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Note

α-Glucosidase inhibitory activity of *Syzygium cumini* (Linn.) Skeels seed kernel in vitro and in Goto–Kakizaki (GK) rats

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Abstract—*Syzygium cumini* seed kernel extracts were evaluated for the inhibition of α-glucosidase from mammalian (rat intestine), bacterial (*Bacillus stearothermophilus*), and yeast (*Saccharomyces cerevisiae*, baker's yeast). In vitro studies using the mammalian α-glucosidase from rat intestine showed the extracts to be more effective in inhibiting maltase when compared to the acarbose control. Since acarbose is inactive against both the bacterial and the yeast enzymes, the extracts were compared to 1-deoxynojirimycin. We found all extracts to be more potent against α-glucosidase derived from *B. stearothermophilus* than that against the enzymes from either baker's yeast or rat intestine. In an in vivo study using Goto–Kakizaki (GK) rats, the acetone extract was found to be a potent inhibitor of α-glucosidase hydrolysis of maltose when compared to untreated control animals. Therefore, these results point to the inhibition of α-glucosidase as a possible mechanism by which this herb acts as an anti-diabetic agent.

Keywords: α-Glucosidase; Diabetes; *Syzygium cumini*; *Eugenia jambolana*; Goto–Kakizaki rats

Type 2 diabetes is a chronic metabolic disorder characterized by hyperglycemia as a result of diminished insulin secretion and insulin resistance of peripheral tissues including the liver, skeletal muscle, and adipose tissue.[1] More than 171 million people worldwide are currently believed to be afflicted with type 2 diabetes, and it is estimated that the number will rise to 366 million by 2030.[2]

α-Glucosidase (EC 3.2.1.0) and α-amylase (EC 3.2.1.1) are the key enzymes involved in the metabolism of carbohydrates. α-Amylase degrades complex dietary carbohydrates to oligosaccharides and disaccharides, which are ultimately converted into monosaccharides by α-glucosidases. Liberated glucose is then absorbed by the gut and results in postprandial hyperglycemia. Inhibition of intestinal α-glucosidases limits postprandial glucose levels by delaying the process of carbohydrate hydrolysis and absorption, making such inhibitors useful in the management of type 2 diabetes.[3] Plants and microorganisms have been a rich source of α-glucosidase inhibitors. For example, acarbose,[4] 1-deoxynojirimycin,[5] and genistein[6] have all been isolated from natural sources.

In this study, we report the inhibition of α-glucosidase by various extracts of *Syzygium cumini* (also called *Eugenia jambolana*) seed kernel and its hypoglycemic effect in Goto–Kakizaki (GK) rats. *S. cumini* of the Myrtaceae family is a tree found throughout India where it is called Jamun or Jambu. Jamun seeds and bark have been prescribed in Ayurvedic medicine for the treatment of diabetes and are also used as anti-inflammatory, antipyretic, astringent, and antidiarrheal agents.[7] Dried seed kernels of *S. cumini* were obtained and certified by Ganesh Aushadhi Bhandar (Mumbai, India), and a voucher specimen is kept in our laboratory. Initially, the seeds were extracted with 70% ethanol but an

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Table 1. Inhibition of α-glucosidase by extracts of *S. cumini*

Extract	IC_{50} (μg/mL)			
	Baker's yeast	*B. stearothermophilus*	Rat intestinal enzymes	
			Sucrase	Maltase
70% Ethanol	24.6 ± 0.7	4.6 ± 0.1	299.2 ± 3.3	120.9 ± 2.1
Acetone	19.5 ± 0.4	6.6 ± 0.2	261.7 ± 1.3	114.4 ± 1.1
Ethyl acetate[a]	16.6 ± 0.3	8.6 ± 0.3	237.7 ± 0.2	108.3 ± 0.4
1-Butanol[a]	8.3 ± 0.2	2.8 ± 0.1	190.2 ± 0.9	91.7 ± 0.1
1-Deoxynojirimycin	83.4 ± 2.1	0.175 ± 0.003	27.1 ± 0.1	20.2 ± 0.3
Acarbose	NI	NI	210 ± 1.2	233 ± 4.0
Gallic acid	NI	NI	NI	458.9
Ellagic acid	NI	NI	NI	NI

NI: no Inhibition at a concentration on 1 mg/mL.
[a] Obtained by sequential extraction from the acetone extract.

acetone extract was subsequently found to contain fewer interfering compounds, and this extract was used for further solvent fractionation with EtOAc and 1-butanol.

