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Alpha-glucosidase inhibitory effect and enzyme kinetics of coastal medicinal plants

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Abstract

Numerous metabolic disorders and diseases associated with the glucosidase enzyme activity. Therefore, in the present study we evaluated alpha glucosidase inhibitory effect of coastal medicinal plants on *Saccharomyces cerevisiae* α-glucosidase to control postprandial hyperglycemia. The n-hexane extracts of study plants screened for α-glucosidase inhibitory activity using yeast glucosidase. The enzyme kinetics studied Lineweaver-burk plot method. Among the ten plants *Citrullus colocynthis, Aegle marmelos* and *Ipomoea pes-caprae* exhibited potent α-glucosidase inhibitory activity, 85.9 ± 0.1, 72.2 ± 0.3 and 67.9 ± 0.1 respectively. From the enzyme kinetics assay potent plants contain uncompetitive inhibitors of alpha glucosidase. *C. colocynthis, A. marmelos* and *Ipomoea pes-caprae* have potential alpha-glucosidase inhibitors that can exploited for its used to treat diabetes.

Introduction

About 2.5-7% of the world's populations diagnosed with leading endocrine disease diabetes mellitus which causes morbidity and mortality (Seghrouchni et al., 2002). By the act of pancreatic α-amylase, starch has hydrolyzed and absorbed as glucose in the small intestine by α-glucosidases, leads to hyperglycemic condition. Moreover, the use of several approaches, α-glucosidase inhibitors is one of the alternative therapeutic approaches. Dietary carbohydrates such as maltose and sucrose absorbed by different types of α-glucosidases viz. maltase, sucrase, glucoamylase and isomaltase present in the intestine. Thus, inhibition of these enzymes can definitely decrease the postprandial hyperglycemia and could be a key tactic in the managements of diabetes mellitus (Hirsh et al., 1997).

The α-glucosidase inhibitors have isolated from natural sources, such as plants, foodstuffs and microbes (Kumar et al., 2011).

Coastal medicinal plants also engage in recreation to treat diabetes, particularly where most people have limited resources and do not have access to modern treatment (Ramanathan, 2000). The main interest of studies in to medicinal plants with hypoglycemic effect is to understand the metabolic activities of human intestinal tract. *Citrullus colocynthis* used for constipation, edema, bacterial infections, cancer and diabetes.

The aerial part of *C. colocynthis* showed antimicrobial and anti-inflammatory, anti-oxidant and local anesthetic effect reported by Gurudeeban et al., (2010). *Suaeda monoica*, *S. maritime* and *S. nudiflora* are traditional medicines for wound healing and hepatitis. The aerial part of this family plant has triterpenoids, sterols which have antiviral activity (Bandaranayake, 2002). *Aegle*

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marmelos, edible plant which are useful in ophthalmia, deafness, in ammations, catarrh, diabetes and asthmatic complaints, diarrhea, dysentery and stomachalgia, ophthalmia, ulcers, dropsy, cholera and cardio tonic effect like digitalis (Nadkarni, 1976). *Sesuvium portulacastrum* has essential oils used as a remedy for fever and scurvy (Magwa et al., 2006). *Clerodendrum inerme* used to treat of skin diseases, veneral infections, elephantiasis and asthma (Anonymous, 2001).

Among these conditions, 10 coastal medicinal plants used to manage diabetes in India namely *C. colocynthis*, *Ipomoea pes-caprae*, *S. monoica*, *S. maritime, S. nudiflora*, *Sesuvium portulacastrum*, *A. marmelos*, *Clerodendrum inerme, Ruta graveolens* and *Casuarina equisetifolia*. These medicinal plants are widely explored for their therapeutic potential. However no reports are available on the mechanism of α-glucosidase inhibitory effects. Therefore, the present study aimed to evaluate their effects on reduced glucose utilization effect.

Materials and Methods

Source of explants

The aerial parts of the 10 medicinal plants collected from the Southeast coast of India and kept under shade net (50%) house environment. The specimen certified by the Herbaria of C. A. S. in Marine Biology, Annamalai University and Botanical Survey of India (BSI) Coimbatore, Tamil Nadu.

Preparation of extract

The aerial parts of the plants extracted with *n*-hexane using a Soxhlet assembly for 48 hours, filtered and last traces of the solvent evaporated under reduced pressure in a rotary evaporator. The resulting extract filtered through filter paper (Whatman No. 1). The experimental chemicals purchased from Sigma-Aldrich Mumbai.

