Original article

In vitro evaluation of selected Indian spices for α-amylase and α-glucosidase inhibitory activities and their spice-drug interactions

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Abstract

India is believed to be crowned as the diabetes capital of the world by 2030. Considering the same, the present study was undertaken to elucidate the antidiabetic potential of selected Indian spices, *viz., Cinnamomum zeylanicum* Blume, *Cuminum cyminum* L., *Laurus nobilis* L., *Piper nigrum* L. and *Elettaria cardamomum* (L.) Maton. Phytochemical constituents of the spices were qualitatively identified and enzyme inhibitory assays performed. The study used 50% hydroacetone extracts of the above-mentioned five spices to evaluate *in vitro* α-amylase and α-glucosidase inhibitory activities. The IC_{50} for α-amylase inhibitory activity were 706.04 ± 0.07, 517.51 ± 0.09, 349.41 ± 0.12, 573.21 ± 0.08 and 376.18 ± 0.12 g/ml, while those for α-glucosidase inhibitory activity were 243.92 ± 0.13, 386.73 ± 0.11, 203.01 ± 0.15, 269.92 ± 0.14 and 182.85 ± 0.16 g/ml for *C. zeylanicum, C. cyminum, L. nobilis, P. nigrum* and *E. cardamomum* respectively. Statistical analysis suggests that the highest α-amylase inhibitory activity was exhibited by *L. nobilis* and *E. cardamomum* while the highest α-glucosidase inhibitory activity was observed for *E. cardamomum*. Diabetes is a disorder associated with many complications and diabetics generally consume spices as nutraceuticals along with their drug regime. Thus, the spice-drug interaction with two commonly used drugs, *viz.*, Acarbose-an antidiabetic and Losartan-an antihypertensive were also studied. The results suggest that spice-drug interactions significantly enhanced the inhibitory activities of α-amylase and α-glucosidase. The maximum increase in percent α-amylase inhibitory activity with Acarbose supplementation was observed in *C. zeylanicum* (74.52%) with minimum for *E. cardamomum* (50.53%) while in presence of Losartan, maximum and minimum enhancement were observed for *C. cyminum* (48.34%) and *C. zeylanicum* (28.19%), respectively. As for modulation of percent α-glucosidase inhibitory activity with Acarbose, *C. cyminum* (52.42%) showed the highest enrichment while no change was observed in the case of *P. nigrum*, while with Losartan maximal enhancement was with *C. zeylanicum* (19.85%) and minimum with *C. cyminum* (5.4%). Such interactions are a double-edged sword having an advantage but can also result in fatal consequences. Thus, for developing functional food having antidiabetic properties, the study of such interactions becomes significant.

Key words: Spice-drug interactions, α-amylase inhibitory activity, α-glucosidase inhibitory activity, Indian spices, antidiabetic

1. Introduction

Currently, the chronic metabolic disease, diabetes, associated with hyperglycemia, caused due to insufficient or inefficient insulin secretion, is a concern worldwide. Due to elevated blood glucose levels, the risks of micro/macro vascular damage are high and the disorder, further, is associated with reduced life expectancy and diminished quality of life (American Diabetes Association, 2010). In India, the disease is turning to be an epidemic with more than 68 million diabetic individuals, diagnosed with the disease (Wild *et al.,*

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2004). As per a 2017 ICMR report, the prevalence of diabetes, in India, is increasing at an alarming rate, especially in people of lower economic strata from urban areas of economically developed states. This ramification of the disease in economically disadvantaged sections of Indian society is disturbing, thus requiring concern. As per published data, the number of diabetics in India is expected to increase from 31.7 million in the year 2000 to 79.4 million in 2030 (ICMR, 2017; Whiting *et al.,* 2011). Prevention and control of diabetes can be easily achieved by incorporating more physical activity, less calorie intake and avoiding sedentary habits, but it is still a major challenge, as individuals find changing lifestyle difficult and look for a less cumbersome alternative. A simpler alternative is consumption of an antidiabetic medication, readily available, now at subsidized rates in the country. Studies in the past have shown that antidiabetic drugs as sulphonylureas, biguanides, dipeptidyl peptidase IV (DPP-IV) inhibitors, sodium-glucose co-transporter (SGLT2) inhibitors, Insulin therapy, and GLP-1 agonists, have multiple adverse reactions (Mohanty *et al.,* 2018; Chaudhury *et al.,* 2017).

The regular consumption year after year along with undesirable side effects to various organs is, thus a big apprehension for use of these antidiabetics. Carbohydrate hydrolyzing enzymes, viz., α-amylase and α-glucosidase convert complex carbohydrates, like starch to simpler ones, facilitating the release of glucose into the blood. The inhibition of α-amylase delays the digestion of starch while that of α-glucosidase impairs the digestion of disaccharides (Oboh et al., 2015), thus being an effective tool for the management of diabetes by regulating and controlling the rise of postprandial glucose level. α-glucosidase inhibitors function by being competitive inhibitors of intestinal α-glucosidase enzyme which is present on the microvilli (Lebovitz, 1998). Natural sources of α-amylase and α-glucosidase inhibitors are of great importance in folk medicine for the treatment and management of diabetes (Balaji et al., 2015; Sindhu et al., 2013).

Plant-based medicinal products have been known to mankind since ancient times and traditional herbal medicines are believed to be safe and effective with little or no side effects, providing an alternative way to manage diabetes (Subbulakshmi and Naik, 2001). Cuisines across the globe are enriched with aromas and flavors contributed by various herbs and spices. Indian cooking styles make use of many spices like Cinnamon (C. verum, C. zeylanicum, C. aromaticum and C. burmannii), Cumin (C. cyminum), Clove (S. aromaticum), Pepper (P. nigrum) and Bay leaves (L. nobilis) and ancient Indian literature supports the use of some of these, as antidiabetic (Patil et al., 2013; Upadhyay, 2016).

