Int.J.Curr.Microbiol.App.Sci (2016) *5*(10): 1136-1142

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 5 Number 10 (2016) pp. 1136-1142**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.510.120

A Study on Evaluation of Antidiabetic Potential of *Cuminum cyminum* (Cumin)

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Keywords

Cumin seeds, *Cuminum cyminum*, Type 2 diabetes, Alpha-glucosidase, Alpha-amylase

Article Info

Accepted:
12 September 2016
Available Online:
10 October 2016

A B S T R A C T

Diabetes is a metabolic disorder which leads to hyperglycemia due to impairment of insulin secretion, insulin function or both. Cumin is a spice which is widely used all over the world. The recent research indicated that, cumin has positive and significant effect on blood glucose and total antioxidant capacity in Type 2 Diabetes patients. Hence, in the present study we aimed to assess the antidiabetic properties of ethanolic extract of cumin seeds through evaluation of *in-vitro* alpha-amylase and alpha-glucosidase activities inhibition assay. Results revealed that in an alpha-amylase inhibitory assay with of ethanolic extract of cumin seeds at a concentration range of 5µg/ml, 10µg/ml, 15µg/ml, and 20µg/ml, shown inhibition effect in the percentage of 3.85, 4.58, 6.25, and 7.56 respectively with an IC_{50} value of 12.28 µg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 20.82 µg/ml. Similarly, in an alpha-glucosidase inhibitory assay with ethanolic extract of cumin seeds at a concentration range of 5µg/ml, 10µg/ml, 15µg/ml, and 20µg/ml, shown inhibition effect in the percentage of 4.68, 5.38, 7.18, and 8.45 respectively with an IC_{50} value of 14.58 µg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 21.18 µg/ml. In conclusion, the results of present preliminary study clearly demonstrated that the cumin extract possess antidiabatic properties. Hence, it could be recommended that cumin seeds could be employed for the management of Type 2 Diabetes and could be considered for development of natural anti-diabetic drugs.

Introduction

Diabetes is a metabolic disorder which leads to hyperglycemia due to impairment of insulin secretion, insulin function or both. Type 2 Diabetes affects 90-95 % of people. The signs and symptoms of this disease are the elevated level of blood glucose, decreased peripheral absorption of glucose due to impairment of insulin secretion and peripheral resistance to insulin (Taghizadeh *et al.*, 2015).

Several pathological processes are involved in the development of diabetes. Various long-term complications of diabetes

developed due to the chronic hyperglycemia and insulin resistance. These complications are both macro and micro vascular abnormalities such as autonomic neuropathy, peripheral neuropathy, retinopathy, cardiovascular symptoms and nephropathy (Wold *et al.*, 2005; Rahimi *et al.*, 2005). Oxidative stress is responsible for the development of chronic complications of diabetes mellitus and results from chronic hyperglycemia, increased oxidants and thereby decreased antioxidants (Opara, 2002).

Cumin is a spice which is widely used all over the world. Cumin's scientific name is *Cumin cyminum*. It is a dried seed and it is a member of the *Apiaceae* family. The cumin plant grows to 30–50 cm tall (Figure 1 and Figure 2) (Mnif and Aifa, 2015). It is primarily grown in India, China, Saudi Arabia, and the Mediterranean Sea adjacent countries. Cumin seeds are broadly utilized as culinary spices and aromatic herbs. It is one of India's most popular condiment spices, and it's also frequently used in Ayurvedic medicine to cure dyspepsia and jaundice (Kim and Ryu, 2013).

The recent research indicated that, Cumin has positive and significant effect on blood glucose and total antioxidant capacity in Type 2 Diabetes patients (Andallu and Ramya, 2007; Dhandapani *et al.*, 2002). Hence, the present study was performed to evaluated antidiabetic potential of Cumin (*Cumin cyminum*) seeds.

Materials and Methods

Collection of Cumin seeds

The seeds of cumin were purchased from local market in Chikkaballapura, Karnataka, India. The cumin seeds were sprayed with ethanol, and then shade dried at room temperature for 10 days. The dried seeds were crushed to fine powder with help of electric grinder and stored in airtight containers for further analysis.

Extraction

Approximately 50 g of dried and coarsely powdered seeds of cumin were subjected to successive solvent extraction by continuous hot extraction (Soxhlet) with 550 mL of ethanol. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The extracts were preserved in airtight containers and stored at room temperature until further use (Shi and Burn, 2004).

Alpha-amylase inhibitory assay

The alpha-amylase inhibition assay was carried out by the method of Miller (1959). Cumin extract/acarbose (5 µg/ml, 10 µg/ml, 15 µg/ml and 20 µg/ml) were incubated for 10 minutes at 25℃ with 500 µl of 20 mM sodium phosphate buffer (pH 6.8) with 20 µl of amylase (1 U/ml). After pre-incubation, each tube was added with 1 ml of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) and incubated for 15 min.