Assay for a-glucosidase inhibitory activity

Alpha-glucosidase activity estimated according to the method previously reported by Shibano et al. (1997) with trivial alterations. 50 µL of 0.1M phosphate buffer (with pH of 7.0), 10 µL of test sample and 25 µL of α-glucosidase solution incubated at 37ºC for 30 min and blanks prepared simultaneously. Then the reaction terminated by the addition of 100 µL of 0.2 M sodium carbonate solution. The enzymatic hydrolysis of substrate examine by the amount of p-nitrophenol released in the reaction mixture at 410 nm using microplate reader. Controls conduct in an identical manner replacing the plant extract with methanol and β-D-Glucose used as positive control. All experiments carried out in triplicates. The inhibition percentage of α-glucosidase assess by the following formula: α-glucosidase% = 100X (control-sample)/controlwhereas,

Control = test– blank; Sample = test – blank.

Kinetics of a-glucosidase inhibitor

To determine the plants extracts inhibition against *S. cerevisiae* α-glucosidase, with increasing concentration of PNPG (4-nitrophenyl α-D-glucopyranoside) used as substrate in the absence and presence of plant extracts at different concentrations. The data determined by Lineweaver-Burk plot analysis of the data (Lineweaver

Table I

α-Glucosidase inhibitory effect of coastal medicinal plants (Southeast coast of India)

Plant name	Voucher No.	Family	Plant parts used	% of α-glucosidase inhibition
Aegle marmelos	AUCASMB65	Rutaceae	leaf & fruit	72.2 ± 0.3
Casuarina equisetifolia	AUCASMB67	Casuarinaceae	leaf	24.9 ± 0.4
Citrullus colocynthis	AUCASMB66		whole part	85.9 ± 0.1
Clerodendrum inerme	AUCASMB33	Verbenaceae	leaf	41.0 ± 0.3
Ipomoea pes-caprae	AUCASMB68	Convolvulaceae	whole part	67.9 ± 0.1
Ruta graveolens	AUCASMB69	Rutaceae	leaf & fruit	25.8 ± 0.2
Suaeda monoica	AUCASMB19	Chenopodiaceae	whole part	No activity
Suaeda maritime	AUCASMB20			
Suaeda nudiflora	AUCASMB21			
Sesuvium portulacastrum	AUCASMB22	Aizoaceae		

Table II

Kinetics effect of α-glucosidase inhibition on plant extracts

Concentration (µg/mol)	*Citrullus colocynthis*		*Aegle marmelos*		*Ipomoea pes-caprae*	
	K_m (mM)	V_{max}(mM/min $^{-1}$)	K_m (mM)	V_{max} (mM/min $^{-1}$)	K_m (mM)	V_{max}(mM/min $^{-1}$)
0	1.226	0.883	3.900	0.334	2.633	0.243
4	1.562		4.329	0.365	5.675	0.250
8	0.886	0.851	2.633	0.312	4.248	0.243

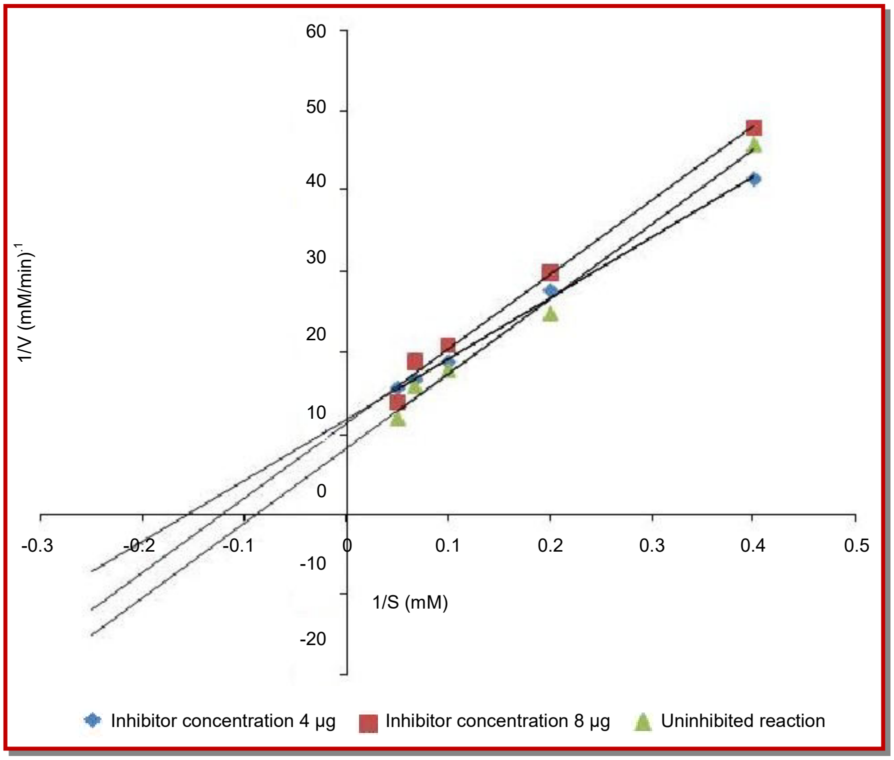

Figure 1 : Lineweaver-Burk plot of *S. cerevisiae* α-glucosidase and PNPG with 4 µg/mL and 8 µg/mL of *citrullus colocynthis extract*

and Burk, 1934), which calculated from the result according to Michaelis-Menten kinetics.

