ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH



# *IN VITRO* HYPOGLYCEMIC AND ANTIMICROBIAL ACTIVITY OF *CUCUMIS CALLOSUS* (ROTTL.) COGN. FRUIT

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#### Received: 14 March 2016, Revised and Accepted: 22 March 2016

# ABSTRACT

**Objective:** This research investigated *in vitro* hypoglycemic and antimicrobial activity of methanolic fruit extract of *Cucumis callosus* (MECC) (Rottl.) cogn.

**Methods:** The *in vitro* antidiabetic ability of MECC was evaluated by inhibition studies of the digestive enzymes including  $\alpha$ -amylase and  $\alpha$ -glycosidase. The antimicrobial activity of extract was further evaluated against 21 laboratory strains belongs to 8 bacterial species and 4 fungal species using cupplate agar diffusion assay and agar dilution assay.

**Results:** Our assay result suggested that the MECC exhibited dose-dependent increase in percentage inhibitory activity on  $\alpha$ -glycosidase (IC<sub>50</sub>405.37 µg/ml) and  $\alpha$ -amylase enzymes (IC<sub>50</sub>721.55 µg/ml). The results of antimicrobial activity also showed that the extract inhibited the growth of 13 strains of 7 bacterial species and all fungal species.

**Conclusion:** The observed inhibitions of  $\alpha$ -amylase and  $\alpha$ -glycosidase and inhibition of some microbial species suggested that the MECC may be useful in the management of diabetes mellitus and treatment of some infectious diseases.

Keywords: Cucumis callosus, Diabetes, α-amylase, α-glycosidase, Antimicrobial.

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## INTRODUCTION

Diabetes mellitus is a pandemic disease, and the world is facing an escalating epidemic of diabetes. The International Diabetes Federation has estimated that in 2010, 285 million people around the world have diabetes, representing nearly 7% of the adult world population [1]. Type 2 diabetes mellitus (T2DM) accounts for 90-95% of all cases of diabetes. Furthermore, T2DM significantly increases the risk of heart disease, stroke, and bacterial and fungal skin infections. T2DM is a disease characterized by a dual defect: (1) By insulin resistance which prevents cells from using insulin properly and (2) degrees of reduced pancreatic insulin secretion [2]. The side effects associated with the prolonged use of insulin and other hypoglycemic drugs have necessitated the demand for safe and effective drugs, especially of herbal origin [3]. Inhibition of  $\alpha$ -glycosidase (EC 3.2.1.20) and  $\alpha$ -amylase (EC 3.2.1.1), enzymes involved in the digestion of carbohydrates, can significantly decrease the postprandial increase of blood glucose after a mixed carbohydrate diet, and therefore, can be an important strategy in the management of postprandial blood glucose level in Type 2 diabetic patients and borderline patients [4].  $\alpha$ -glycosidase and  $\alpha$ -amylase inhibitors are useful for people taking sulfonylurea medication or metformin, who need an additional medication to keep their blood glucose levels within a safe range. Therefore, the retardation and delay of carbohydrate absorption with a plant-based  $\alpha$ -glycosidase inhibitor offers a prospective therapeutic approach for the management of T2DM and borderline patients.

*Cucumis callous* (Rottl.) Cogn. (*Cucurbitaceae*) commonly called as "Bitter cucumber" in English, "Kachri" in Hindi, is a highly branched very common prostate, perennial herb, distributed throughout India in the arid zones. The fruits are smooth, ovoid, ellipsoid, green variegated stripes and have bitter pulp [5,6]. *C. callosus* is essentially a warm season crop and a long period of warm and humid climate is required [7]. Fruit pulp of *C. callosus* is bitter, acrid, thermogenic, anthelmintic, liver tonic, cardiotonic, appetizer, expectorant, and intellect promoting. Roots are used as emetic and purgative [8]. Traditionally, its fruits and seeds are used for strong memory, remove vertigo, cooling, astringent, bilious disorder,

constipation, piles, urticaria, jaundice, amenorrhea, dysmenorrhea, and calculi [9,10]. The *C. callosus* fruit is used in the treatment of diabetes mellitus by Srilankan Ayurvedic and traditional physicians [11]. The leaf extract of *C. callosus* topically used in wound healing [4].