One ml DNS was added to arrest the reaction. After that, the tubes were kept in a boiling water bath for 5 min and cooled to room temperature. After that, distilled water (10 ml) was added to the reaction mixture, and the absorbance was measured at 540 nm. The test compound was not used in the preparation of the control samples. The following formula was used to determine the percent inhibition of alpha-amylase activity;

% Inhibition = (Abs control – Abs test) / (Abs control)

Alpha-glucosidase Inhibition Assay

The alpha-glucosidase inhibition assay was

performed using the modified method of Kim *et al.*, (2010). The different concentrations of cumin extracts and standard drug acarbose (5 µg/ml, 10 µg/ml, 15 µg/ml and 20 µg/ml) were prepared. Phosphate buffer (1 ml; 100 mM, pH 6.8) and 80 µl of test cumin extracts / acarbose of concentrations (5 µg/ml, 10 µg/ml, 15 µg/ml and 20 µg/ml) were added to 20 µl of alpha-glucosidase and incubated at 37°C for 10 minutes. Later, pNPG- 50 µl (5 mM) was added to the assay mixture to initiate the reaction. Then, the reaction mixture was incubated at room temperature for one hour and arrested the reaction by adding 2.5 ml of 0.1 M Na_2CO_3. The absorbance was measured at 400 nm to determine the activity of alpha-glucosidase activity. The following formula was used to determine the percent inhibition of alpha-glucosidase activity;

% Inhibition = (Abs control – Abs test) / (Abs control)

Results and Discussion

Alpha-amylase inhibitory assay

In an alpha-amylase inhibitory assay with ethanolic extract of cumin seeds at a concentration range of 5 µg/ml, 10 µg/ml, 15 µg/ml, and 20 µg/ml, shown inhibition effect in the percentage of 3.85, 4.58, 6.25 and 7.56 respectively (Table 1 and Figure 3) with an IC_{50} value of 12.28 µg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 20.82 µg/ml.

Alpha-glucosidase inhibitory assay

Similar trend was observed in an alpha-glucosidase inhibitory assay with ethanolic extract of cumin seeds. Ethanolic extract of cumin seeds at a concentration range of 5 µg/ml, 10 µg/ml, 15 µg/ml and 20 µg/ml, shown inhibition effect in the percentage of 4.68, 5.38, 7.18, and 8.45 respectively (Table 2 and Figure 4) with an IC_{50} value of 14.58 µg/ml in comparison with the standard antidiabetic drug acarbose with an IC_{50} value of 21.18 µg/ml. Cumin's biological activities have been established due to occurrence of bioactive compounds such as terpenes, phenols, and flavonoids, according to a plethora of evidence in the literature (Mnif and Aifa, 2015). Alpha-amylase hydrolyses glycosidic bonds of starch and glycogen (Stedman, 1920). It is ubiquitous in humans and other mammals (Voet and Voet, 2005). Alpha-glucosidase, is a glucosidase of brush borders that acts upon α (1→4) bonds, (Flanagan and Forstner, 1978) of starch and disaccharides to release free glucose. In diabetics, inhibiting alpha-amylase is an essential therapeutic target for regulating postprandial blood glucose elevations (Djeridane *et al.*, 2015).

Inhibition of alpha-glucosidase and alpha-amylase can lower the post absorptive rise in blood glucose and hence can be a better strategy in achieving the glycemic goals in diabetic and borderline prediabetics (Kajaria *et al.*, 2013). Therefore, in the present study antidiabetic properties of ethanolic extract of cumin seeds was assessed through evaluation of *in-vitro* alpha-amylase and alpha-glucosidase activities inhibition assay.

The results of our study delineated that ethanolic extract of cumin seeds possess a strong antidiabetic potential which was evident through *in-vitro* alpha-amylase and alpha-glucosidase inhibition assays, wherein ethanolic extract of cumin seeds exhibited IC_{50} value of 12.28 µg/ml and 14. 58 µg/ml in *in-vitro* alpha-amylase and alpha-glucosidase inhibition assays respectively.