E. cardamomum, or green elaichi, has been used not only in cuisines but also as an integral component of betel leaves. In vivo as well as in vitro studies demonstrate the effectiveness of the spice in the treatment of diarrhea, constipation, colic distress and hypertension (Sharma et al., 2011) and as an analgesic, antispasmodic, anti-inflammatory, antioxidant, antimicrobial, anticonvulsant and sedative (Suneetha and Krishnakantha, 2005). The methanolic and aqueous extracts of the spice in vitro are reported to possess α-glucosidase and α-amylase inhibition at 1 mg/ml, thus proving to be useful as an antidiabetic (Ahmed et al., 2017). Further, a 10-week long randomized placebo-controlled clinical trial by Aghasi et al. (2018, 2019) to analyze blood glucose levels in type 2 diabetic patients reported a decrease in HbA1c and insulin level in type 2 diabetes mellitus patients. An ethanolic cardamom leaves extract has been found to have the potential to be used as a functional food component for therapy in the diabetic patient (Winarsi et al., 2014).

Cinnamon has been used for its medicinal properties across the world for as long as 5000 years (Singh et al., 2018). A study by Khan et al. (2003) analyzed the effect of cinnamon on levels of glucose and lipids in type 2 diabetics and recommended the supplementation of 1-6 g of cinnamon to the diet, thereby facilitating a significant decrease in serum glucose levels. The hypoglycemic activity of cinnamon may be attributed to: (a) stimulation of the release of insulin as well as insulin receptor signaling, (b) inhibition of various enzymes involved in metabolism and, thus release of simple carbohydrates in blood (c) alteration of cellular glucose uptake by probably increased glucose transporter-4 receptor synthesis (Ali et al., 2018). Kamble and Rambhimaiah (2013) studied the antidiabetic effect of aqueous extract of C. cassia alone and in combination with glibenclamide in alloxan-induced diabetic rats. The study reported that the combination when given for 15 days

caused a more significant reduction in blood glucose level than either drug is given alone.

Cumin (C. cyminum), family Apiaceae, is used regularly in every Indian household. It has been prescribed for its potential as a stomachic, diuretic, carminative, emmenagogic, antispasmodic, antimicrobial as well as a fungicide (Joshi, 2000). Srivastava et al. (2013), studied the in vivo effects of the ethanolic extract of cumin seeds and found it to possess α-glucosidase inhibitory activity with IC_{50} of 100 μg/ml as compared to a known α-glucosidase inhibitor drug Acarbose that has an IC_{50} close to 25 μg/ml. Administration of C. cyminum supplement to type 2 diabetics leads to a fall in the serum levels of insulin, FBS and glycosylated hemoglobin along with a decrease in the inflammatory indices of TNF-α and hsCRP accompanied by an increase in the adiponectin serum levels, thus also facilitating better control of the complications that arise due to the same (Jafari et al., 2017). The antidiabetic effect of cumin seeds can be attributed to the enhancement of insulin secretion and the amelioration of diabetes-induced oxidative stress by exhibiting free radical scavenging activity in streptozocin induced diabetic rats (Joshi, 2000).

Traditionally, black pepper is widely used for treating diarrhea, dyspepsia, cholera, and gastric ailments. The spice is rich in various active phytochemicals such as flavones, steroids, terpenes, and alkaloids of which, piperine is the major alkaloid (Khaliq et al., 2015). According to the study of Kavitha et al. (2018), a 30% ethanolic extract exhibited 67.93% α-amylase inhibitory activity at 1000 μg/ml. In vivo studies have suggested that the antidiabetic effect of black pepper is attributed to either inhibition of the increase in insulin output or that of glucose intestinal absorption or by enhancing glucose metabolism or combination of all (Bandigari, 2018).

Bay leaf, L. nobilis, member of Lauraceae family, is a very popular culinary spice. The use of this spice as a herbal medicine with pharmacological activities as antibacterial, antifungal, antidiabetic and anti-inflammatory (Fang et al., 2005). Khan et al. (2009) demonstrated that daily consumption for 30 days (1-3 g/day), decreases the risk for diabetes and is beneficial for type 2 diabetes. Abdulrahim Aljamal (2011) showed that 4 weeks of bay leaves supplementation improves plasma glucose levels in type 2 diabetics. The extract also exhibits potential anti-α-amylase activity in combination with soursop leaves (Berawi et al., 2017).

The present study uses hydroacetone extracts of the above-mentioned five spices to evaluate in vitro α-amylase and α-glucosidase inhibitory activities. The consumption of these nutraceuticals for their antidiabetic potential is generally not monitored by physicians and further, the regular drug regime of the individual is not compromised. The complexity of the human organization could lead to interactions between the spice and the drug, being hazardous via adverse drug events, or, advantageous through enhancement of drug pharmacodynamics (Gupta et al., 2017a; Kamath and Adiga, 2014). This field has not been largely explored, especially with spices consumed in our country.

The patients under medication prescribed by physicians, along with the use of herbal remedies may enhance the possibility of the occurrence of polypharmacosis. The interactions could be spice-drug or food-drug in nature. The food-drug interactions can affect

the bioavailability of a drug, while the mechanism of spice-drug interaction remains to be elucidated.

In the current study, Acarbose, an antidiabetic drug that functions as an α-glucosidase inhibitor, is used for studying spice-drug interactions. Furthermore, diabetes and hypertension frequently co-exist and so interaction with an antihypertensive drug as Losartan, an ACE inhibitor, is evaluated as well. To maximize the efficacy of herbal remedies, prior knowledge about additive, synergistic, antagonist, or unidentified effect is desirable. This information can be of great significance to develop functional food having antidiabetic properties.

