In vitro hypoglycemic potential of spices: Cinnamon and Cumi

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Abstract: Present study evaluates the anti-hyperglycemic potential of two Indian spices *Cinnamomum zeylanicum*(CZ) and *Cumin cyminum*(CC) (whole powder and aqueous extracts) using *in vitro* techniques like glucose adsorption assay, amylolysis kinetics and *ex vivo* assays like amylase, Sucrase and α-glucosidase assay. CZ displayed higher glucose adsorption and glucose diffusion retardation than CC, as shown by glucose adsorption and amylolysis kinetics assay. CZ showed lower inhibition of α-amylase and sucrase where as CC has no effect on both the enzymes. In case of α-glucosidase, CC had better inhibition than CZ. Further research is needed to understand the mechanism through which both the spices act to regulate the hyperglycemia.

Keywords: *Cinnamomum zeylanicum, Cumin cyminum,* α-glucosidase, α-amylase, sucrase, adsorption.

INTRODUCTION

Diabetes is one of the most leading killer disorders persisting in the world population; one in every 20 deaths is due to diabetes. Presently 1.71 billion of the world population is suffering from diabetes and by 2030 it is estimated to reach 3.66 billion (Allogot *et al.,* 2003; WHO report on diabetes 2011). The well known risk factors for diabetes are age, ethnicity, family history with diabetes, obesity, smoking, and physical inactivity (Deshpande *et al.,* 2008). Diabetes itself is a risk factor for many diseases such as cardiovascular diseases (Stegmayr *et al.,* 1995), nephropathy (Timothy *et al.,* 2000), peripheral neuropathy (Gerard Said *et al.,* 2007), retinopathy (Benson*et al.,* 1994; David *et al.,* 1999), etc. The patho-physiology of the disease is mainly due to prolonged hyperglycemia, which results in glycation of proteins, increased oxidative stress and production of pro-inflammatory agents; which in turn would badly mark the cause for diabetic complications (Michael Brownlee 2001). Such culprits are the root cause for the patho-physiology of the secondary complications. Controlling hyperglycemia forms the most important target to treat diabetes. Hyperglycemia can be controlled either by increasing the glucose uptake into body cells or by decreasing glucose absorption in the intestine. Glucose absorption can be controlled either by inhibiting carbohydrate digestion in the intestine or inhibiting glucose transport across the intestinal epithelial cells or by both. Inhibitors of carbohydrate digesting enzymes are being promoted as anti-hyperglycemic and thus as anti-diabetic drugs. Chemical drugs are efficient in controlling hyperglycemia but on the contrary they have many side effects and its higher costs and less availability make it less accessible to the common people in developing countries.

Plants have been recognized for their medicinal value since ancient times. Medicinal plants form the indispensible and most commonly used treatment in many human ailments. Wild plants serve as sources for about eighty percent of all medicinal drugs. In fact, 25 percent of all prescriptions written annually contain the chemicals originated in plants. Diabetes was called by name "Madhumeha" in ancient times and was treated using plants; many such plants have been cited in Ayurvedic literature. As these are used since long time with marginal side effects but efficient curing ability, medicinal plants form alternative but efficient medicine to treat Diabetes.

Spices, in addition to enhancing flavor in food preparations, have many medicinal values like anti-hyperglycemic, hypoglycemic, anti-allergic, anti-arthritic etc. Spices were used in ancient treatments to treat diabetes. Many studies have also shown the anti-diabetic potential of spices in the animal models (Ibegbulem *et al.,* 2012; Arun *et al.,* 2002; Yadav *et al.,* 2002), but the mechanism through which plants combat the disease lies largely debated. The medicinal history of two spices *Cinnamomum zeylanicum* and *Cumin cyminum* suggests their efficacy in controlling diabetes but the mechanism of their action is not reported (Ping *et al*., 2010; Willatgamuwa *et al.,* 1998; Kim *et al.,* 2006). Cinnamon (*Cinnamomum zeylanicum*) has been used since long, in the management of hyperglycemia, however, there is lack of enough data to understand the mechanism of beneficial effect (Dugoua *et al.,* 2007).