Table.1 Effect of ethanolic extract of cumin seeds on alpha-amylase inhibition activity

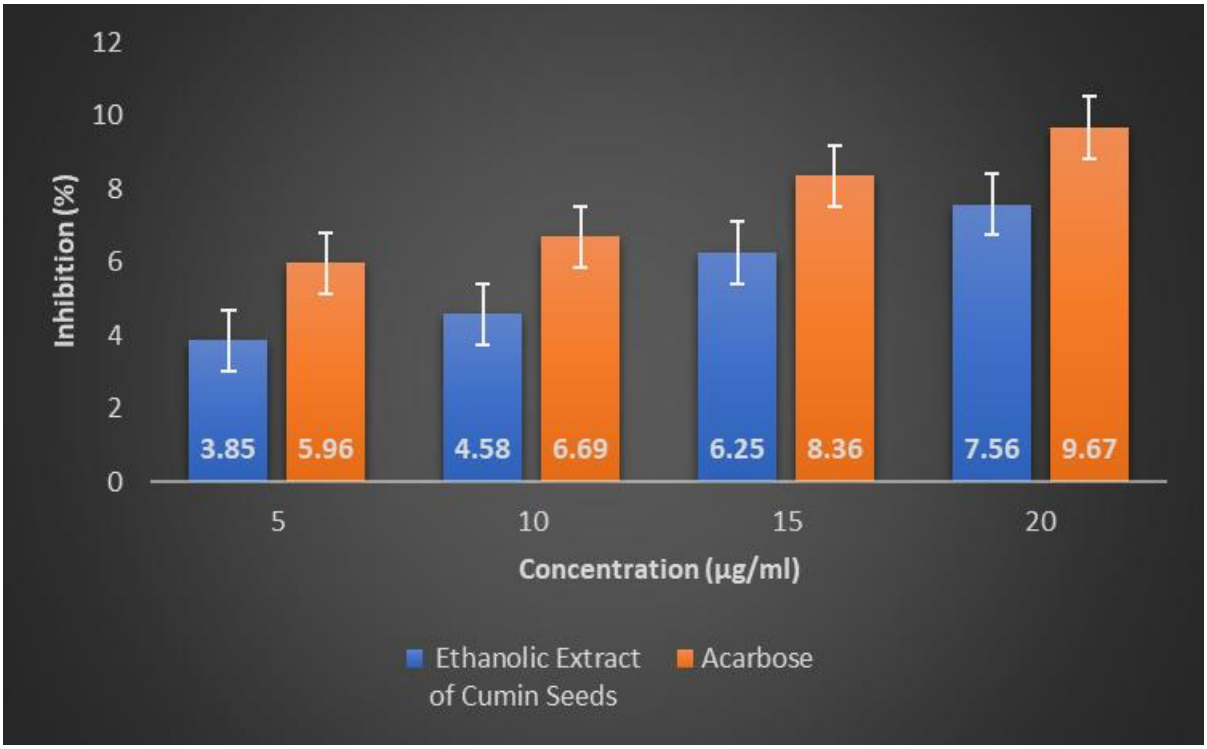
Conc. of Ethanolic Extract of Cumin Seeds (µg/ml)	Inhibition (%)	Conc. of Acarbose (µg/ml)	Inhibition (%)
5	3.85 ± 0.08	5	5.96 ± 0.03
10	4.58 ± 0.11	10	6.69 ± 0.08
15	6.25 ± 0.06	15	8.36 ± 0.05
20	7.56 ± 0.08	20	9.67 ± 0.04

Values were expressed Mean ± SD; n=3

Fig.1 Showing *Cuminum cyminum* plant

Fig.2 Showing seeds of *Cuminum cyminum*

Fig.3 Effect of ethanolic extract of cumin seeds on alpha-amylase inhibition activity