2. Materials and Methods

2.1 Preparation of spice extract

The spices were procured locally and authenticated by Dr. Bindu Gopalakrishnan, Department of Botany, Mithibai College (Autonomous), Mumbai. The spices selected were *Cinnamomum zeylanicum* Blume (stem bark), *Cuminum cyminum* L. (seeds), *Laurus nobilis* L. (leaves), *Piper nigrum* L. (seeds) and *Elettaria*

cardamomum (L.) Maton (seeds inside the pods) with Herbarium numbers: MIT0130, MIT0113, MIT0135, MIT0109, MIT0076, respectively. The spices were oven-dried to remove any moisture at 45°C and thereafter, finely powdered and sieved (sieve pore size=0.3 mm x 0.5 mm). 0.2 gm of this powder was extracted in 5 ml 50% hydroacetone on a rotary shaker for 24 h. The extract was filtered and the filtrate dried over water bath at 45°C. The dried extract was weighed and the percent yield was calculated using the formula:

Weight of dried spice extract/weight of spice powder * 100

Lastly, the extract was reconstituted in a minimum amount of dimethylsulphoxide (DMSO) and dilutions of 10 µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml and 160 µg/ml were prepared for the study.

2.2 Phytochemical and biomolecule analysis

The hydroacetone extracts of the spices were tested for their phytochemical and biomolecule constituents qualitatively using modification of the methods by Soni and Sosa (2013), Modi *et al.* (2018) and Dahanayake *et al.* (2019).

Phytochemical / biomolecule test	Procedure
Molisch test	Spice solution was mixed with 10% methanolic α-naphthol solution and then 4 to 5 drops of concentrated H_2SO_4 was added along the side of the test tube. A violet ring indicates the presence of glycoside or sugar.
Barfoed's test	To spice extract was added Barfoed's reagent and it was heated in a boiling water bath for 2 minutes. The formation of a red precipitate indicates the presence of monosaccharides.
Fehling's test	*Free sugars*: To spice extract was added Fehling's reagent, boiled in the water bath for 5 minutes. The formation of red-colored precipitate indicates a positive test. *For Combined sugars*: 5 ml of dilute HCl was added to the spice extract and heated in a boiling water bath for 5 minutes. Then it was neutralized using NaOH. Fehling's reagent was added to this test tube. Reddish colored precipitate indicates the test is positive.
Soluble starch	The spice extract was boiled with 1 ml 5% KOH. After cooling, sulphuric acid is added to it. A yellow coloration indicates the presence of starch.
Proteins	The spice extract was mixed with biuret reagent, and purple coloration indicates a positive test.
Alkaloids	Spice extract was diluted in acidic solution like 1-5% HCl and the solution thus obtained used for the detection of alkaloids using various reagents.
Mayer's test	Spice extract was mixed with Mayer's reagent. The formation of white or buff precipitates confirms the presence of alkaloids in the sample.
Hager's test	Spice extract was mixed with Hager's reagent. A yellow precipitate confirms the presence of alkaloids in the sample.
Flavonoids	Spice extract was heated on a water bath and the filtrate was treated with 10% ferric chloride ($FeCl_3$) and the presence of bluish-green color indicates flavonoids.
Steroid and sterol	Conc. H_2SO_4 was added into spice solution along the side of the test tube, wherein formation of two phases and development of red color indicates the presence of sterol.
Phenols	Spice extract was mixed with 1 ml of 5% $FeCl_3$ in 90% methanol. It was observed for blue, blue-black, or blue-green color which indicates the presence of polyphenols.
Saponin	Powdered spice was dissolved in water and shaken well. The formation of foam, stable for 15 min or more indicates the presence of saponin.
Tannins	Spice extract was dissolved in water and heated on a boiling water bath for 1 h. The filtrate was treated with $FeCl_3$ and observed for the formation of a dark green color.
Terpenoids	To spice extract was added a few ml of chloroform and concentrated sulphuric acid from the sides of the test tube. The formation of a reddish-brown colored layer indicates the test is positive.

2.3 Enzyme inhibitory assays

2.3.1 α-amylase inhibitory assay

The assay was conducted as per the modification of Narkhede *et al.* (2011) method. 0.5 ml of spice extract (various dilutions) and 0.5 ml of fungal α-amylase enzyme were mixed in test tubes and incubated at 25°C for 30 minutes. One ml of 0.5% starch solution was added and further incubated at 25°C for 30 min. The reaction was terminated by the addition of 0.5 ml of 2N NaOH and finally 0.5 ml 3,5 dinitrosalicylic acid (DNSA) was added and the tubes were placed in a boiling water bath for 5 min. On cooling, the contents were diluted with 6 ml distilled water and extinction measured at 540 nm. To the control and blank tubes of each concentration, plant extract and enzyme were omitted, respectively.

2.3.2 Calculations for α-amylase inhibitory activity

100 x (Ext_{540} 'Control − Ext_{540}' Test) / Ext_{540} 'Control.

The extinction of blank was subtracted from that of the test to nullify the effect of naturally occurring reducing sugars in the spice extract.

2.3.3 α-glucosidase inhibitory assay

The assay was performed by modification of the method of Geng *et al.* (2007). For the same, 3 test tubes, *viz.*, blank, control and test were prepared. To the tubes were added 2% yeast (200 μl), 0.02 M phosphate buffer (pH-6.8) (100 μl), spice extract (50 μl of different concentrations) and distilled water (50 μl in control and 100 μl in blank). The tubes were incubated for 10 min. After incubation, 200 μl of maltose was added, and the tubes were again incubated at 37°C for 30 min. The tubes were kept in a boiling water bath for 5 min and then cooled in an ice bath. To the individual concentration, control and blank tubes, plant extract and enzyme, respectively were omitted. For glucose estimation, GOD/POD method was used and the extinction read at 540 nm.

2.3.4 Calculations for α-glucosidase inhibitory activity

Amount of glucose in control: Ext_{540} 'Control / Ext_{540} 'Standard x 100 = A μg/ml.

Amount of glucose present in the test: Ext_{540} 'Test/ Ext_{540} 'Standard x 100 = B μg/ml.

(Ext_{540} 'Test = Ext_{540} 'Test − Ext_{540} 'Blank)

The extinction of blank was subtracted from that of the test to nullify the effect of naturally occurring reducing sugars in the spice extract.

α-glucosidase inhibitory activity was calculated as: A-B/A x 100

where A = Amount of glucose in control and B = Amount of glucose in the test

For estimating the α-amylase and α-glucosidase inhibitory activities of drugs, the same procedure was used, with the spice extract being replaced by the drug, while to study the spice-drug interaction, both spice and drug were simultaneously used.

