respectively. A. indicum root methanolic fraction had the highest inhibitory effect on rat alpha-glucosidase activity compared to 1-deoxynojirimycin with the IC₅₀ of 0.08 and 0.11 mg/ml respectively. M. coromandelianum, the dichloromethane fraction of roots and the methanolic fraction of stems, showed the strongest effect on alpha-amylase inhibition compared to acarbose with the IC₅₀ of 0.07, 0.07 and 2.7 mg/ml respectively.

Conclusion: S. acuta, A. indicum and M. coromandelianum dichloromethane and methanolic fractions of the root, stem and leaf parts demonstrated an appreciable inhibitory activity on alpha-amylase from porcine, alpha-glucosidase from Saccharomyces cerevisiae and from rat intestine compared to 1-deoxynojirimycin and acarbose.

Keywords: Alpha-amylase, Alpha-glucosidase, Enzyme inhibition, Malvaceous weed

INTRODUCTION

After meal ingestion, the digestion of Starch begins firstly by salivary α-amylase and pancreatic α-amylase to produce maltose, maltotriose and α-limit dextrin which are further completely hydrolysed to glucose by α-glucosidases in the brush border of intestinal epithelial cells or enterocytes [1]. The inhibition of α-glucosidases as well as α-amylase is one of the powerful interventions to decrease glucose absorption. Natural or synthetic α-glucosidase inhibitors are of therapeutic interest to delay postprandial hyperglycemia in type 2 diabetes. Sida acuta Burm. f. is a Malvaceous weed cosmopolitan in distribution, especially in tropics and sub-tropics. It is commonly found on abandoned areas i.e. wastelands. The leaf, root, and whole plant have been ethnomedicinally used for treatments of wound, dysentery, helminthiasis, hemorrhoid and malarial fever [2]. Arya et al. reported that S. acuta leaf alcoholic extract exhibited a slight decrease in blood glucose levels after 2 and 4 h of oral administration in normal rats [3]. Okwusa et al. found that the aqueous extract and methanolic extract of S. acuta leaves significantly increased the tolerance for glucose in glucose fed normal rabbits and also decreased blood glucose of alloxan-induced diabetic rabbits [4]. Furthermore, Abutilon indicum (Linn.) Sweet and Malvastrum coromandelianum (Linn.) Garcke are another Malvaceous weeds previously reported of the hypoglycemic potential in animal model [5-7]. This study aimed to investigate in vitro inhibitory activities on the starch digesting enzymes of the fractional extracts of the roots, leaves and stems of S. acuta, A. indicum and M. coromandelianum, the Malvaceous weeds used in traditional medicine.

MATERIALS AND METHODS

Chemicals

Rat intestinal acetone powders, Saccharomyces cerevisiae alpha-glucosidase, porcine pancreatic α-amylase, p-nitrophenyl-α-D-glucopyranoside, 2-chloro-4 nitrophenol-α-D-maltotrioside, 1-deoxynojirimycin and acarbose were obtained from Sigma-Aldrich, USA. The chemicals were analytical grade. The ultrapure water was prepared by Ultra-pure water purification system, Heal Force, China.

Plant collection

S. acuta, A. indicum and M. coromandelianum were authenticated by Nijsiri Ruangrungis. Voucher specimens (ND/PH/300115, ND/PH/300215 and ND/PH/300315) were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. After sorting out any foreign matters, the stems, roots and leaves were dried in hot air oven at 50 °C then pulverized for extraction.

Plant extraction

The stems, roots and leaves of S. acuta, A. indicum and M. coromandelianum were exhaustively extracted with dichloromethane and methanol respectively using Soxhlet apparatus. The fractional extracts were filtered through Whatman number 1 filter paper then evaporated to dryness in vacuo. The extracts were dissolved in 10% DMSO and diluted with water to obtain concentrations of 0.625-10 mg/ml.

Yeast alpha-glucosidase inhibition assay

The activity of alpha-glucosidase from Saccharomyces cerevisiae was assayed using 1 mmol of p-nitrophenyl-α-D-glucopyranosides substrate. The reaction mixture including 50 μl of 0.1M sodium phosphate buffer (pH 6.9), 50 μl of the substrate, 50 μl of sample and 50 μl of α-glucosidase (0.5 U/ml) was incubated at 37 °C for 20 min then added with 100 μl of 1M sodium carbonate to stop the reaction. Enzymatic activity was quantified by measuring the absorbance of p-nitrophenol at 405 nm. 1-Deoxynojirimycin was used as positive control. Each test was done in triplicate.
Rat intestinal alpha-glucosidase inhibition assay

Thirty milligrams of rat intestinal acetone powders were suspended in 1 ml of 0.1 M sodium phosphate buffer (pH 6.9), sonicated for 20 min and centrifuged at 3000 rpm for 30 min. The supernatant was used as α-glucosidase enzyme. The reaction mixture consisted of 100 µl of 1 mmol of p-nitrophenyl-α-D-glucopyranoside as substrate, 50 µl of sample and 50 µl of the enzyme. The mixture was incubated at 37 °C for 30 min. The absorbance was measured at 405 nm. All tests were done in triplicate. 1-Deoxynojirimycin was used as a positive control. Each test was done in triplicate.