Statistical analysis

All assays performed at three times with triplicates samples. Values represented as mean ± Standard deviation.

Results and Discussion

Coastal plants are most promising source for recent drug development in marine pharmacological research. Earlier reports revealed the basic reason for diabetic mellitus, that the enzyme α-glucosidase participated in biological process like digestion, biosynthesis of glycolprotein and mainly involve in the cleavage of glycosidic bond, as a result increase in blood glucose level leads to chronic hyperglycemia. One of the therapeutic approaches for decreasing postprandial hyperglycemia is to retard absorption of glucose by carbohydrate hydrolyzing enzymes, α-amylase and α-glucosidase inhibition, in the digestive organs (Deshpande et al., 2009). Hence, we evaluated 10 coastal plants to develop such eminent α-glucosidase inhibitors from marine floral resources for reducing wide range of side effects. Among the 10 study plants *A. marmelos*, *C. colocynthis* and *I. pes-caprae* showed moderate α-glucosidase inhibitory activity. *Ruta graveolens* and *Casuarina equisetifolia* showed less inhibitory activity and salt marsh plants *S. monoica*, *S. maritime, S. nudiflora* and *Sesuvium portulacastrum* did not posses any inhibition

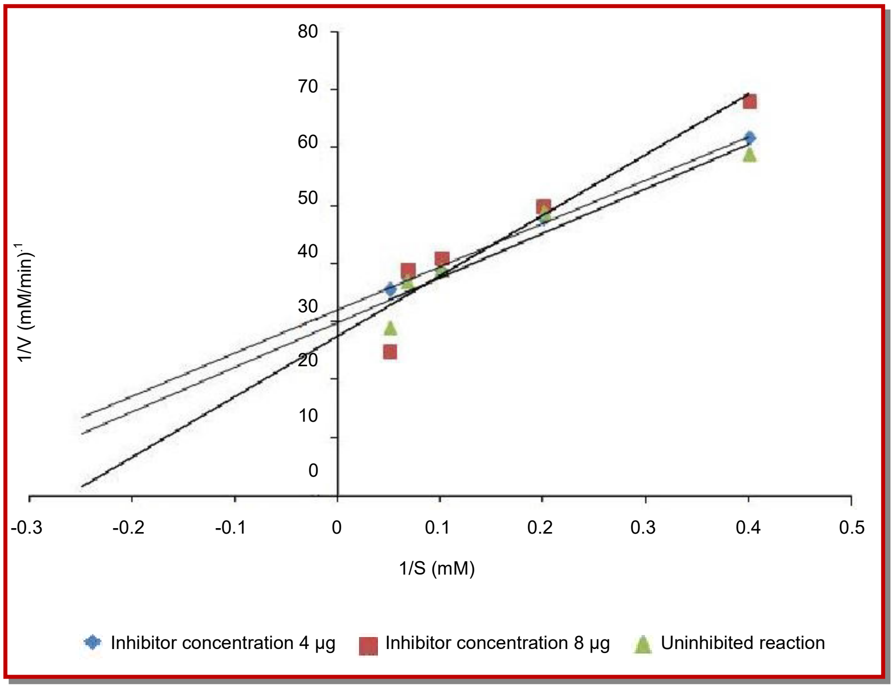

Figure 2 : Lineweaver-Burk plot of *S. cerevisiae* α-glucosidase and PNPG with 4 µg/mL and 8 µg/mL of *Aegle marmelos extracts*

against S. cerevisiae α-glucosidase. Acarbose used as positive control which showed low α-glucosidase inhibitory potential with IC50 value of 133 µg/mL (Table I).

Earlier studies, most mammalian α-glucosidase inhibitors did not effectively inhibit microbial α-glucosidases (Oki et al., 1999), because plants and mammalian enzymes are maltostructure, it hydrolyze homogeneous substrate whereas bacterial, yeast, and insect enzymes are maltooligosaccharides show higher activity toward heterogeneous substrates such as sucrose and pNPG, and no activity toward homogeneous substrates (Kimura, 2002; Kimura et al., 2004). Our present result exhibits the strong enzymatic inhibitory activity against yeast α- glucosidases shown by three plant extracts (*A. marmelos*, *C. colocynthis* and *I. pes-caprae*) is obviously enhanced than the commercial inhibitor acarbose at low concentration. This will favor that *A. marmelos*, *C. colocynthis* and *I. pes-caprae* extracts can decrease blood glucose level.