2.4 Statistical analysis

The IC_{50} were calculated using the IC_{50} software. The data were expressed as mean ± standard error (SE) for n = 5. Statistical analysis of the results was performed using the Student's t-test. $p<0.05$ was considered to be statistically significant. Computation of two way ANOVA was performed to determine the spice amongst the ones studied, possessing a better enzyme inhibitory activity.

3. Results

3.1 Percent yield

The percent yield was calculated by considering the weight of dried extract obtained post 24 h extraction, filtration and drying with respect to the spice powder. The yields of extracts obtained are depicted in Table 1.

3.2 Phytochemical and biomolecule analysis

The current study was conducted with a 50% hydroacetone extract of the spices. The results of the phytochemical and biomolecule analysis are depicted in Table 2.

Table 1: Yield of plant extracts post 24 h extraction process

Spice	% Yield
C. zeylanicum	4.89 ± 0.05
C. cyminum	5.21 ± 0.10
L. nobilis	4.93 ± 0.02
P. nigrum	5.10 ± 0.02
E. cardamomum	4.51 ± 0.06

The values represented are mean ± SE, where n = 5.

3.3 Enzyme inhibitory activities

Ancient Indians, since time immemorial, have been using spices and other herbs and plants for therapy of various disorders and dysfunction of the human physiology. Various herbal extracts have been cited as potent antidiabetic. The current study evaluated five common Indian spices for their disruption of carbohydrate metabolism *via* α-amylase and α-glucosidase inhibitory activity.

α-amylase inhibitory activity

A dose-dependent increase in the inhibitory activity of hydroacetone extracts towards the carbohydrate metabolizing enzyme, α-amylase, was observed, between the concentration range 10-160 g/ml for spices (Table 3, Figure 1). Between the lowest and the highest concentrations of spice studied (10 g/ml and 160 g/ml), the percent increase was 54.98%, 80.71%, 82.94%, 89.72% and 78.47% for *C. zeylanicum*, *C. cyminum*, *L. nobilis*, *P. nigrum*, and *E. cardamomum*, respectively. The IC_{50} for the spices were 706.04, 517.51, 349.41, 573.21 and 376.18 μg/ml, respectively and are presented in Table 5. Statistical analysis suggests that amongst the spices studied, better inhibitory activities were observed for *L. nobilis* and *E. cardamomum*.

α-glucosidase inhibitory activity

For hyrdroacetone extract, *C. zeylanicum*, *C. cyminum*, *L. nobilis*, *P. nigrum*, and *E. cardamomum*, yeast α-glucosidase inhibitory activity, was investigated and the results are shown in Table 4, Figure 2. The characteristic increase in blood glucose levels in diabetes can be regulated by the inhibition of the above-mentioned enzyme, thus facilitating sudden fluctuations in plasma glucose levels. A dose-dependent increase was observed and the percent increase between the extreme concentrations studied was 56.15%, 74.63%, 58.09%, 62.14% and 59.39% for *C. zeylanicum*, *C. cyminum*, *L. nobilis*, *P. nigrum*, and *E. cardamomum*, respectively. The IC_{50} for the spices were 243.92, 386.73, 203.01, 269.92 and 182.85 μg/ml, respectively and are presented in Table 5. Statistical analysis suggests that amongst the spices studied, better inhibitory activity was observed for *E. cardamomum*.

Table 2: Phytochemical and biomolecule analysis of 50% hydroacetone extract of spices

Phytochemicals and biomolecules	*C. zeylanicum*	*C. cyminum*	*L. nobilis*	*P. nigrum*	*E. cardamomum*
A) Carbohydrates	+	+	+	+	+
i) Reducing sugars	+	-	-	-	-
ii) Combined sugars	-	+	-	-	-
iii) Soluble starch	-	+	+	-	-
B) Protein	-	–	-	-	-
C) Alkaloids	-	+	-	+	+
i) Mayer's test	-	+	-	+	+
ii) Hager's test	-	+	-	+	+
D) Flavonoids	+	+	+	+	+
E) Steroid & Sterols	+	-	+	-	-
F) Phenols	+	+	-	-	+
G) Saponins	-	-	-	-	-
H) Tanins	+	+	+	+	+
I) Terpenoids	+	+	-	+	-

Legend: + represents presence of the phytochemical
       - represents absence of the phytochemical.

Table 3: Percent α-amylase inhibitory activity of spices

	Percent α-amylase inhibitory activity				
Concentration (μg/ml)	*C. zeylanicum*	*C. cyminum*	*L. nobilis*	*P. nigrum*	*E. cardamomum*
10	1.63 ± 0.01%	3.27 ± 0.04%	4.19 ± 0.35%	1.58 ± 0.13%	5.26 ± 0.34%
20	2.32 ± 0.04%	4.83 ± 0.13%	11.72 ± 0.69%	4.01 ± 0.30%	9.68 ± 0.28%
40	3.89 ± 0.25%	8.08 ± 0.06%	15.59 ± 0.58%	8.05 ± 0.11%	12.14 ± 0.36%
80	6.73 ± 0.16%	12.49 ± 0.13%	23.04 ± 0.11%	11.56 ± 0.58%	16.55 ± 0.35%
160	12.03 ± 0.09%	16.95 ± 0.12%	24.57 ± 0.19%	14.72 ± 0.72%	24.43 ± 0.08%

The values represented are mean ± SE, where n = 5.