In our laboratory many medicinal plants, reported for their anti-diabetic potential in animal models or those used in folklore medicine, are being investigated for their proposed anti-diabetic mechanism using simple *in vitro* and *ex vivo* assays which mimic the physiological environment (Ahmed *et al.,* 2009; 2010; 2011; Asna *et al.,* 1998; Harish *et al.,* 2011; Sudha *et al.,* 2012) The

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present study uses simple *in vitro* assays to understand the mechanism of anti-diabetic activity of *Cinnamomum zeylanicum* and *Cumin cyminum*.

MATERIALS AND METHODS

p-Nitrophenyl-α-D-glucopyranoside was obtained from Sisco Research Laboratory, India. α-amylase (2000 IU/mg solid) was purchased from Sigma Aldrich, India. A glucose oxidase/peroxidase assay kit (GOD-POD) was purchased from Aggappe Diagnostics, India. All the purchased chemicals and reagents used in the study were of analytical grade.

Processing of the sample

Cinnamomum zeylanicum (CZ) and *Cumin cyminum* (CC) were purchased from local market in Mysore. The samples used are commonly used spices, however it was confirmed by the faculty of Postgraduate studies in Botany, University of Mysore. The samples were powdered, passed through 60 mesh and stored in airtight container at ambient temperature till further use.

Preparation of water extract

50 g powder of both the spices were transferred into two conical flasks containing 100mL of deionized water and placed on a mechanical shaker for 6 h. One set from both spices was extracted at room temperature and other at 60°C. The dark brown colored solution of cinnamon and the greenish yellow colored solution of the cumin were centrifuged to allow the water insoluble components to settle. The supernatant of both the solutions were filtered through Whatman filter paper, the solution collected were freeze dried (Thermo scientific, freeze drier). Thus obtained freeze dried extracts were named as CCR for CC room temperature extract CCH for CC hot water extract, CZR for CZ room temperature extract, CZH for CZ hot water extract and were transferred into air tight containers and stored at 5°C until further use.

Extraction of crude enzymes from rat small intestine

Male rats of Wistar strain (140-160 g) were obtained from central animal house, Department of Zoology, University of Mysore. The rats were sacrificed by cervical dislocation, the intestine was immediately excised and washed with Maleate buffer of pH 6 (0.1 M). The brush border was carefully scraped and homogenized in maleate buffer (5:1 w/v) in cold condition. The homogenate was centrifuged at 1000×g for 15 min at 4°C. The supernatant was collected and stored at -50°C for further use as crude enzyme source of α-glucosidase and sucrose (Dahlqvist *et al*., 1962).

Glucose adsorption capacity

Glucose adsorption capacity of CZ and CC were determined according to the method defined earlier (Ou *et al*., 2001). Samples Powder (1%) were added to 25mL of four different concentrations (5, 10, 15, 20 mM) glucose solution, the mixture was stirred well and incubated in a shaker water bath for 6h, centrifuged at 4000×g for 20 min and the glucose content in the supernatant was determined using the GOD /POD enzymatic kit and the glucose bound was calculated using the following formula.

$$\text{Glucose adsorbed} = \frac{\text{G1-G6}}{\text{Weight of the sample}} \times \text{Volume of solution (mL)}$$

Where, GI: glucose concentration of original solution, G6: glucose concentration after 6h.

Amylolysis kinetics

Potato starch solution (1%) was prepared in phosphate buffer (0.05 M, pH 6.5). 25mL of starch solution was added with α-amylase (100 mg) and test samples (CZ & CC) at 3 concentrations (0.5, 1 and 2%) in dialysis bags of 12 KD cutoff, and dialyzed against 200mL of deionized water at 37°C in a shaker water bath. The glucose diffused into the dialysate was determined at 60, 120 and 180 min using GOD /POD diagnostic kit. The values are indices of glucose diffusion retardation index (GDRI). A control, without samples, was also run in the same conditions.

$$\text{GDRI}=100 \quad \frac{\text{Glucose content with addition of sample}}{\text{Weight of the sample}} \times 100$$