Porcine alpha-amylase inhibition assay

The activity of porcine pancreatic α-amylase inhibition was performed on 96 well plates using 1 mmol of 2-chloro-4-nitrophenol-α-D-maltotrioside as substrate. Various concentrations of S. acuta, M. coromandelianum, and A. indicum fractional extracts (50 µl) were added into 0.5 µl of 0.5 U/ml of porcine pancreatic α-amylase prepared in 0.1 M sodium phosphate buffer pH 6.9. The plate was preincubated at room temperature for 10 min and 50 µl of substrate were added into each well and incubated at 37 °C for 20 min. The absorbance was measured at 405 nm. Each test was done in triplicate. Acarbose was used as a positive control.

Enzyme inhibitory activity calculation

The enzyme inhibitory activity was calculated from the absorbance of p-nitrophenol liberated at 405 nm with and without the inhibitor.

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\text{Inhibition} (\%) = \left( 1 - \frac{A_{405 \text{ Inhibitor}}}{A_{405 \text{ Negative Control}}} \right) \times 100
\]

\(\text{IC}_{50}\) values denoted the concentration of sample required to inhibit 50% of enzyme activity.

RESULTS

The fractional extracts of the stems, roots and leaves of three selected Malvaceous plants were performed by dichloromethane and methanol, respectively. The percent yields were shown in table 1.

| Plant                  | Part used | Yield (g/100g) |
|------------------------|-----------|----------------|
|                        |           | DCM\(^a\) fraction | M\(^b\) fraction |
| *Sida acuta* Burm. f   | Stem      | 3.93           | 12.55          |
|                        | Root      | 1.46           | 4.62           |
|                        | Leaf      | 1.65           | 21.43          |
| *Abutilon indicum* (Linn) Sweet. | Stem | 1.34           | 8.20           |
|                        | Root      | 3.07           | 25.90          |
|                        | Leaf      | 1.60           | 15.40          |
| *Malvastrum coromandelianum* (L.) Garcke. | Stem | 3.50           | 13.15          |
|                        | Root      | 3.48           | 13.00          |
|                        | Leaf      | 9.08           | 24.12          |

\(^a\)Dichloromethane \(^b\)Methanol

| Plant                  | Part used | IC\(_{50}\) (mg/ml) Yeast alpha-glucosidase | IC\(_{50}\) (mg/ml) Rat intestinal alpha-glucosidase |
|------------------------|-----------|------------------------------------------|---------------------------------------------------|
|                        |           | DCM\(^a\) fraction | M\(^b\) fraction | DCM\(^a\) fraction | M\(^b\) fraction |
| *Sida acuta* Burm. f   | Stem      | 1.56           | 5.88           | 3.03           | 2.53           |
|                        | Root      | 1.46           | 8.12           | 3.96           | 1.08           |
|                        | Leaf      | 1.66           | 2.38           | 2.43           | 0.19           |
| *Abutilon indicum* (Linn) Sweet. | Stem | 0.45           | 1.69           | 4.67           | 1.11           |
|                        | Root      | 0.36           | 1.38           | 3.19           | 0.08           |
|                        | Leaf      | 1.07           | 4.21           | 2.69           | 1.38           |
| *Malvastrum coromandelianum* (L.) Garcke. | Stem | 0.48           | 0.48           | 6.50           | 1.35           |
|                        | Root      | 0.71           | 0.74           | 0.90           | 1.88           |
|                        | Leaf      | 1.07           | 1.70           | 1.55           | 3.61           |

1-Deoxynojirimycin

| Plant                  | Part used | IC\(_{50}\) (mg/ml) |
|------------------------|-----------|-------------------|
|                        |           | Yeast alpha-glucosidase | Rat intestinal alpha-glucosidase |
|                        |           | DCM\(^a\) fraction | M\(^b\) fraction | DCM\(^a\) fraction | M\(^b\) fraction |
| *Sida acuta* Burm. f   | Stem      | 1.71           | 2.65           |
|                        | Root      | 0.33           | 0.66           |
|                        | Leaf      | 1.88           | 2.08           |
| *Abutilon indicum* (Linn) Sweet. | Stem | 1.97           | 1.35           |
|                        | Root      | 0.90           | 1.89           |
|                        | Leaf      | 1.55           | 3.61           |
| *Malvastrum coromandelianum* (L.) Garcke. | Stem | 2.12           | 0.07           |
|                        | Root      | 0.07           | 0.28           |
|                        | Leaf      | 0.81           | 1.71           |