The aqueous and alcoholic extract of *C. callosus* (seed) has been reported for its antioxidant activity [12,13]. The tribal people of Balasore and Baripada (Odisha, India) use fruits of *C callosus* during worship, as vegetable and for curing diabetes, epilepsy and diarrhea [14]. Hence, the present study was aimed at evaluating the hypoglycemic and antimicrobial activity of methanolic fruit extract of *C. callosus* (MECC).

## **METHODS**

#### **Collection of plant**

The fruits of *C. callosus* were collected in the month of July 2014 from village area of Kendrapara and Balasore district, Odisha (India). The plant was authenticated by M. S. Mondal, Botanical Survey of India, Kolkata, India, and a voucher specimen (CNH/1-1(196)/2007/ Tech-II/160) has been preserved in the Pharmacology Research Laboratory, Jadavpur University, Kolkata, for future reference.

#### Extraction

The fruits of *C. callosus* were shade dried and then powdered with a mechanical grinder. The powder (500 g) was defatted with petroleum ether at 40°C in a Soxhlet extraction apparatus and then extracted similarly with methanol. The solvents were completely removed under reduced pressure to obtain a dry mass. The yields of the petroleum ether and methanol extracts were found to be 2.8% w/w and 9% w/w, respectively. The extracts were stored in vacuum desiccators for further use. The preliminary phytochemical screening of the MECC showed the presence of mainly flavonoids, saponin, and terpenoids.

## Chemicals

Pyrocatechol,  $\alpha$ -amylase, and  $\alpha$ -glycosidase enzymes were purchased from SRL Pvt. Ltd., Mumbai. Glucose assay kit from Agape Diagnostic

Pvt. Ltd., Kerala, and Acarbose was obtained from Bicon Pvt. Ltd. All other chemicals used in the study were obtained commercially and were of analytical grade.

# Bacterial strains and fungal strains

Gram-positive strains: *Staphylococcus aureus, Bacillus subtilis,* and *Bacillus pumilus*.

Gram-negative strains: Escherichia coli, Vibrio cholerae, Shigella boydi, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Salmonella choleraesuis, Shigella dysenteriae, and Proteus vulgaris.

Fungal strains: *Candida albicans* MTCC 183, *Aspergillus flavus* MTCC 1973, *Aspergillus niger* MTCC 16404, *Cryptococcus albidus* MTCC 183.

Microorganisms were obtained from the stock culture of Division of Microbiology, Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India.

#### In vitro methods employed in antidiabetic studies

# Inhibition of $\alpha$ -amylase enzyme

About 0.05 ml of test samples (100-1000  $\mu$ g/ml) and 0.05 ml standard drug (100-1000  $\mu$ g/ml) were separately added to 0.05 ml of 0.1 M phosphate buffer (pH 6.9) containing 0.1 ml of  $\alpha$ -amylase (0.5 mg/ml) solution in test tube and were incubated at 25°C for 10 minutes. After these, 0.05 ml of 1% starch solution in 0.1 M phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 minutes. The reaction was stopped with the addition of 2.0 ml of 3, 5 dinitrosalicylic acid color reagent. The test tubes were then incubated in a boiling water bath for 5 minutes, cooled to room temperature. The reaction mixture was then diluted with 10 ml distilled water and absorbance was measured at 540 nm. The generation of maltose was quantified by the reduction of 3, 5 dinitrosalicylic acid to 3-amino-5-nitro salicylic acid. Control represent 100% enzyme activity and was conducted in a similar way by replacing extract with vehicle [15].

## Inhibition of $\alpha$ -glycosidase enzyme

α-glycosidase inhibitory activity of the extract was assayed according to Kim *et al.*, with slight modification. The reaction was initiated with the preparation of reaction mixture taking 0.05 ml each of the samples (test and standard) at different concentrations (100-1000 μg/ml) in 0.05 ml of 0.1 M phosphate buffer (pH 6.9) followed by incubation at 37°C for 15 minutes. After which 0.1 ml of enzyme solution was immediately added to the mixture and incubated at 37°C for 15 minutes. Then, 0.25 ml of substrate 3 mM *p*-nitro phenyl-*α*-D-glycopyranoside (pNPG) was added to the mixture and again incubated at 37°C for 15 minutes, after which the reaction was stopped by the addition of 4 ml of 0.1 mM Na<sub>2</sub>CO<sub>3</sub>. α-glycosidase inhibitory activity was determined by measuring the release of p-nitrophenol from pNPG at 405 nm. The control contained all reagents without the tested sample. The reactions were conducted in triplicate. The percentage of inhibition was calculated as:

% inhibition =	$\Delta Abs_{control} - \Delta Abs_{sample} \times 100$	
	$\Delta Abs_{control} \times 100$	

Where

 $Abs_{sample}$  is the absorbance of sample extract  $Abs_{control}$  is the absorbance of the control

The concentration of  $\alpha$ -glycosidase inhibitor required to inhibit 50% of  $\alpha$ -glycosidase activity under the assay conditions is defined as the IC<sub>50</sub> value [16].

## In vitro methods employed in antimicrobial studies

#### Minimum inhibitory concentration (MIC)

The MIC was determined by the agar dilution method in Mueller-Hinton agar medium (Oxoid). Before gelling, 20 ml of agar medium was added to each of the Petri dishes containing a specific concentration of plant extract, or purified compounds, and the Petri dishes were swirled carefully until the agar began to set. Concentrations ranging from 200 to 2000  $\mu$ g/ml were used for extract. Subsequently, 2  $\mu$ l of bacteria (10<sup>8</sup> colony-forming units [CFU]/ml) and 2  $\mu$ l fungi (10<sup>4</sup> CFU/ml) were inoculated using a Steers replicator on the Mueller-Hinton agar surface [17].

# Zone of inhibition

The zone of inhibition was determined by the cup-plate method in Mueller-Hinton agar medium (Oxoid). The antimicrobial activity of MECC was determined using the disk diffusion method. Petri dishes containing 20 ml of Mueller-/Hinton agar medium (Oxoid, Hampshire, England) were seeded with a 24 hrs culture of the microbial strains in Trypticase soy broth (TSB, Oxoid). The inoculum size was adjusted to approximately  $10^8$  CFU/ml. The solutions of the plant extract were applied to sterile filter paper disks (Whatman Number 1; 5 mm in diameter) to give the final concentrations of 2500 and 3000 µg/ml and placed on the surface of the inoculated medium. The plates were incubated at  $35^{\circ}$ C for 24 hrs. Antimicrobial activity was determined by measuring the diameter of the inhibited zone formed around the disk [18].

## Total phenol estimation

Total phenolic compound of methanolic fruit extract was determined by Folin-Ciocalteu method. For the preparation of the calibration curve, 1 ml aliquot of 0.025, 0.05, 0.075, 0.1, 0.2, and 0.3 mg/ml methanolic gallic acid solution was mixed with 5 ml of Folin-Ciocalteu reagent (10 times diluted) and 4 ml sodium carbonate (75 g/L). The absorbance at 765 nm was measured after 1 hr of incubation at 20°C in biochemical oxygen demand incubator, and the calibration curve was drawn. To the similar reagent, 1 ml methanolic fruit extract (4 mg/ml) was mixed as described above and after 1 hr of incubation the absorbance was measured at 765 nm. The total phenol content was expressed in milligrams of gallic acid equivalents (GAE)/g of the extract [19].

## The analysis of total flavonoid contents

The total flavonoid content of extract was determined according to the colorimetric method as described by Moreno *et al.* To do so, 0.5 ml of each extract was added to test tubes containing 0.1 ml of 10%  $Al(NO_3)_3$  (w/v), 0.1 ml of 1 M potassium acetate, and 4.3 ml of 80% ethanol. After incubation for 40 minutes at room temperature, the absorbance was determined at 415 nm. The total flavonoid content was expressed in milligrams of quercetin equivalents (QE)/g of the extract [20].

#### RESULTS

The methanolic fruit extract was found to contain noticeable amount of total phenol and total flavonoid. The total phenolic content and flavonoid content of MECC were found to be 117.63 mg GAE/g of extract

Table 1: α-amylase inhibitions by methanol extract of *Cucumis* callosus

Sample	Concentration (µg/ml)	% inhibition	IC <sub>50</sub> (μg/ml)
MECC	100	18.66±0.24	721.55
	200	30.41±0.14	
	400	36.33±0.27	
	800	53.49±0.27	
	1000	63.34±0.11	
Acarbose	100	33.15±0.69	345.52
(standard)			
	200	48.71±0.33	
	400	58.33±0.14	
	800	66.97±0.39	
	1000	73.38±0.27	

All determinations were carried out in triplicate manner and values are expressed as the mean±SEM. The  $IC_{s0}$  value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions. *C. callosus: Cucumis callosus,* MECC: Methanolic fruit extract of *Cucumis callosus* 

and 116.3 mg QE/g of extract, respectively, which play a major role in antidiabetic and antimicrobial activity. These quantitative determinations were done using the standard curve of gallic acid and quercetin.

