International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 12, Issue 4, 2020

Original Article

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

NANTAPORN DINLAKANONT[1], CHANIDA PALANUVEJ[1*], NIJSIRI RUANGRUNGSI[1,2]

[1]College of Public Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand, [2]College of Pharmacy, Rangsit University, Pathum Thani 12000, Thailand
Email: chanida.p@chula.ac.th

Received: 17 Mar 2020, Revised and Accepted: 14 May 2020

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 i.e. *Sidaacuta* Burm. f., *Abutilon indicum* (Linn.) Sweet and *Malvastrumcoromandelianum* (Linn.) Garcke.

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-α-D-glucopyranoside and 2-chloro-4 nitrophenol-α-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 IC_{50} 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 IC_{50} 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 IC_{50} 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

DOI: http://dx.doi.org/10.22159/ijcpr.2020v12i4.39088. Journal homepage: https://innovareacademics.in/journals/index.php/ijcpr

INTRODUCTION

After meal ingestion, the digestion of Starchbeginsfirstly 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. *Sidaacuta* Burm. f. is a Malvaceous weed cosmopolitan in distribution, especially in tropics and sub-tropics. It is commonly found on abandoned areas i.e. roadsides and 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]. Okwuosa *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 *Malvastrumcoromandelianum* (Linn.) Garckeare 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 Ruangrungsi. 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-glucopyranosideas 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.

$$\text{Inhibition (\%)} = (1 - A_{405}\text{Inhibitor}/A_{405}\text{Negative Control}) \times 100$$

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.

Table 1: Extract yield from selected malvaceous plants

Plant	Part used	Yield (g/100g)	
		DCM[a] fraction	M[b] fraction
Sidaacuta 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
Malvastrumcoromandelianum (L.) Garcke.	Stem	3.50	13.15
	Root	3.48	13.00
	Leaf	9.08	24.12

[a]Dichloromethane [b]Methanol

Table 2: IC_{50} of *S. acuta, A. indicum, M. coromandelianum* extracts on yeast and rat alpha-glucosidase inhibition

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
Sidaacuta 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
Malvastrumcoromandelianum (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		0.58		0.11	

[a]Dichloromethane [b]Methanol

Table 3: IC_{50} of *S. acuta, A. indicum, M. coromandelianum* extracts on alpha-amylase inhibition

Plant	Part used	IC_{50} (mg/ml)	
		DCM[a] fraction	M[b] fraction
Sidaacuta 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
Malvastrumcoromandelianum (L.) Garcke.	Stem	2.12	0.07
	Root	0.07	0.28
	Leaf	0.81	1.71
Acarbose		2.7	

[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 strong inhibitory effect on yeast alpha-glucosidase compared to 1-deoxynojirimycin with the IC_{50} 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 IC_{50} of 0.08 and 0.11 mg/ml, respectively. The dichloromethane fraction of *M. coromandelianum* stems showed the weakest effect with the IC_{50} 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 *Saccharomyces cerevisiae* and from rat intestine compared to 1-deoxynojirimycin and acarbose. 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-ferulate 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. coromandelia*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-6´(p-coumaroyl)-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.

ACKNOWLEDGEMENTS

The authors are thankful to College of Public Health Sciences, Chulalongkorn University fornecessary assistance and instrumental support. N. D. wishes to express gratitude to Graduate School, Chulalongkorn University, for providing her research scholarship.

FUNDING

The 90th Anniversary Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund)

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Gray GM. Starch digestion and absorption in nonruminants. J Ntur 1992;122:172-7.
2. Dinda B, Das N, Dinda S, Dinda M, Silsarma I. The genus *Sida* L.-A traditional medicine: its ethnopharmacological, phytochemical and pharmacological data for commercial exploitation in herbal drugs industry. J Ethnopharmacol 2015;176:135-76.
3. Arya A, Abdullah MA, Haerian BS, Mohd MA. Screening for hypoglycemic activity on the leaf extracts of nine medicinal plants: *in vivo* evaluation. J Chem 2012;9:1196-205.
4. Okwuosa CN, Azubike NC, Nebo II. Evaluation of the anti-hyperglycaemic activity of crude leaf extracts *Sidaacuta* in normal and diabetic rabbits. Indian J Novel Drug Delivery 2011;3:206–13.
5. Krisanapun C, Lee SH, Peungvicha P, Temsiririrkkul R, Baek SJ. Antidiabetic activities of *Abutilon indicum* (L.) sweet are mediated by enhancement of adipocyte differentiation and activation of the GLUT1 promoter. Evid Based Complement Alternat Med 2011. DOI:10.1093/ecam/neq004.
6. Peungvicha P, Krisanapun C, Temsiririrkkul R, Wongkrajang Y. Aqueous extract of *Abutilon indicum* sweet inhibits glucose absorption and stimulates insulin secretion in rodents. Nutr Res 2009;29:579-87.
7. Deore AB, Chavan PN, Sapakal VD, Naikwade NS. Antidiabetic and antihyperlipidemic activities of *Malvastrumcoromandelianum* Linn leaves in alloxan-induced diabetic rats. Int J Pharmtech Res 2012;4:351-7.
8. Arciniegas A, Perez Castorena AL, Nieto Camacho A, Kita Y, Vivar AR. Anti-hyperglycemic, antioxidant, and anti-inflammatory activities of extracts and metabolites from *Sidaacuta* and *Sidarhombifolia.* Quimica Nova 2017;40:176-81.
9. Aderogba MA, Madikizela B, Mcgaw LJ. Bioactive constituents from *Malvastrumcoromandelianum* (L.) garcke leaf extracts. S Afr J Bot 2019;126:371-6.
10. Mengting Ni, Xing Hu, Deming Gong, Guowen Zhang. Inhibitory mechanism of vitexin on α-glucosidase and its synergy with acarbose. Food Hydrocoll 2020;105:105824.