α-amylase inhibitory activity

α-Amylase inhibitory activity of untreated CZ and CC powder and Hot and cold-water extracts of both spices was studied using the previously described method (Ou *et al*., 2001). The concentrations used were 0.5%, 1% and 2% for powder and 0.5, 1 and 2 mg for hot and cold water extracts. To the 25mL of the 1% starch solution (prepared in phosphate buffer (0.05 M, 6.5 pH) α-amylase enzyme (100 mg) and samples were added in 50mL centrifuge tubes, stirred vigorously and incubated at 37°C for 60 min. The enzymic reaction was terminated by adding 2mL of 0.1 M NaOH and heated on a boiling water bath for 15 min. The contents were centrifuged (3000×g, 15 min) and the glucose content in the supernatant was determined by Standard Glucose oxidase peroxidase method.

$$\%\ \text{Inhibition} \quad \frac{\text{Abs. of Control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

α-glucosidase inhibitory activity

α-glucosidase inhibitory activity of CZ and CC samples were assayed according to the previously described method (Honda and Hara 1998). Crude enzyme solution (10 µL), sample (10 µL), and Maleate buffer (0.05 M, pH 6.5) were mixed in a test tube and incubated at 37°C for 10 min. The enzyme reaction was started by adding 200µL of p-nitrophenyl-a-D-glucopyranoside solution (2 mM) and incubated at37°C. The reaction was terminated

by heating the mixture with boiling water for 5 min after 30min. Then, 1mL of 0.1M disodium hydrogen phosphate was added, absorbance of the liberated p-nitrophenolwas read at 400 nm. An untreated enzyme solution was used as the control. The activity, as percent inhibition of α-glucosidase was calculated using the formula below.

$$\% \text{ Inhibition} \quad \frac{\text{Abs. of Control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

Sucrase inhibition activity

Sucrase inhibition activityof the sampleswas examined by previously described method (Honda and Hara). The enzyme solution (10µL), sample (10µL) and 180µL of Maleate buffer (pH 6.0) were mixed in a test tube and incubated for 10 min at 37°C. The enzyme reaction was started by adding 100µL of sucrose solution (60 mM) and incubated at 37°C for 30min. The reaction was terminated after 30 min by heating on a boiling water bath for 5 min. Glucose released is measured by GOD-POD glucose estimation kit. The absorbance of the solution was read at 540nm. An untreated enzyme solution was used as control. The percent inhibition of sucrase was calculated using the following formula.

$$\text{GDRI}=100 \quad \frac{\text{Glucose content with addition of sample}}{\text{Glucose content of the control}} \times 100$$

STATISTICAL ANALYSIS

All the values are the mean of triplicates and the data were subjected to one-way analysis of variation followed by Turkey's multiple comparisons test for difference using SPSS 11.5 software. The values were considered significant at significance of 0.05.

RESULTS

Glucose adsorption capacity

The glucose adsorption capacity of samples expressed as mmol/dL is shown in table.1. Between the two samples CZ exhibited higher glucose adsorption. CZ showed significant increase in glucose adsorption with increases in both the sample and glucose concentration upto of the sample and glucose. There was no increase in glucose adsorption with further increase in the sample concentration beyond 3%. CZ has shown good glucose adsorbing capacity, which was proportional to both the glucose and sample concentration. CC did not show similar results.

Amylolysis kinetics

The results of glucose diffusion are shown in table 2. Compared to the positive control, CZ significantly retarded the glucose diffusion as indicated by the GDRI values. CZ shows significant increase in glucose retardation with increase in concentration and retarding capacity sustained upto 180 min as shown by GDRI values. CC did not retard glucose diffusion at all concentration and time intervals.

Inhibition of α-amylase activity

α-Amylase inhibitory activity of powder and water extract of CZ and CC is shown in table 3. CZ powder (1 %) showed 14 % inhibitory activity and there was no effect of the increase in sample concentration on enzyme activity. The aqueous extract showed a maximum inhibition of 15.1% at 1.5mg/mL concentration but there was no increase in inhibitory activity with further increase in sample concentration. Both the hot and room temperature extracts showed similar degree of activity. Whereas CC has no inhibitory effect on alpha amylase in powder as well as extract form. Nature of extraction of the extract (extraction at hot and ambient temperature) has no effect on the activity of the enzyme.