# Evaluation of *in vitro* $\alpha$ -amylase inhibitory activity using MECC

There was a dose-dependent increase in percentage inhibitory activity against  $\alpha$ -amylase enzyme. The extract 100 µg/ml showed a percentage inhibition of 18.66 ± 0.24 and 63.34 ± 0.11 at 1000 µg/ml (Table 1). The concentration of extract required for 50% inhibition of  $\alpha$ -amylase (IC<sub>50</sub>) was found to be 721.55 µg/ml, and the IC<sub>50</sub> value of standard drug acarbose against  $\alpha$ -amylase was found to be 345.52 µg/ml.

# Evaluation of *in vitro* α-glycosidase inhibitory activity of MECC

The MECC revealed a significant inhibitory action on  $\alpha$ -glycosidase enzyme. The percentage inhibition of MECC at 100 µg/ml showed 30.63

Table 2: $\alpha$ -glycosidase inhibitions by methanol extract of
Cucumis callosus

Sample	Concentration (µg/ml)	% inhibition	IC <sub>50</sub> (μg/ml)
MECC	100	30.63±0.30	405.37
	200	39.17±0.46	
	400	56.52±0.23	
	800	64.38±0.27	
	1000	86.04±0.45	
Acarbose	100	40.5±0.13	236.20
(standard)			
	200	49.12±0.49	
	400	63.11±0.24	
	800	72.07±0.56	
	1000	91.32±0.18	

All determinations were carried out in triplicate manner and values are expressed as the mean $\pm$ SEM. The IC<sub>50</sub> value is defined as the concentration of inhibitor to inhibit 50% of its activity under the assayed conditions. *C. callosus: Cucumis callosus*, MECC: Methanolic fruit extract of *Cucumis callosus* 

 $\pm$  0.30 and 86.04  $\pm$  0.45 at 1000 µg/ml (Table 2). The concentration of extract required for 50% inhibition of  $\alpha$ -glycosidase (IC\_{50}) was found to be 405.37 µg/ml, whereas the IC\_{50} value of standard drug acarbose against  $\alpha$ -glycosidase was found to be 236.20 µg/ml.

# Evaluation of in vitro antimicrobial activity of MECC

The antibacterial and antifungal activities of MECC against 20 laboratory bacterial strains belong to 6 Gram-positive and 14 Gramnegative species and 4 fungal species were tested using agar dilution method as well as by disc diffusion method. The results showed that methanol extract of *C. callosus* has antibacterial activity against three strains of Bacillus species, four strains of *S. aureus*, one strain of *Klebsiella pneumoniae* 114, *Vibrio cholerae* 117, *S. typhimurium* NCTC74, *S. dysenteriae* 7, *E. coli* DH5 $\alpha$ , and *S. boydi* (RC). It inhibited the growth of 13 bacterial species and all fungal species at a concentration of 2000 µg/ml and did not show antibacterial activity against the remaining pathogenic bacterial species. The methanol extract of *C. callosus* fruit showed stronger and broader spectrum of antimicrobial activity.

## DISCUSSION

Although there are citations of *in vitro* antioxidant activity of alcoholic extract and aqueous extract of seeds of *C. callosus*, there are no previous reports on the activity of this extract on *in vitro*  $\alpha$ -amylase and  $\alpha$ -glycosidase activity [12,13]. Our *in vitro* studies demonstrated an appreciable  $\alpha$ -glycosidase and  $\alpha$ -amylase inhibitory activity of MECC.