| Plant                  | Part used | IC\(_{50}\) (mg/ml) |
|------------------------|-----------|-------------------|
|                        |           | Yeast alpha-glucosidase |
|                        |           | DCM\(^a\) fraction | M\(^b\) fraction |
| *Sida acuta* Burm. f   | Stem      | 1.71           | 2.65           |
|                        | Root      | 0.33           | 0.66           |
|                        | Leaf      | 1.88           | 2.08           |
| *Abutilon indicum* (Linn) Sweet. | Stem | 1.97           | 1.35           |
|                        | Root      | 0.90           | 1.89           |
|                        | Leaf      | 1.55           | 3.61           |
| *Malvastrum coromandelianum* (L.) Garcke. | Stem | 2.12           | 0.07           |
|                        | Root      | 0.07           | 0.28           |
|                        | Leaf      | 0.81           | 1.71           |

Acarbose

\(^a\)Dichloromethane \(^b\)Methanol
The yeast and rat intestinal alpha-glucosidase inhibition of the fractional extracts (0.625-10 mg/ml) and 1-deoxynojirimycin (0.03-1.5 mg/ml) were demonstrated in table 2. All of the extracts could inhibit yeast alpha-glucosidase activity especially the dichloromethane fraction of the roots and stems from A. indicum and also dichloromethane as well as methanolic fractions of the stems of M. coromandelianum. They showed the strongest inhibitory effect on yeast alpha-glucosidase compared to 1-deoxynojirimycin with the IC50 of 0.36, 0.45, 0.48, 0.48 and 0.58 mg/ml respectively. The inhibitory activities against rat intestinal alpha-glucosidase were shown that A. indicum root methanolic fraction had the highest inhibitory effect on rat alpha-glucosidase activity compared to 1-deoxynojirimycin with the IC50 of 0.08 and 0.11 mg/ml, respectively. The dichloromethane fraction of M. coromandelianum stems showed the weakest effect with the IC50 of 6.50 mg/ml. All of the extracts inhibited alpha-amylase activity, especially the dichloromethane fraction of M. coromandelianum roots and methanolic fraction of M. coromandelianum stems. They showed the strongest effect on alpha-amylase inhibition compared to acarbose with the IC50 of 0.07, 0.07 and 2.7 mg/ml respectively (table 3).

**DISCUSSION**

The retardation of postprandial glucose absorption is beneficial in diabetes mellitus prevention and care. Inhibition of alpha-amylase and alpha-glucosidase, the enzymes involved in the starch digestion and absorption, is one of the therapeutic approaches for reducing postprandial hyperglycemia. In this in vitro study, S. acuta, A. indicum and M. coromandelianum dichloromethane and methanolic fractions of the root, stem and leaf parts demonstrated an appreciable inhibitory activity on alpha-amylase from porcine, alpha-glucosidase from S. acuta and from rat intestine compared to 1-deoxynojirimycin at 1 mg/ml. Polar compounds were abundant in these plant materials due to higher yields of methanolic fractions than dichloromethane fractions. However, dichloromethane fractions showed stronger yeast alpha-glucosidase inhibition than methanolic fraction. For rat intestinal alpha-glucosidase, most methanolic fractions showed stronger inhibitory activity except M. coromandelianum root and leaf parts. Arciniegas et al. showed that the acetone extracts of S. acuta and S. rhombifolia aerial parts had potent inhibitory activities on alpha-glucosidases from Saccharomyces cerevisiae and rat intestine. Para-hydroxyphenethyl trans-fenulate and beta-sitosteryl glucopyranoside isolated from these Sida spp. were reported as active compounds [8]. For alpha-amylase, most dichloromethane fractions had stronger inhibitory activity than methanolic fractions except the stems of A. indicum and M. coromandelianum. The phytochemical study of M. coromandelianum leaf by Aderogba et al. was performed using 80% methanol extraction then successive partitioning with n-hexane, dichloromethane, ethyl acetate, n-butanol and water respectively. The ethyl acetate and n-butanol fractionation afforded apigenin-7-O-β-D-glucopyranoside and apigenin-8-C-glucopyranoside (vitexin) [9]. Vitexin was revealed for potent alpha-glucosidase inhibitory activity in vitro [10].

**CONCLUSION**

This in vitro studies of S. acuta, A. indicum, M. coromandelianum fractional extracts demonstrated the appreciable inhibitory activities on porcine α-amylase, yeast α-glucosidase and rat intestinal α-glucosidase enzymes involved in starch absorption. The results contributed the use in traditional medicine and provided scientific information to continually validate the potential of these Malvaceous plants.

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**AUTHORS CONTRIBUTIONS**

All the authors have contributed equally.

**CONFLICT OF INTERESTS**

Declared none

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