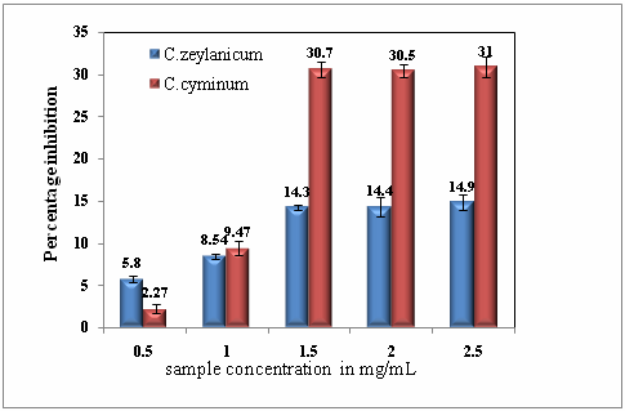

Fig. 1: Effect of aqueous extracts of samples on α-glucosidase activity

Expressed values are expressed in percent to the activity of the control and all the values are expressed in the standard deviation of the mean of values (n=3)

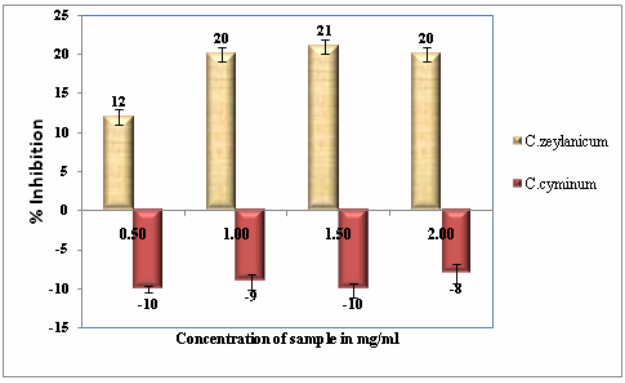

Fig. 2: Effect of aqueous extracts of samples on Sucrase

Negative values indicate the activity of the sucrose enzyme increased with addition of the samples, when compared to control.

Inhibition of α-glucosidase activity

Effect of water extract of CZ and CC on α-glucosidase enzyme activity is shown in fig. 1. Both the samples

Table 1: Glucose adsorption capacity of samples at varying concentrations of glucose

Sample concentration (%)		Glucose adsorbed (mM)		
		5 mM	10 mM	15 mM
CZ	1	1.3(±0.8)	4(±0.6)	6(±1.4)
	2	1.9(±0.4)	4.8(±0.6)	7.1(±1)
	3	1.8(±0.5)	4.9(±0.9)	6.9(±0.8)
	4	1.9(±0.5)	4.7(±0.7)	7.0(±0.5)
CC	1	0.1(±0.5)	-1(±0.3)	-1(±0.3)
	2	-1(±0.4)	-2(±0.4)	-2(±0.2)
	3	-2(±0.5)	-1.5(±0.4)	-1(±0.3)
	4	-1.7(±0.9)	-3(±0.8)	-1.8(±0.9)

CZ= Cinnamon powder; CC= Cumin powder. All the values for CZ in columns and rows differ significantly with ≤0.05 significance. Values in the parenthesis are the standard deviation from the mean values.

Table 2: Effect of samples on amylolysis kinetics

Sample concentration (%)		Glucose content in the dialysate (mM)		
Control		60min	120min	180min
		0.67	0.86	1.03
CZ	0.5	0.50 (25)	0.65 (24)	0.79(23)
	1.0	0.41 (39)	0.53 (38.4)	0.65(37)
	2.0	0.36 (46)	0.47 (45)	0.55(47)
CC	0.5	0.68 (-2)	0.85 (1)	1.20 (-17)
	1.0	0.71 (-6)	0.88(-2)	1.22 (-18)
	2.0	0.73 (-9)	0.88 (-2)	1.25 (-21)

CZ= cinnamon powder; CC= cumin powder; Values are the amount of glucose diffused in mmol and the values in parenthesis denote Glucose dialysis retardation index (GDRI). All the values for CZ in columns and rows differ significantly with ≤0.05 significance. Values are the mean of the triplicates.