The inhibition kinetics of *A. marmelos*, *C. colocynthis* and *I. pes-caprae* analyzed by Lineweaver-Burk plot analysis, which indicated that it is a non-competitive inhibitor with respect to PNPG for S. cerevisiae α-glucosidase (Table II). Inhibitor concentration (µg/mL) plotted on the X-axis and 1/V (mM/min)-1 values obtained from the Lineweaver-Burk plot plotted on the Y-axis. The aerial extract of *C. colocynthis* has uncompetitive inhibitor (s) of alpha-glucosidase (Figure 1) and reduction of Vmax from 1.2 mM/min-1 to 0.8 mM/min-1 and Km from 1.6 mM to 0.9 mM. The leaf and fruit extract of *A. marmelos* has mixed inhibitors of alpha glucosidase (Figure 2) and there is a reduction in Vmax from 3.7 mM/min-1 to 3.1 mM/min-1 and Km from 4.3 mM to 2.6 mM. The aerial extract of *I. pes-caprae* (Figure 3) has uncompetitive inhibitors of alpha-glucosidase and there is reduction of Vmax from 0.3 mM/min-1 to 0.2 mM/min-1 and the Km from 5.7 mM to 4.2 mM. The commercial alpha-glucosidase inhibitor, acarbose is a competitive inhibitor of the enzyme and it required at higher concentration to reduce the post-prandial glucose level. The uncompetitive inhibitors bind to the enzyme-substrate complex, lowering the Km and the maximum enzyme activity (Vmax).

Conclusion

Inhibition of α-glucosidase is one of the therapeutic approaches for preventing post postprandial hyperglycemia. Hence, the explore for α-glucosidase inhibitors in coastal medicinal plants is imperative because these inhibitors could control the postprandial hyperglycemia of diabetic patients.

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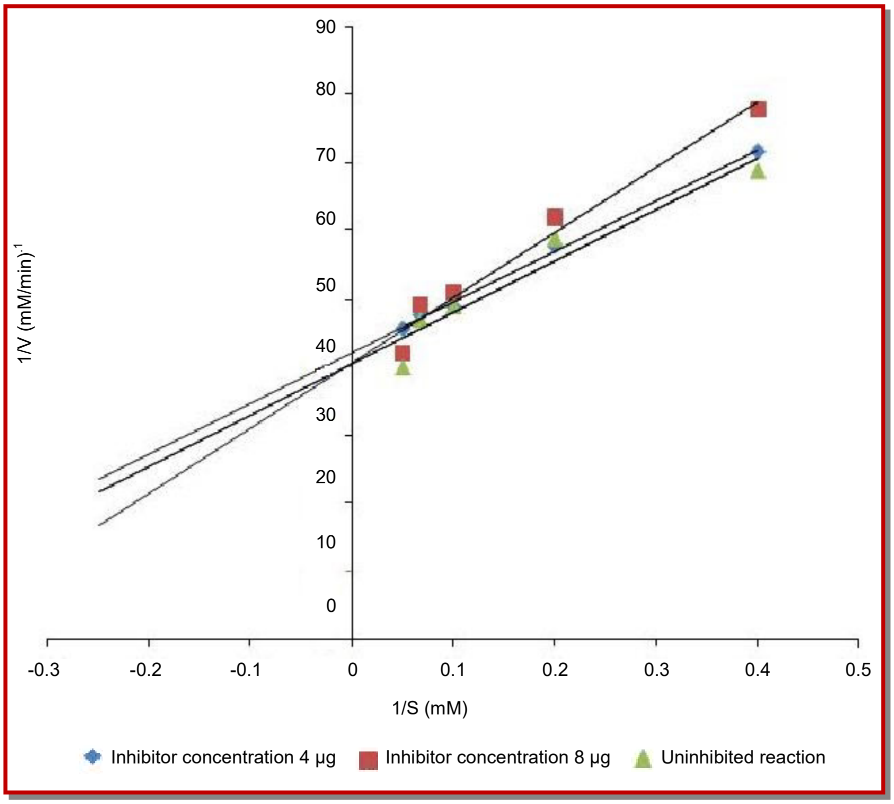

Figure 3 : Lineweaver-Burk plot of *S. cerevisiae* α-glucosidase and PNPG with 4 µg/mL and 8 µg/mL of *Ipomoea pes-caprae extracts*

Conflict of Interest

Authors declare no conflict of interest

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