Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 7, 2014

Original Article

EFFECT OF *COSTUS PICTUS* D. DON ON CARBOHYDRATE HYDROLYZING ENZYMES

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Received: 14 Apr 2014 Revised and Accepted: 18 May 2014

ABSTRACT

Objective: *Costus pictus* D. Don is a potent anti-diabetic plant used in folk as well as Indian traditional medicine. In the present study, 4 extracts namely, Fresh-aqueous, Fresh-hydroalcoholic, Dry-Aqueous and Dry-hydroalcoholic prepared from fresh as well as dried leaves of *C. pictus* were evaluated for their effect on alpha-amylase and alpha-glucosidase enzymes using *In vitro* assays.

Methods: Alpha-Amylase inhibitory activity of *C. pictus* extracts was evaluated using Porcine pancreatic alpha-amylase (PPA) with starch as a substrate, whereas, alpha-glucosidase inhibitory activity was evaluated using p-nitrophenyl-α-D-Glucopyranoside (PNPG) as a substrate.

Results: Only Fresh-aqueous extract of *C. pictus* revealed potent alpha-amylase inhibitory (IC_{50}= 9.90mg/ml) as well as alpha-glucosidase inhibitory (IC_{50}= 2.51mg/ml) activities. In general, it exhibited strong inhibitory effect on alpha-glucosidase than alpha amylase.

Conclusion: Present study does seem to justify usage of fresh leaves of *C. pictus* as a munching dilatory supplement for treatment of diabetes as Fresh-aqueous extract exhibited potent inhibitory effect on carbohydrate hydrolyzing enzymes.

Keywords: Alpha-amylase, Alpha-glucosidase, *Costus pictus*, Antidiabetic.

INTRODUCTION

One of the anti-diabetic therapeutic strategies is inhibition of carbohydrate digesting enzymes such as α-amylase and α-glucosidase [1]. Alpha-amylase hydrolyzes complex starches to oligosaccharides, while, alpha-glucosidase hydrolyzes oligosaccharides to glucose and other monosaccharides. Inhibition of these enzymes produces postprandial anti-hyperglycemic effect by reducing the rate and extent of glucose absorption [2]. The most prevalent form of diabetes affecting 90-95% of diabetics is type-2 which is associated with elevated postprandial hyperglycemia (PPHG) [3] and India is considered as the "Diabetes Capital" of the world [4]. Currently, there are 5 classes of conventional anti-diabetic drugs; however, these drugs are associated with various side-effects [5]. Hence, there is urgent need to identify and explore natural sources with fewer side-effects for such inhibitors. In the present study, four extracts of *Costus pictus* D. Don were evaluated for their effect on alpha-amylase and alpha-glucosidase enzymes. *C. pictus* is a potent anti-diabetic plant used in folk as well as Indian traditional medicine [6]. Fresh raw leaves are used as a munching dietary supplement for the treatment of diabetes in southern India [7]. The probable mechanism behind this has been evaluated in the present *In vitro* study.

MATERIALS AND METHODS

Collection of the material

Costus pictus was collected from Goa by Dr. R. Y. Ambaye. It was identified by Dr. Rajendra D. Shinde, Associate Professor, Department of Botany, St. Xaviers College, Mumbai. Fresh specimen was deposited at the Blatter Herbarium of St. Xaviers College, which matched with the Blatter Herbarium specimen AM-1.

Preparation of extracts

Finely chopped fresh leaves were used to prepare aqueous as well as hydroalcoholic (HA) extracts. Similarly, the leaves were shade dried, powdered and then subjected to aqueous and hydroalcoholic extractions. Aqueous extracts were obtained by hot decoction method, while, hydroalcoholic extracts were prepared by cold maceration.

Hot decoction

To the material distilled water (100^{0}c) was added in a stoppered flask, shaken well and allowed to stand for 10 min. The mixture was then filtered and evaporated to dryness on a boiling water bath.