Table 4: Percent α-glucosidase inhibitory activity of spices

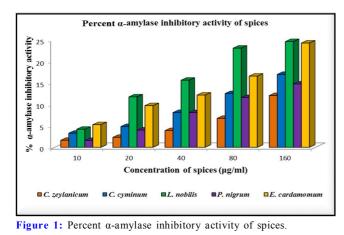|	Percent α-glucosidase inhibitory activity				
Concentration (μg/ml)	*C. zeylanicum*	*C. cyminum*	*L. nobilis*	*P. nigrum*	*E. cardamomum*
10	16.05 ± 0.33%	6.27 ± 0.53%	17.52 ± 1.33%	13.26 ± 0.48%	17.83 ± 0.50%
20	20.09 ± 0.50%	12.29 ± 0.62%	21.48 ± 1.50%	16.80 ± 0.25%	25.10 ± 0.80%
40	27.20 ± 1.13%	16.88 ± 0.29%	29.30 ± 1.10%	20.40 ± 0.34%	31.49 ± 0.78%
80	33.32 ± 0.42%	19.58 ± 0.41%	33.60 ± 1.07%	23.73 ± 0.51%	37.85 ± 0.42%
160	36.60 ± 0.50%	24.71 ± 0.26%	41.80 ± 0.32	35.02 ± 0.10%	43.91 ± 0.42%

The values represented are mean ± SE, where n = 5.

Table 5: IC_{50} for α-amylase and α-glucosidase inhibitory activity (µg/ml) on Acarbose and Losartan supplementation

Spice	IC_{50} of α-amylase inhibitory activity in µg/ml			IC_{50} of α-glucosidase inhibitory activity in µg/ml		
		Supplementation			Supplementation	
	Spice	Acarbose	Losartan	Spice	Acarbose	Losartan
C. zeylanicum	706.04 ± 0.07	179.91 ± 0.13*	507.01 ± 0.08*	243.92 ± 0.13	150.04 ± 0.10*	195.50 ± 0.15*
C. cyminum	517.52 ± 0.09	193.33 ± 0.12*	267.33 ± 0.12*	386.73 ± 0.11	184.00 ± 0.11*	365.85 ± 0.12
L. nobilis	349.41 ± 0.12	162.20 ± 0.19*	230.26 ± 0.14*	203.01 ± 0.15	151.98 ± 0.10*	162.30 ± 0.15*
P. nigrum	573.22 ± 0.08	247.82 ± 0.08*	334.02 ± 0.12*	269.92 ± 0.14	270.23 ± 0.11	248.57 ± 0.14
E. cardamomum	376.18 ± 0.12	186.74 ± 0.16*	235.25 ± 0.15*	182.85 ± 0.16	163.72 ± 0.13	153.49 ± 0.14

The values represented are mean ± SE, where n = 5.
Mean values superscripted by * are statistically significant at $p < 0.05$.

Figure 1: Percent α-amylase inhibitory activity of spices.
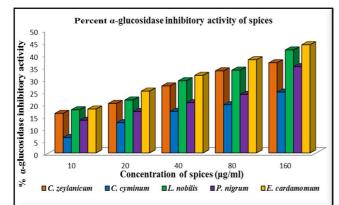

Figure 2: Percent α-glucosidase inhibitory activity of spices.

3.4 Spice-drug interactions

The spices within the preview of the current study possess antidiabetic activity as indicated by their potential to mediate carbohydrate metabolism by inhibiting the two major enzymes in carbohydrate metabolism, *viz.*, α-amylase and α-glucosidase. Since herbal remedies are believed to be 'safe' with 'no adverse reactions', they are consumed without any medical advice.

In our study, we investigated the effect on the inhibitory activities of the two enzymes on supplementation with drugs. The drugs under study are Acarbose, an antidiabetic and Losartan, an antihypertensive. The results suggest a significant increase in the inhibitory activities of the enzymes on drug supplementation. The results are indicated in Table 6-10a and b and Figure 3-7a and b.

Table 6a: Percent α-amylase inhibitory activity of *C. zeylanicum* and its interaction with Acarbose and Losartan

Concentration (μg/ml)	*C. zeylanicum*	*C. zeylanicum* + Acarbose supplementation	*C. zeylanicum* + Losartan supplementation
10	1.63 ± 0.01%*	20.31 ± 0.73%*	8.05 ± 0.31%*
20	2.32 ± 0.04%*	31.29 ± 0.18%*	12.24 ± 0.28%*
40	3.89 ± 0.25%*	35.33 ± 0.31%*	16.94 ± 0.26%*
80	6.73 ± 0.16%*	39.41 ± 0.18%*	20.74 ± 0.26%*
160	12.03 ± 0.09%*	45.11 ± 0.20%*	21.17 ± 0.88%*

The values represented are mean ± SE, where n = 5.
Mean values superscripted by * are statistically significant at $p<0.05$.
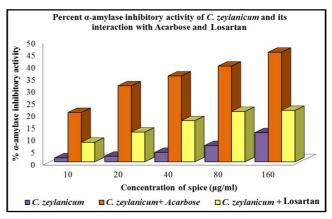

Figure 3a: Percent α-amylase inhibitory activity of *C. zeylanicum* and its interaction with Acarbose and Losartan.

Table 6b: Percent α-glucosidase inhibitory activity of *C. zeylanicum* and its interaction with Acarbose and Losartan

Concentration (μg/ml)	*C. zeylanicum*	*C. zeylanicum* + Acarbose supplementation	*C. zeylanicum* + Losartan supplementation
10	16.05 ± 0.33%*	32.09 ± 0.23%*	18.73 ± 0.28%*
20	20.09 ± 0.50%*	37.94 ± 0.17%*	22.90 ± 0.22%*
40	27.20 ± 1.13%*	41.01 ± 0.18%*	31.83 ± 0.17%*
80	33.32 ± 0.42%*	46.19 ± 0.35%*	37.24 ± 0.18%*
160	36.60 ± 0.50%*	49.08 ± 0.27%*	42.18 ± 0.18%*

The values represented are mean ± SE, where n = 5.
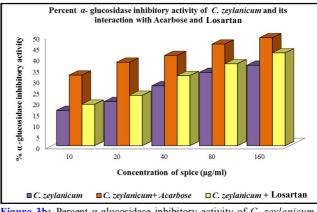Mean values superscripted by * are statistically significant at $p<0.05$.

Figure 3b: Percent α-glucosidase inhibitory activity of *C. zeylanicum* and its interaction with Acarbose and Losartan.

