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Alpha-amylase inhibitory activities of ascidians in the treatment of diabetes mellitus

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Abstract

Extracts of ten selected ascidians with a reputation of usefulness in treating diabetes were examined for alpha-amylase inhibition using an *in vitro* model. The extract with the highest activity was selected for further characterization. From the results ethyl acetate showed predominant amylase inhibition activity for all species and the maximum level of inhibition was recorded in *Phallusia mammillata* (68%) at 300 µg/mL and the lowest activity was noted in *Microcosmus squamiger* (12%) at 200 µg/mL. After preliminary results, the methanolic extract of *P. mammallita* were further assayed for confirmation of enzyme inhibition and the maximum results (82%) were obtained at 250 µg/mL and the IC_{50} value of *P. mammillata* were evidenced at 145.0 ± 0.4 µg/g. In the present study, *P. mammillata* indicated the maximum α-amylase activity without toxic effects. Similarly, α-glucosidase and α-amylase inhibitor bromophenol, C_6H_5BrO was produced by *P. mammillata*.

Introduction

Type 2 diabetes is one of the primary threats to human health due to increasing prevalence, chronic course and disabling complications (Bhandari et al., 2008). Hence, retardation of starch digestion by inhibition of enzymes such as α-amylase plays a key role in the control of diabetes. Inhibitors of pancreatic α-amylase delay carbohydrate digestion causing a reduction in the rate of glucose absorption and lowering the post-prandial serum glucose levels (Chopra et al., 1986). Some inhibitors currently in clinical use are acarbose and miglitol which inhibit glycosidases such as β-glucosidase and α-amylase while others such as voglibose inhibit α-glucosidase. However, many of these synthetic hypoglycemic agents have their limitations, are non-specific, produce serious side effects and fail to alleviate diabetic complications.

The search for new pharmacologically active agents obtained by screening natural sources or their extracts can lead to potent and specific inhibitors for α-amylase (Grover et al., 2002). Pharmacological properties of α-glucosidase inhibitors such as acarbose that can also inhibit pancreatic α-amylase revealed that the complications of diabetes mellitus such as onset of renal, retinal, lens and neurological changes and the development of ischemic myocardial lesions are prevented or delayed (Kotowaroo et al., 2006).

Long-term day-to-day management of diabetes, with acarbose is well tolerated and can improve glycaemic control as monotherapy, as well as in combination therapy (Kostova and Dinchev, 2005). The major adverse effects of acarbose are abdominal distention, flatulence, meteorism and possibly diarrhea (Klein et al., 2007). The search for safer, specific, and effective hypoglycemic agents has continued to be an important area of investigation with natural extracts from readily available traditional medicinal plants offering great potential for discovery of new anti-diabetic drugs (Klein et al., 2007).

Materials and Methods

Extraction

The extraction of samples was carried out using the standard method (Pearce et al., 2007). The samples of freshly collected ascidians (*Phallusia mammillata, P. arabica, Microcosmus squamiger, Microcosmus* sp. *Didemnum vexillum, Tridemnum savignii, Polyclinum aurantium, Polyclinum* sp. *Ascidia ahodori* and *Ascidia* sp.) were kept in ice chest at 5°C and immediately transported to laboratory. After thorough cleaning, the samples were dissected and the mantle body was separated and freeze dried before being used. The freeze dried ascidian (150 g each) was separately extracted with methanol and acetone (1/5, w/v). The extracts were filtered and concentrated under vacuum on a rotary evaporator at 40°C to produce 9.96, 10.2 and 7.5 g of methanol extract, acetone extract and ethyl acetate extract respectively. The residues were further reconstituted in an appropriate buffer or normal saline for further assay.

Extraction of α-amylase inhibitors

100 g of sample was dissolved in 300 mL of 0.2 M NaCl and kept for 1 hour at room temperature. After centrifugation at 17,400 rpm for one hour, the supernatant was heated in boiling water bath at 80°C for 10 min and again centrifuged. The soluble material after heating is called heat soluble proteins. This material was subjected to ammonium sulfate precipitation. The fractions that precipitated at 0.5, 1.0, 1.5 and 2.5 M ammonium sulfate were designated as AS 0.5, 1.0, 1.5 and 2.5 respectively. Ammonium sulfate precipitates were suspended in 0.2M phosphate buffer (pH 6.8) and dialyzed thoroughly against the same buffer for two days at 4°C (cold room). After centrifugation the supernatants were filtered through Whatman No. 1 filter paper. The filtrate was kept at -20°C in a deep freezer and then lyophilized using lyodel lyophilizer. The buffer reconstituted (10 mg/100 mL) lyophilized powder was used as a source of α-amylase inhibitor for further studies.

Enzyme and inhibitor assay

One unit of α-amylase (diluted) and two mL (200 mg of partially purified inhibitor) of inhibitor sample to be assayed were mixed in 0.2 mL of 0.2M phosphate buffer (pH 6.8) and incubated at 37°C for 15 min, the reaction was stopped by adding 1 mL of DNSA reagent. According to the standard graph, drawn from different concentrations of enzymes, the maltose formed was calculated. One unit of enzyme activity was defined as the amount of enzyme causing the release of 1 µmol of maltose in 1 min under the assay condition.

One inhibitor activity unit (I.U) was defined as the amount of inhibitor that caused 50% of inhibition of one unit of α-amylase enzyme.