Table 3: Effect of samples on α- amylase activity

In aqueous extracts (mg/mL)					
Samples		0.5	1	1.5	2
CZ	CZH	12.2(±2)	14.3(±1.8)	15.1(±1.3)	15(±2)
	CZR	11.2(±1.4)	14.1(±1.2)	14.8(±1.7)	14.9(±0.9)
CC	CCH	-1.3(±0.4)	-8.2(±0.8)	-8.2(±0.9)	-7.4(±0.9)
	CCR	-0.4(±0.2)	-2.9(±0.5)	-3(±1.3)	-6.4(±1.3)
In dehydrated samples (%)					
	1	2	3	4	
CZ	14(±1.2)	12(±2)	13(±1.2)	12.5(±0.9)	
CC	- 27(±2)	-27(±1.5)	-29(±1.6)	-30.7(±2)	

CZ= Cinnamon dehydrated powder; CC= cumin dehydrated powder; CZH = cinnamon hot water extract; CZR= Cinnamon room temperature extract; CCH = cumin hot water extract; CCR = Cumin room temperature extract. Values are % inhibition to control and the values in parenthesis are the standard deviation for the mean values (n=3)

inhibited the enzyme in dose dependent manner, but the inhibitory effect increased with concentration, which was more prominent in case of CC. The inhibitory activity ranged between 5.8-14.3% for CZ and between 2.27-30.7% for CC sample, respectively. Maximum activity was observed at 1.5mg/mL concentration; however, there was no significant increase in enzyme inhibition was observed with increase in sample concentration. There was no difference in activity between the hot water and room temperature extracts of both the samples.

Inhibition of sucrase activity

The sucrase inhibitory activity values of both the spices are shown in fig. 2. CZ shows a maximum of 21% inhibition at 1.5mg/mL and there was no increase in inhibitory activity with further increase in sample concentration. CC has no inhibitory effect on sucrase. There was no difference in activity between the hot water and room temperature extracts of both the samples was observed.

DISCUSSION

Although, results of *in vitro* screening cannot be extrapolated to *in vivo* hypoglycemic potential, the *in vivo* simulating model systems used in this study indicate possible mechanisms by which a medicinal plant/ sample may act to regulate postprandial glucose levels.

Glucose adsorption is a physical phenomenon where glucose molecules get adsorbed onto the surface of sample powder. This mechanism may retard glucose uptake by intestinal epithelial cells. The results indicates *in vivo* glucose binding capacity of sample CZ, which can help in slow release of glucose into blood stream and thus may help in controlling sudden rise in postprandial glucose. The glucose binding capacity of the CZ may be attributed to its fiber content as soluble and insoluble fiber can contribute to glucose adsorption (Adiotomre *et al.*, 1990), where as CC has no glucose adsorptive property and the negative values in the results may indicate the release of glucose by the CC powder itself. Water extract and the dry powder of CZ shows prominent inhibitory activity on amylase enzyme which is dose dependent. The extraction was done in hot water and warm water to see the effect of temperature on the stability of the components showing inhibitory potency, the results indicated that inhibitory components are stable in hot water. CC showed no inhibitory capacity on the amylase enzyme in both powder and water extract form. The retardation of glucose diffusion (GDRI) by CZ may be due to its free glucose binding capacity and partly by amylase digestion inhibitory potency but the CC showed negative GDRI values due to its least glucose adsorbing and amylase inhibitory capacity. Sucrase and α-glucosidase are the enzymes involved in digestion of sucrose and glycosidic bonds of oligosaccharides, respectively. CC showed negative values, which indicate the sucrose digestion is more than the normal control, because it may enhance the activity of the sucrase enzyme. CZ inhibited both the Sucrase and α-glucosidase enzymes partly, which can help in release of minimum required amount of glucose. CZ has been shown to reduce postprandial glucose level in type-2 diabetes patients (Mang *et al*., 2006). The safety and efficacy of the CZ has also been evaluated, which shows the CZ as safe anti-diabetic additive, but the mechanism mediated to exert its anti-diabetic property has not been reported to our knowledge. Hence, this study adds up to the data on anti-diabetic potency of the CZ; the results and properties shown by CZ in the assays used in the present study indicate the hypoglycemic effect of CZ, at least to some extent, may be mediated through the retardation in digestion of carbohydrate polymers into glucose monomers and also by slow release of glucose into blood stream and thus contributing towards hypoglycemic property as shown by previous studies (Brijendra *et al.*, 2010).

CONCLUSION

These results suggest that cinnamon might exert its anti-diabetic effect by suppressing carbohydrate absorption from intestine, and thereby reducing the post-prandial increase of blood glucose, whereas the cumin has less prominent anti-hyperglycemic potency at the intestinal level.

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