Table 7a: Percent α-amylase inhibitory activity of *C. cyminum* and its interaction with Acarbose and Losartan

Concentration (μg/ml)	*C. cyminum*	*C. cyminum* + Acarbose supplementation	*C. cyminum* + Losartan supplementation
10	3.27 ± 0.04%*	23.56 ± 0.32%*	15.69 ± 0.41%*
20	4.83 ± 0.13%*	31.16 ± 0.35%*	20.89 ± 0.19%*
40	8.08 ± 0.06%*	35.14 ± 0.33%*	24.58 ± 0.43%*
80	12.49 ± 0.13%*	39.89 ± 0.30%*	31.26 ± 0.23%*
160	16.95 ± 0.12%*	44.04 ± 0.18%*	35.16 ± 0.25%*

The values represented are mean ± SE, where n = 5.
Mean values superscripted by * are statistically significant at $p<0.05$.
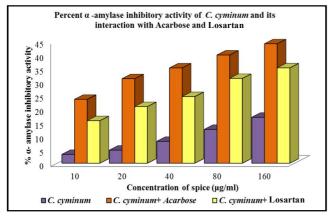
Figure 4a: Percent α-amylase inhibitory activity of *C. cyminum* and its interaction with Acarbose and Losartan.

Table 7b: Percent α-glucosidase inhibitory activity of *C. cyminum* and its interaction with Acarbose and Losartan

Concentration (g/ml)	*C. cyminum*	*C. cyminum* + Acarbose supplementation	*C. cyminum* + Losartan supplementation
10	6.27 ± 0.53%*	28.46 ± 0.36%*	11.87 ± 0.33%*
20	12.29 ± 0.62%*	31.16 ± 0.35%*	14.27 ± 0.42%*
40	16.88 ± 0.29%*	36.32 ± 0.37%*	19.01 ± 0.14%*
80	19.58 ± 0.41%*	40.93 ± 0.33%*	21.21 ± 0.17%*
160	24.71 ± 0.26%*	45.84 ± 0.42%*	28.31 ± 0.39%*

The values represented are mean ± SE, where n = 5.
Mean values superscripted by * are statistically significant at $p<0.05$.
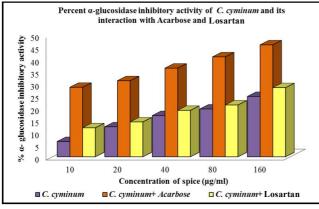

Figure 4b: Percent α-glucosidase inhibitory activity of *C. cyminum* and its interaction with Acarbose and Losartan.

Table 8a: Percent α-amylase inhibitory activity of *L. nobilis* and its interaction with Acarbose and Losartan

Concentration (g/ml)	*L. nobilis*	*L. nobilis* + Acarbose supplementation	*L. nobilis* + Losartan supplementation
10	4.19 ± 0.35%*	15.64 ± 0.23%*	15.79 ± 0.24%*
20	11.72 ± 0.69%*	22.58 ± 0.28%*	18.91 ± 0.85%*
40	15.59 ± 0.58%*	30.97 ± 0.26%*	26.85 ± 0.28%*
80	23.04 ± 0.11%*	38.99 ± 0.19%*	31.64 ± 0.13%*
160	24.57 ± 0.19%*	46.62 ± 0.12%*	38.12 ± 0.16%*

The values represented are mean ± SE, where n = 5.
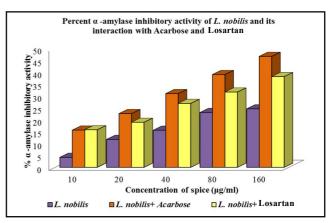Mean values superscripted by * are statistically significant at $p<0.05$.

Figure 5a: Percent α-amylase inhibitory activity of *L. nobilis* and its interaction with Acarbose and Losartan .

Table 8b: Percent α-glucosidase inhibitory activity of *L. nobilis* and its interaction with Acarbose and Losartan

Concentration (g/ml)	*L. nobilis*	*L. nobilis* + Acarbose supplementation	*L. nobilis* + Losartan supplementation
10	17.52 ± 1.33%*	32.12 ± 0.22%*	22.11 ± 0.27%*
20	21.48 ± 1.50%*	38.03 ± 0.18%*	28.70 ± 0.28%*
40	29.30 ± 1.10%*	40.98 ± 0.32%*	36.06 ± 0.26%*
80	33.60 ± 1.07%*	45.96 ± 0.32%*	40.85 ± 0.23%*
160	41.80 ± 0.32%*	48.94 ± 0.25%*	47.32 ± 0.21%*

The values represented are mean ± SE, where n = 5.
Mean values superscripted by * are statistically significant at $p<0.05$.
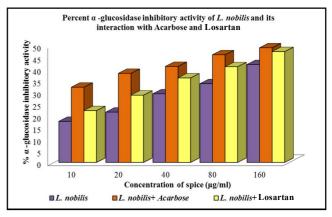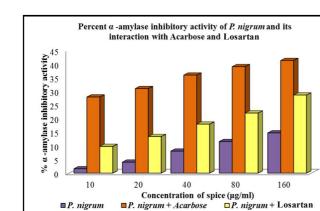

Figure 5b: Percent α-glucosidase inhibitory activity of *L. nobilis* and its interaction with Acarbose and Losartan.
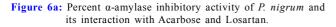
Table 9a: Percent α-amylase inhibitory activity of *P. nigrum* and its interaction with Acarbose and Losartan
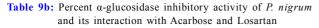
Concentration (g/ml)	*P. nigrum*	*P. nigrum* + Acarbose supplementation	*P. nigrum* + Losartan supplementation
10	1.58 ± 0.13%*	27.86 ± 0.25%*	9.76 ± 0.28%*
20	4.01 ± 0.30%*	30.95 ± 0.28%*	13.38 ± 0.46%*
40	8.05 ± 0.11%*	35.87 ± 0.26%*	17.92 ± 0.18%*
80	11.56 ± 0.58%*	38.97 ± 0.19%*	21.98 ± 0.25%*
160	14.72 ± 0.72%*	41.22 ± 0.24%*	28.58 ± 0.44%*

The values represented are mean ± SE, where n = 5.
Mean values superscripted by * are statistically significant at $p<0.05$.