Cold maceration

The material was macerated with 20% ethanol in a stoppered flask for 48 hrs at 4^{0}C with occasional stirring. The mixture was then filtered and evaporated to dryness on a boiling water bath. Percentage yield of individual extract was calculated (Table 1). Extracts were kept at 4^{0}C until further use. Thus there were 4 extracts, namely, Fresh-Aqueous, Fresh-HA, Dry-Aqueous and Dry-HA. Hydroalcoholic extracts were reconstituted in 20% ethanol, whereas, aqueous extracts were reconstituted in distilled water.

Table 1: Percentage yield of extracts

Extract	Material (gm)	Solvent (ml)	Yield (%)
Fresh- Aqueous	30	300	1.56
Fresh- HA	25	250	1.66
Dry- Aqueous	2.8	140	15.64
Dry- HA	6	300	12.98

Alpha-amylase assay

Effect of extracts on alpha-amylase was evaluated according to the method of Sudha et al [8] with slight modification. In a 96-well plate, reaction mixture containing 50µl phosphate buffer (50mM, pH= 6.8), 10µl alpha-amylase (10U/ml) [SRL] and 20µl of varying concentrations of extracts was pre-incubated at 37^{0}C for 10 min. Then 20µl soluble starch (0.05%) [HiMedia] was added as a substrate and incubated further at 37^{0}C for 15 min. The reaction was stopped by adding 20µl 1N HCl, followed by addition of 100µl iodine reagent (5mM I_2 and 5mM KI, stored in amber colored bottle). The absorbance was read at 620nm using Multimode Reader (Synergy HT, BioTek). Each experiment was performed in triplicates, along with appropriate blanks. Acarbose at various concentrations (10-100 µg/ml) was included as a standard. Acarbose was provided by Mr. Dnyaneshwar Nagmoti, Institute of Chemical Technology, Mumbai. Negative control without extracts was set up in parallel. The result is expressed as percentage inhibition, which was calculated as, Inhibition (%)= $A_{Test} - A_{Negative\ control}$ / A_{Test} X 100, where, A is absorbance. The result is also expressed as IC_{50} value.

Alpha-glucosidase assay

Effect of extracts on alpha-glucosidase was assessed according to the method of Bachhawat et al [9] with slight modification. In a 96-well

plate, reaction mixture containing 50µl phosphate buffer (50mM, pH= 6.8), 10µl alpha-glucosidase (1U/ml) [SRL] and 20µl of varying concentrations of extracts was pre-incubated at 37^{0}C for 15 min. Then 20µl p-nitrophenyl-α-D-Glucopyranoside (PNPG) (1mM) [SRL] was added as a substrate and incubated further at 37^{0}C for 30 min. The reaction was stopped by adding 50µl sodium carbonate (0.1M). The yellow color produced was read at 405nm using Multimode Reader (Synergy HT, BioTek). Each experiment was performed in triplicates, along with appropriate blanks. Acarbose at various concentrations (200-1000 µg/ml) was included as a standard. Negative control without extracts was set up in parallel. The result is expressed as percentage inhibition, which was calculated as,

Inhibition (%)= $A_{\text{Negative control}} - A_{\text{Test}}/A_{\text{Negative control}}$ X 100, where, A is absorbance. The result is also expressed as IC_{50} value.

Statistical analysis

All the determinations were done in triplicate. Means, standard deviations and IC_{50} values were calculated using a Microsoft Excel program. Statistical analysis was done using one-way ANOVA followed by Dunnett's multiple comparison test using GraphPad Prism 5 software and P<0.05 was regarded as significant.

RESULTS AND DISCUSSION

Recently, the status of diabetes has changed from being considered as a mild disorder of elderly to one of the major causes of morbidity and mortality [10] mainly due its chronic complications [11]. Nature is the best combinatorial chemist and has possible answers to all diseases of mankind [12] and plants hold definite promises in the management of Diabetes mellitus [13,14]. Hence in the present study, four extracts prepared from *C. pictus* were evaluated for their effect on carbohydrate digesting enzymes.

Porcine pancreatic alpha-Amylase (PPA) is closely related to human alpha-Amylase [8]. Hence PPA was used to evaluate inhibitory activity of *C. pictus* extracts with starch as a substrate. The assay was based on starch-iodine color complex formation, whereas, alpha-glucosidase inhibitory activity was evaluated using p-nitrophenyl-α-D-Glucopyranoside (PNPG) as a substrate which was based on development of yellow color of p-nitro phenol. In the present study, only Fresh-aqueous extract exhibited potent alpha-amylase inhibitory activity, whereas, rest of the extracts showed no inhibitory effect even at a concentration of 20 mg/ml (Table 2).