 $\alpha$ -amylase and  $\alpha$ -glycosidase are two main enzymes in the human body that are responsible for the breakdown of polysaccharides to more simple sugars. The  $\alpha$ -amylase hydrolyzes complex polysaccharides to produce oligosaccharides and disaccharides which are then hydrolyzed by  $\alpha$ -glycosidase to monosaccharides. The monosaccharides are absorbed through the small intestine into the hepatic portal vein and increase postprandial glucose level [21]. Slowing the digestion and absorption of starch by amylase

Bacteria	Gram-positive/ Gram-negative	Control	200 µg/ml	400 μg/ml	800 µg/ml	1200 µg/ml	1600 μg/ml	2000 μg/ml
S. aureus ML 351	G+	+	+	+	+	+	-	-
S. aureus ML 314	G+	+	+	+	+	+	+	-
S. aureus ML 264	G+	+	+	+	+	+	+	-
S. aureus ML 267	G+	+	+	+	+	+	+	-
B. subtilis UC 564	G+	+	+	+	-	-	-	-
B. pumilus 8241	G+	+	+	+	+	+	-	-
B. cereus PI 11778	G+	+	+	+	+	+	-	-
V. cholerae 117	G-	+	+	+	+	+	+	-
K. pneumoniae 114	G-	+	+	+	+	+	+	-
K. pneumoniae 1	G-	+	+	+	+	+	+	+
K. pneumoniae 725	G-	+	+	+	+	+	+	+
<i>E. coli</i> DH 5α	G-	+	+	+	+	-	-	-
S. dysenteriae 7	G-	+	+	+	+	-	-	-
S. boydi 8	G-	+	+	+	+	+	+	+
S. boydi RC	G-	+	+	+	-	-	-	-
S. boydi ET 6552 (9)	G-	+	+	+	+	+	+	+
S. choleraesuis (36)	G-	+	+	+	+	+	+	+
S. typhimurium 74	G-	+	+	+	+	+	+	-
P. vulgaris 21	G-	+	+	+	+	+	+	+
P. aeruginosa	G-	+	+	+	+	+	+	+
ATCC-27853								
C. albicans MTCC 183		+	+	+	+	+	+	-
A. flavus MTCC 1973		+	+	+	+	+	-	-
A. niger MTCC 16404		+	+	+	+	-	-	-
C. albidus MTCC 183		+	+	+	+	+	+	-

+ : Growth of microorganisms, – : No growth of microorganisms. MIC: Minimum inhibitory concentration, MECC: Methanolic fruit extract of *Cucumis callosus*, ATCC: American Type Culture Collection, MTCC: Microbial Type Culture Collection. *S. aureus: Staphylococcus aureus*, *B. subtilis: Bacillus subtilis*, *B. pumilus: Bacillus upmilus*, *B. cereus: Bacillus cereus*, *V. cholera: Vibrio cholera*, *K. pneumoniae: Klebsiella pneumoniae*, *E. coli: Escherichia coli*, *S. dysenteriae: Shigella dysenteriae*, *S. boydi: Shigella boydi*, *S. choleraesuis: Salmonella choleraesuis*, *S. typhimurium: Salmonella typhimurium*, *P. vulgaris: Proteus vulgaris*, *P. aeruginosa: Pseudomonas aeruginosa*, *C. albicans: Candida albicans*, *A. flavus: Aspergillus flavus*, *A. nige: Aspergillus niger*, *C. albidus: Cryptococcus albidus* 

Bacteria	Gram-positive/	2500 μg/ml	3000 μg/ml	Standard	Standard	Standard
	drain negative			pentennin	emorampitemeor	Griscoluivin
S. aureus ML 351	G+	14	15	28	31	
S. aureus ML 314	G+	13	16	34	38	
S. aureus ML 264	G+	13	15	21	28	
S. aureus ML 267	G+	12	12	30	34	
B. subtilis UC 564	G+	10	12	18	25	
B. pumilus 8241	G+	8	11	17	26	
B. cereus PI 11778	G+	10	12	14	20	
V. cholerae 117	G-	12	13	24	32	
K. pneumoniae 114	G-	12	14	27	30	
<i>E. coli</i> DH 5α	G-	10	11	34	38	
S. dysenteriae 7	G-	13	15	24	30	
S. boydi RC	G-	18	20	33	34	
S. typhimurium 74	G-	10	12	17	30	
C. albicans MTCC 183		10	16			34
A. flavus MTCC 1973		15	17			30
A. niger MTCC 16404		15	16			30
C. albidus MTCC 183		17	18			28