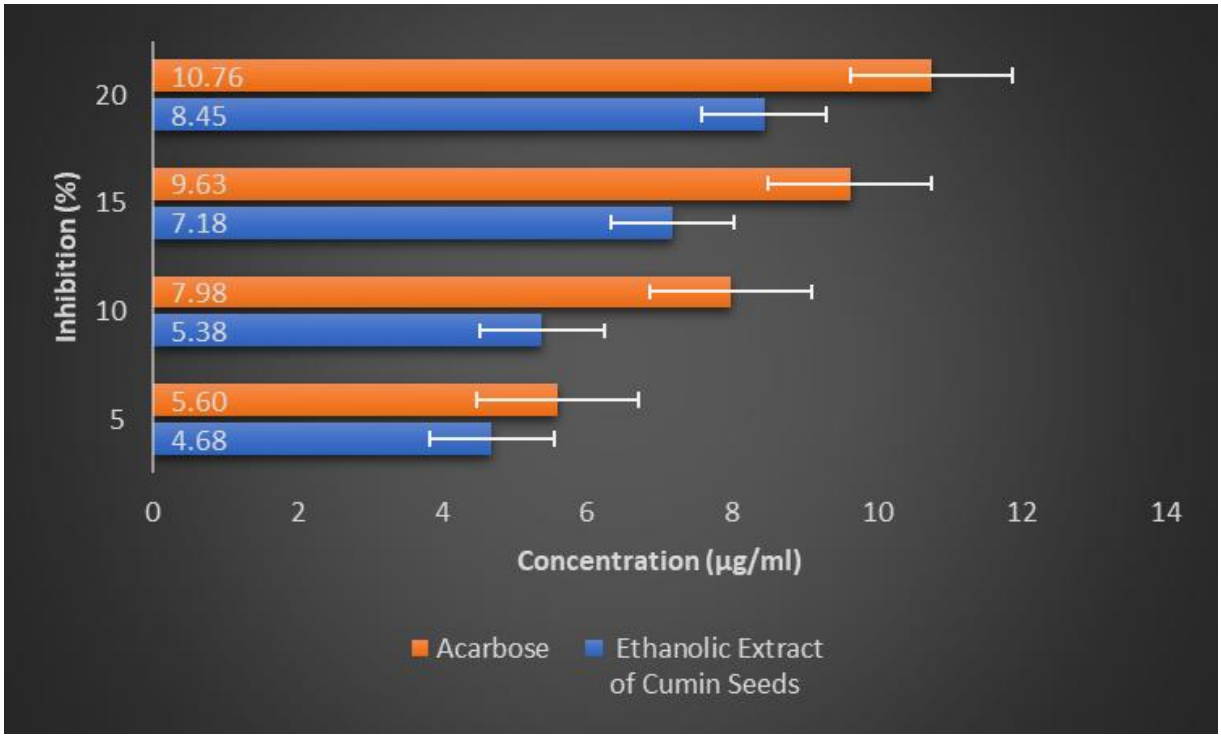
Values were expressed Mean; n=3

Table.2 Effect of ethanolic extract of cumin seeds on alpha-glucosidase inhibition activity

Conc. of Ethanolic Extract of Cumin Seeds (µg/ml)	Inhibition (%)	Conc. of Acarbose (µg/ml)	Inhibition (%)
5	4.68 ± 0.05	5	5.60 ± 0.03
10	5.38 ± 0.11	10	7.98 ± 0.08
15	7.18 ± 0.09	15	9.63 ± 0.05
20	8.45 ± 0.15	20	10.76 ± 0.04

Values were expressed Mean ± SD; n=3

Fig.4 Effect of ethanolic extract of cumin seeds on alpha-glucosidase inhibition activity



Values were expressed Mean ± SD; n=3

The IC_{50} values of ethanolic extract of cumin seeds was at par with IC_{50} exhibited by standard acarbose *viz.* 20.82 µg/ml and 21.18 µg/ml in *in-vitro* alpha-amylase and alpha-glucosidase inhibition assay respectively.

The findings of our study are comparable with reports published in literature by various other research investigators (Andallu and Ramya 2007). reported that, a significant ($p<0.05$) decrease in fasting glucose levels and was observed in experimental diabetics i.e., treated with cumin seeds while such decrease was not observed in control subjects treated with the drug (Andallu and Ramya, 2007). Black cumin had an anti-diabetic effect which appeared to improve insulin sensitivity (Benhaddou-Andaloussi *et al.*, 2011). From various researchers, it has been proved that cumin has antidiabetic property (Taghizadeh *et al.*, 2017).

Plant-based alternative medications and functional foods that modulate physiological effects in preventing and treating diabetes and obesity are becoming more popular. The plant kingdom could be a vast resource for finding natural, effective oral hypoglycemic medicines with little or no adverse effects. Over 1200 plant species have been identified as being utilized effectively for hypoglycemic action over the world (Tundis *et al.*, 2010). As a result, natural α-glucosidase and α-amylase inhibitors derived from the plant kingdom offer promising leads for hyperglycemia management (Jijith, 2017).

Fiber present in cumin seeds might have contributed to the observed effect as fiber were reported to slow down stomach emptying, delay and attenuate the post prandial raise in blood glucose. In addition, ascorbic acid, niacin, copper and manganese present in cumin seeds were reported to exhibit anti-diabetic effect (Andallu and Ramya, 2007).

The results of present preliminary study clearly demonstrated that the ethanolic extract of cumin seeds possess antidiabatic properties. Hence, it could be recommended that cumin seeds could be employed for the management of Type 2 Diabetes and could be considered for development of natural anti-diabetic drugs. However, further *in-vivo* studies are recommended to evaluate the antidiabetic potentials of cumin seeds to reaffirm the antidiabetic activities of ethanolic extract of cumin seeds in *in-vivo* models.

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How to cite this article:

Keshamma, E. and Kamal Kant Patra. 2016. A Study on Evaluation of Antidiabetic Potential of *Cuminum cyminum* (Cumin). *Int.J.Curr.Microbiol.App.Sci.* 5(10): 1136-1142.
doi: http://dx.doi.org/10.20546/ijcmas.2016.510.120