Table 2: Effect of *C. pictus* on alpha-Amylase

Extract	Conc. (mg/ml)	% Inhibition (Mean ± SD)	IC_{50} (mg/ml)
	9.4	12.61 ± 9.9	
Fresh-Aqueous	9.6	34.34 ± 4.5**	9.90
	9.8	46.23 ± 0.7***	
	10	69.12 ± 2.8***	
	10.5	75.52 ± 0.3***	
Fresh- HA	-	No	-
Dry- Aqueous	-	No	-
Dry- HA	-	No	-
Acarbose (Standard)	-	-	34 µg/ml

**P<0.01 and ***P<0.001 (as compared with negative control)

In case of alpha-glucosidase, Fresh-aqueous and Fresh-HA extracts revealed potent inhibitory activity (Table 3). However, Dry-aqueous and Dry-HA extracts showed 18.84% and 14.13% inhibition respectively at a concentration of 20mg/ml. In general, Fresh-aqueous extract of *C. pictus* revealed potent inhibitory effect on both the carbohydrate digesting enzymes. Further, it exhibited strong inhibitory activity on alpha-glucosidase than alpha-amylase (Table 2 and Table 3). According to Ani and Naidu [15], effective strategy for type-2 diabetes management is mild inhibition of alpha-amylase and strong inhibition of alpha-glucosidase and our study supports this finding.

Table 3: Effect of *C. pictus* on alpha-Glucosidase

Extract	Conc. (mg/ml)	% Inhibition (Mean ± SD)	IC_{50} (mg/ml)
	2	10.14 ± 4.4*	
Fresh-Aqueous	2.4	44.54 ± 7.5***	2.51
	2.8	71.21 ± 5.4***	
	3	89.35 ± 2.2***	
	3.2	98.03 ± 1.3***	
	2.8	21.13 ± 4.3***	
Fresh- HA	3	34.37 ± 4.6***	3.18
	3.4	75.27 ± 1.2***	
	3.6	86.40 ± 3.5***	
	3.8	95.19 ± 1.9***	
Dry- Aqueous	-	-	>20
Dry- HA	-	-	>20
Acarbose (Standard)	-	-	468.92 µg/ml

*P<0.05 and ***P<0.001 (as compared with negative control)

The role of oxidative stress in diabetes and diabetic complications has been reported [16] and antioxidants play important role in management of diabetes. The aqueous extract of *C. pictus* was found to have strong antioxidant activity and to lower the TBARS levels in liver and kidney of treated rats [7]. Our study also exhibited antioxidant potential of *C. pictus* (result not included). A study by Suganya et al [17] also reported to prevent diabetic complications due to oxidative stress in diabetic rats by *C. pictus*. The preliminary phytochemical investigation of *C. pictus* by Shiny et al [18] revealed presence of steroids, alkaloids, phenols, glycosides, quinones, coumarins and flavonoids. Among these phytochemicals, phenols and flavonoids are well known antioxidants. Our study also confirmed presence of phenolics and flavonoids. The natural phenolic antioxidants often act as reducing agents, terminate the free radical chain reaction and chelate transition metals, thus inhibit oxidation reactions. Likewise, flavonoids are believed to be good scavengers of free radicals [19]. Furthermore, Phenolic components have shown effective inhibition of alpha-amylase and alpha-glucosidase enzymes in the past [20,21]. Hence alpha-amylase and alpha-glucosidase inhibitory activities of *C. pictus* could be attributed to presence of phenolics.

Present study does seem to justify usage of fresh leaves of *C. pictus* as a munching dilatory supplement for treatment of diabetes as Fresh-aqueous extract exhibited potent inhibitory effect on carbohydrate hydrolyzing enzymes.

CONCLUSION

Costus pictus can be effective in treatment of diabetes not only by inhibition of alpha-amylase and alpha-glucosidase enzymes, but also through its antioxidant property.

CONFLICT OF INTERESTS

Declared None

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