Figure 6a: Percent α-amylase inhibitory activity of *P. nigrum* and its interaction with Acarbose and Losartan.

Table 9b: Percent α-glucosidase inhibitory activity of *P. nigrum* and its interaction with Acarbose and Losartan

Concentration (μg/ml)	*P. nigrum*	*P. nigrum* + Acarbose supplementation	*P. nigrum* + Losartan supplementation
10	13.26 ± 0.48%*	22.47 ± 0.27%*	11.8 ± 0.32%*
20	16.80 ± 0.25%*	25.63 ± 0.25%*	18.94 ± 0.28%*
40	20.40 ± 0.34%*	28.86 ± 0.32%*	23.78 ± 0.34%*
80	23.73 ± 0.51%*	31.26 ± 0.31%*	28.80 ± 0.25%*
160	35.02 ± 0.10%*	38.68 ± 0.41%*	35.74 ± 0.21%*

The values represented are mean ± SE, where n = 5.
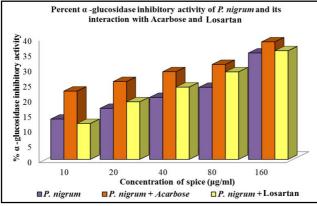Mean values superscripted by * are statistically significant at $p<0.05$.

Figure 6b: Percent α-glucosidase inhibitory activity of *P. nigrum* and its interaction with Acarbose and Losartan.

Table 10a: Percent α-amylase inhibitory activity of *E. cardamomum* and its interaction with Acarbose and Losartan

Concentration (μg/ml)	*E. cardamomum*	*E. cardamomum* + Acarbose supplementation	*E. cardamomum* + Losartan supplementation
10	5.26 ± 0.34%*	15.04 ± 0.16%*	13.86 ± 0.24%*
20	9.68 ± 0.28%*	22.92 ± 0.20%*	16.86 ± 0.17%*
40	12.14 ± 0.36%*	31.01 ± 0.30%*	22.13 ± 0.30%*
80	16.55 ± 0.35%*	36.97 ± 0.25%*	28.96 ± 0.27%*
160	24.43 ± 0.08%*	42.66 ± 0.17%*	37.19 ± 0.13%*

The values represented are mean ± SE, where n = 5.
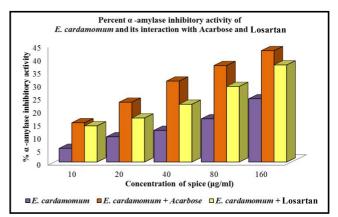Mean values superscripted by * are statistically significant at $p<0.05$.

Figure 7a: Percent α-amylase inhibitory activity of *E. cardamomum* and its interaction with Acarbose and Losartan.

Table 10b: Percent α-glucosidase inhibitory activity of *E. cardamomum* and its interaction with Acarbose and Losartan

Concentration (μg/ml)	*E. cardamomum*	*E. cardamomum* + Acarbose supplementation	*E. cardamomum* + Losartan supplementation
10 μg/ml	17.83 ± 0.50%*	27.69 ± 0.25%*	26.82 ± 0.22%*
20 μg/ml	25.10 ± 0.80%*	30.78 ± 0.26%*	32.02 ± 0.24%*
40 μg/ml	31.49 ± 0.78%*	36.70 ± 0.26%*	37.03 ± 0.26%*
80 μg/ml	37.85 ± 0.42%*	42.18 ± 0.27%*	42.80 ± 0.27%*
160 μg/ml	43.91 ± 0.42%*	47.75 ± 0.20%*	49.07 ± 0.29%*

The values represented are mean ± SE, where n = 5.
Mean values superscripted by * are statistically significant at $p<0.05$.

Figure 7b: Percent α-glucosidase inhibitory activity of *E. cardamomum* and its interaction with Acarbose and Losartan.

The depression of IC_{50}, on drug supplementation, is presented in Table 5.

Further, the data was analyzed to find the percent enhancement of enzyme inhibitory activities and is represented in Table 11. The same was calculated as:

$$\frac{IC_{50} \text{ of spice} - IC_{50} \text{ on drug supplementation}}{IC_{50} \text{ of spice}} \times 100$$

Table 11: Percent enhancement of effectiveness of α-amylase and α-glucosidase inhibitory activities on Acarbose and Losartan supplementation

Spices	% Enhancement of effectiveness of α-amylase inhibitory activity		% Enhancement of effectiveness of α-glucosidase inhibitory activity	
	Acarbose supplementation	Losartan supplementation	Acarbose supplementation	Losartan supplementation
C. zeylanicum	74.52%	28.19%	38.49%	19.85%
C. cyminum	62.64%	48.34%	52.42%	5.40%
L. nobilis	53.58%	34.10%	25.14%	20.05%
P. nigrum	56.77%	41.73%	-0.11%	7.91%
E. cardamomum	50.36%	37.46%	10.46%	16.06%

4. Discussion

Selected antidiabetic properties, namely α-amylase and α-glucosidase inhibitory activities for *C. zeylanicum*, *C. cyminum*, *L. nobilis*, *P. nigrum*, and *E. cardamomum* were evaluated using widely established, sensitive, specific, validated and internationally accepted bioassays *in vitro*. As per conclusions drawn by Adisakwattana *et al.* (2011), α-amylase and α-glucosidase are the key enzymes involved in starch digestion. Thus, in the management of diabetes, inhibitors of these enzymes can play a key therapeutic role.

Active phytochemicals, such as alkaloids, tannins, phenols, saponins, terpenoids, flavonoids, steroids, and sterols, themselves or in conjunction, mediate the medicinal properties of a spice/plant. Varied methods and solvents can be employed for the extraction of these phytochemicals, which govern the pharmacokinetics and pharmacodynamics of the spice. Most of the phytochemicals are extracted well in organic solvents as compared to water. Widely used organic solvents for extraction include methanol, ethanol, dichloromethane, chloroform and acetone (Premakumara *et al.*, 2017; Salve and Mule, 2018; Upadhyay, 2016). However, all of them are associated with toxic effects on human physiology. Thus, for the current study, 50% hydroacetone was the 'solvent of choice' as the toxicity associated with this solvent system is minimal. The scientific literature regarding the applicability of hydroacetone is largely limited and this highlights the uniqueness of our study.