Values are zone of inhibition (mm, including the diameter of the bore [6 mm]). Penicillin (100 µg/ml), chloramphenicol (100 µg/ml). Griseofulvin (100 µg/ml). MECC: Methanolic fruit extract of *Cucumis callosus*, ATCC: American Type Culture Collection, MTCC: Microbial Type Culture Collection. *S. aureus: Staphylococcus aureus*, *B. subtilis: Bacillus subtilis*, *B. pumilus: Bacillus pumilus*, *B. cereus: Bacillus cereus*, *V. cholera: Vibrio cholera*, *K. pneumoniae: Klebsiella pneumoniae*, *E. coli: Escherichia coli*, *S. dysenteriae: Shigella dysenteriae*, *S. boydi: Shigella boydi*, *S. choleraesuis: Salmonella choleraesuis*, *S. typhimurium: Salmonella typhimurium*, *P. vulgaris: Proteus vulgaris*, *P. aeruginosa: Pseudomonas aeruginosa*, *C. albicans: Candida albicans*, *A. flavus: Aspergillus flavus*, *A. niger: Aspergillus niger*, *C. albidus: Cryptococcus albidus* 

inhibitors may have the beneficial effects on insulin resistance and glycemic index control in people with diabetes [22,23]. Preliminary phytochemical analysis of MECC indicated the presence of phenolic compounds, flavonoids, saponin, and terpenoids. Natural polyphenols have been reported to inhibit the activity of  $\alpha$ -amylase and  $\alpha$ -glycosidase [22].  $\alpha$ -amylase and  $\alpha$ -glycosidase inhibitory activity were also related only for oleanane, ursane, and lupeol type terpenoids [24]. The high content of phenolic compounds, saponin, terpenoids, and flavonoids in the extract may also be a contributing factor towards antidiabetic activity. The mechanism by which fruit extract suppressed postprandial hyperglycemia may be due to its action on carbohydrate binding regions of  $\alpha$ -amylase and  $\alpha\text{-glycosidase}$  enzymes that catalyze hydrolysis of the internal  $\alpha\text{-}1,$ 4 glycosidic linkages in starch and other related polysaccharides. Therefore, this study buttresses the claim that natural inhibitors from dietary plants have  $\alpha$ -amylase and  $\alpha$ -glycosidase inhibitory activity and could be used as effective therapy for the management of postprandial hyperglycemia with minimal side effects.

The tested fruit extract was more active against Gram-positive bacteria compared to Gram-negative. In general, Gram-negative bacteria are more resistant than Gram-positive bacteria [25,26]. The higher sensitivity of Gram-positive bacteria could be due to the exposure of the outer peptidoglycan layer while Gram-negative bacteria bear an extra outer membrane which includes the asymmetric distribution of the lipids with phospholipids and lipopolysaccharide located in the inner and outer leaflets, respectively, can act as additional barrier which hinders the movement of foreign substance into the cell [27,28]. The methanol is a better solvent for more consistent extraction of antimicrobial substances from medicinal plants compared to other solvents, such as water, ethanol, hexane, and chloroform [29,30]. Methanol extract was more active than the aqueous extract against all the human pathogenic organisms [31]. Antibacterial compounds isolated from a large number of plant species throughout the world play a role in plant defense, polyphenolic compounds being known to have multiple functions. Flavonoids, such as naringenin, flavone, and flavonol, including kaempferol, morin, and quercetin and constitute a large group of secondary plant metabolites that have been reported to have antimicrobial activities [32,33]. The high content of phenolic compounds, saponin, and flavonoids in the extract may also be a contributing factor towards antimicrobial activity. This is the first study to provide data that the MECC possess potential antibacterial and antifungal activities.

#### CONCLUSION

The results of this study support the folkloric use of this plant in the management of controlling diabetes and post-diabetic bacterial and fungal infections. However, further detailed scientific studies are essential to find out the fundamental mechanisms of antihyperglycemic and antimicrobial activity as well as isolation of active components responsible for this pharmacological property.

# ACKNOWLEDGMENTS

The author Siva Prasad Panda is grateful to AICTE, New Delhi, India, Department of Pharm Tech., Jadavpur University, Kolkata, India, and College of Pharmaceutical Sciences, Puri, for providing financial support to this work.

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