Results

Ethyl acetate showed predominant amylase inhibition activity for all species and the maximum level of inhibition is recorded at *P. mammillata* (68%) at 300 µg/mL the lowest activity was noted in *M. squamiger* (12%) at 200 µg/mL (Table I). The second predominant

Table I

α-Amylase inhibition activity of different solvent extracts of *Ascidians* species

Names	%Inhibition of methanol extracts (µg/mL)			%Inhibition of acetone extracts (µg/mL)			%Inhibition of ethyl acetate extracts (µg/mL)		
	100	200	300	100	200	300	100	200	300
Phallusia mammillata	65 ± 0.2	78 ± 0.0	81 ± 0.9	-	-	-	40 ± 0.7	12 ± 1.0	35 ± 0.0
Phallusia arabica	45 ± 0.6	65 ± 0.7	68 ± 0.7	-	-	-	45 ± 0.6	60 ± 0.8	65 ± 0.1
Microcosmus squamiger	40 ± 0.7	60 ± 0.4	68 ± 0.5	-	-	-	65 ± 0.2	55 ± 0.6	68 ± 0.0
Microcosmus sp.	48 ± 0.1	50 ± 0.5	60 ± 1.2	29 ± 0.3	50 ± 0.6	53 ± 0.1	48 ± 0.0	33 ± 0.5	57 ± 0.9
Didemnum vexillum	48 ± 0.6	62 ± 0.3	74 ± 0.1	-	-	-	48 ± 0.6	54 ± 0.3	61 ± 1.0
Tridemnum savignii	-	-	-	-	-	-	-	30 ± 0.3	53 ± 0.6
Polyclinum aurantium	-	4 ± 0.2	12 ± 0.1	-	-	-	-	52 ± 0.3	65 ± 0.2
Polyclinum sp.	-	-	-	-	-	-	44 ± 0.2	-	-
Ascidia ahodori	44 ± 0.2	63 ± 0.3	71 ± 0.6	-	-	-	-	29 ± 0.5	45 ± 0.1
Ascidia sp.	-	-	-				44 ± 0.9	52 ± 0.6	58 ± 0.1

Table I

The maximum results of inhibition of *Phallusia mammillata*

Concentration (µg/g)	Inhibition (%)
16	8 ± 0.0
31.3	13 ± 0.2
62.5	28 ± 0.8
125	45 ± 0.4
250	82 ± 0.2
IC_{50}	145 ± 0.4

inhibition activity was observed in methanol extracts and showed superior activity for 9 species except *T. savignii* and the maximum activity was noted in *P. mammillata* (81%) at 300 µg/mL and the least activity is noted in *P. aurantium* at 200 µg/mL. Very poor amylase inhibition activity is recorded in acetone extracts and only *Microcosmus* sp. showed inhibition activity (53%) at 200 µg/mL and the other 9 ascidian species failed to display any results. From the above results, the premier enzyme inhibition results were recorded in methanolic extract of *P. mammallita* at 300 µg/mL. After preliminary results, the methanolic extract of *P. mammallita* was further assayed for confirmation of enzyme inhibition and the results are shown in Table II. The maximum results were obtained (82%) at 250 µg/mL and the IC_{50} value of *P. mammillata* were evidenced at 145 ± 0.4 µg/g.

Discussion

In the present study, *P. mammillata* indicated the maximum α-amylase activity without toxic effects. Similarly, α-glucosidase and α-amylase inhibitor bromophenol, C_6H_5BrO, is produced by *P. mammillata*. It has been used in the therapy of type II diabetes mellitus, in order to enable patients to control blood sugar contents while living with starch-containing diets. Other species of ascidians also produce various other components which inhibit α-glucosidase and α-amylase, of which component is hard to separate during extraction and purification, which is one of the most modern work-up processes developed to date (Kim et al., 2010).

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References

Bhandari MR, Jong-Anurakkun N, Hong G, Kawabata J. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*, Haw.). Food Chem. 2008; 106: 247-52.

Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. Council of Scientific and Industrial Research, New Delhi, India, 1986.

Kim K, Himmeldirk Y, Chen X. Evidence-based complementary and alternative medicine. J Mol Biol. 2007; 4: 401-07.

Klein G, Kim J, Himmeldirk K, Cao Y, Chen X. Evidence-based complementary and alternative medicine. J Mol Biol. 2007; 4: 401–07.

Kostova I, Dinchev D. Saponins in *Tribulus terrestris*: Chemistry and bioactivity. Phytochem Rev. 2005; 4: 111–37.

Kotowaroo MI, Mahomoodally MF, Gurib-Fakim A, Subratty AH. Traditional medicinal herbs and food plants have the potential to inhibit key carbohydrate hydrolyzing enzymes *in vitro* and reduce postprandial blood glucose peaks *in vivo*. Phytothera Res. 2006; 20: 228-31.

Pearce AN, Chia EW, Berridge MV, Clark GR, Harper JL, Larsen L, Maas EW, Page MJ, Perry NB, Webb VL, Copp BR. Anti-inflammatory Thiazine Alkaloids Isolated from the New Zealand ascidian *Aplidium* sp. Inhibitors of the neutrophil respiratory burst in a model of gouty arthritis. J Nat Products. 2007; 70: 111-13.

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