Amongst the reported phytochemicals, the antidiabetic effects of a few are mentioned by Emeka and Asuzu (2018). Flavonoids constitute a family of soluble polyphenols and their antidiabetic properties are attributed partly to its antioxidant potentials and partly to its ability to modulate cell signaling. Flavonoids such as naringenin and cyanidin are reported to inhibit amylase and glucosidase (Li *et al.*, 2006; Priscilla *et al.*, 2014; Akkarachiyasit *et al.*, 2010). Saponins are major plant metabolites that naturally occur as surface-active glycosides and induce insulin production, amelioration of oxidative stress and advanced glycation end products (AGEs) formation (Jiang *et al.*, 1999). Also, saponins promote insulin release from the pancreas (Ali and Adanlawo, 2012) and inhibit α-amylase and α-glucosidase (Hemlatha *et al.*, 2010). Alkaloids contain basic nitrogen atoms and cause significant elevation of plasma insulin, decrease in serum lipids and lipid peroxide formation (Khalil *et al.*, 2007). It has been reported by Sharma *et al.* (2009), that there also is a significant improvement of GLUT 4, glucokinase activity, attenuation of glucose-6-phosphatase activity and improvement in the hepatic glycogen content. Tannins are polyphenolic biomolecules, exhibiting antidiabetic effects mainly by inhibiting the α-amylase and α-glucosidase activities (Kunyanga *et al.*, 2011) and stimulating

transportation of glucose (Liu *et al.*, 2005). Terpenoids have demonstrated hypoglycemic potentials (Zhang *et al.*, 2009) affecting translocation of GLUT 4 (Huang *et al.*, 2010) and also inhibition of α-glucosidase activities (Matsuda *et al.*, 1999).

As per the phytochemical and biomolecule analysis of the spices under consideration, using hydroacetone as the solvent, all the spices demonstrated the presence of flavonoids as well as tannins, both of which possess the ability to inhibit the carbohydrate metabolizing enzymes amylases and glucosidases, *in vitro*. The α-amylase inhibitory activities as demonstrated by the spices studied were better for *E. cardamomum* and *L. nobilis*, while for α-glucosidase inhibitory activity, the spice depicting better activity was *E. cardamomum*.

Complementary and Alternative Medicine (CAM) is being used by around 80% of the present-day global population as an alternative to conventional medicine (Malagi *et al.*, 2014). Chang *et al.* (2007) reported the use of herbal medicine, dietary supplements, and CAM therapies by about 72.8% diabetics, along with the mainstream treatments (Liu *et al.*, 2015). The same can be beneficial, as well as, can possess potential risks in effective management of the disorder. For diabetics, a combination therapy is suggested to ameliorate the other complications associated with it. (Curtis, 2006; Murthy *et al.*, 2013; Gupta *et al.*, 2017b).

This prompted us to consider this exclusive study on the antidiabetic effect of spices in conjugation with two of the most commonly used drugs, *viz.*, Acarbose and Losartan. The commercially available drugs were used for the same. The drugs were screened for their α-amylase and α-glucosidase inhibitory activities at various concentrations and it was observed that beyond 5000 μg/ml Acarbose, the α-amylase inhibitory activity reached saturation of 22% while α-glucosidase inhibitory activity was saturated at 45%. In the case of Losartan at 1600 g/ml, the α-amylase and α-glucosidase inhibitory activities saturated at 22% and 41%, respectively. Thus, these concentrations were used for supplementation with the spices, as the aim of the study was to evaluate the modulation of inhibitory activity of the spices under the normal drug regime.

The results on statistical analysis depict a significant increase in inhibitory activities of both the enzymes in the presence of the drugs. The results suggest that for the spices, *viz.*, *C. zeylanicum*, *C. cyminum*, *L. nobilis*, *P. nigrum* and *E. cardamomum*, the percent enhancement in presence for Acarbose was 74.52, 62.65, 53.58, 56.76 and 50.35%, respectively for the α-amylase inhibitory activity while it was 28.19, 48.34, 34.1, 41.73 and 37.46%, respectively in presence of Losartan. Further, for the α-glucosidase inhibitory activity

of the spices as mentioned above, the enhancement of inhibitory activity was 38.48, 52.42, 25.14, -0.11, 10.46%, respectively on Acarbose supplementation and 19.85, 5.40, 20.05, 8.12, 16.06% on Losartan supplementation. Thus, modulation of inhibitory activity generally is higher for Acarbose than Losartan for both enzymes considered, except in case of *P. nigrum* where negligible difference was observed in presence of Acarbose for α-glucosidase inhibitory activity.

Such interactions have largely been neglected and such spice-drug interactions can lead to hypoglycemic conditions which could be fatal.

5. Conclusion

The current results indicate that all the five-spice extracts showed good inhibitory activity towards α-amylase and α-glucosidase, and hence, the spices can be used as dietary supplements to control postprandial increase in blood glucose levels. The spices essentially contain herbal bioactive compounds having enzyme inhibitory potential and thus, further structural elucidation and characterization of the bioactive constituents is required. The present study is a stepping stone towards the development of nutraceuticals with minimum adverse reactions. Further studies with focus on investigating effects *in vivo* are being considered.

Currently, the medical academic curriculum lacks data on spice-drug or a larger perspective of plant-drug interactions. To enable better therapy, a merger of ancient scientific knowledge along with the use of medicines is the need of the hour. This study opens up the possibility for the search of such interactions, which may be the two sides of a coin, leading to benefits and/or health impairment.

Conflict of interest

The authors declare that there are no conflicts of interest in the course of conducting the research. All the authors had final decision regarding the manuscript and decision to submit the findings for publication.

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