α-Glucosidase inhibitory activity was evaluated against the enzyme obtained from three different sources: mammalian (rat intestine), bacterial (*Bacillus stearothermophilus*) and yeast (*Saccharomyces cerevisiae*, baker's yeast). Both 70% ethanol and acetone extracts showed inhibition of all three sources of enzyme with the following order of activity: mammalian < yeast < bacterial when compared to 1-deoxynojirimycin as the control (Table 1). Specifically, the 70% ethanol extract was approximately five times more active against α-glucosidase obtained from *B. stearothermophilus* ($IC_{50} = 4.6 \pm 0.1$ μg/mL) than baker's yeast ($IC_{50} = 24.6 \pm 0.7$ μg/mL) and approximately 60 and 30 times more active against the mammalian enzymes, sucrase ($IC_{50} = 299 \pm 3.3$ μg/mL) and maltase (120.9 ± 2.1 μg/mL), respectively. Similarly, the acetone extract was three times more active against *B. stearothermophilus* ($IC_{50} = 6.6 \pm 0.2$ μg/mL) than baker's yeast ($IC_{50} = 19.5 \pm 0.4$ μg/mL) and approximately 40 and 15 times more active against the mammalian enzymes, sucrase ($IC_{50} = 261.7 \pm 1.3$ μg/mL) and maltase (114.4 ± 1.1 μg/mL), respectively.

The acetone extract was sequentially fractionated with EtOAc and 1-butanol. The EtOAc fraction showed only a small improvement in activity against baker's yeast, whereas inhibition against *B. stearothermophilus* decreased. Its IC_{50} for sucrase improved slightly, changing from 261.7 μg/mL to 237.7 μg/mL, and there was no dramatic increase in activity against maltase. The 1-butanol fraction showed a slight improvement in activity against rat intestinal sucrase and maltase and a two-fold improvement against the bacterial and yeast enzymes. This was undoubtedly the most active extract in this series.

When compared to acarbose as the control, only the mammalian enzyme was inhibited. This was expected since acarbose has been shown to be a potent inhibitor of mammalian sucrase and maltase and inactive against yeast and bacterial forms.[8] All the extracts showed a two to three times higher maltase inhibition than the acarbose control, whereas all the extracts demonstrated equivalent sucrase inhibition (Table 1). Ellagic and gallic acids were isolated from the acetone extract of *S. cumini*. Weak inhibition of maltase was observed for gallic acid, whereas ellagic acid was inactive under the assay conditions.

Herbs are often contaminated with heavy metals such as lead (Pb) and mercury (Hg),[9] which are known to denature enzymes. The crude, powdered herb was screened for the presence of toxic heavy metals using scanning electron microscopy (SEM) coupled with X-ray microanalysis. Crude *S. cumini* was found to contain the metals magnesium (Mg), zirconium (Zr), rhodium (Rh), and calcium (Ca); however, the heavy metals Pb and Hg were not detected in the sample. Therefore, the inhibition of α-glucosidase found in our experiments could not be due to the presence of the heavy metals Pb and Hg.

In an effort to evaluate the in vivo α-glucosidase inhibitory activity of this herb, we studied the effect of the acetone extract of *S. cumini* on GK rats. Following a maltose challenge (2 g/kg), both the untreated and *S.*

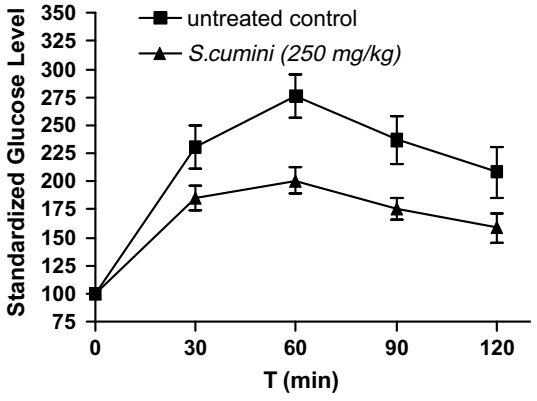

Figure 1. Standardized glucose levels of control and *S. cumini* treated GK rats following a maltose challenge.

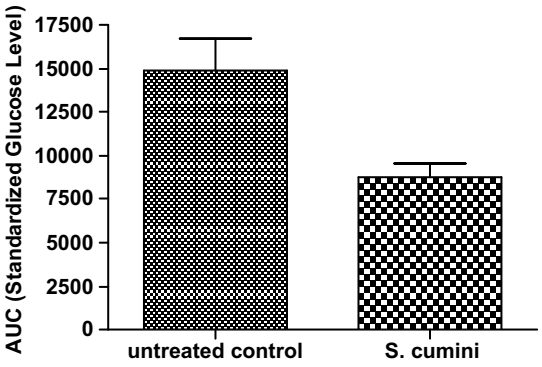

Figure 2. Standardized glucose AUC of control and *S. cumini* treated GK rats following a maltose challenge.

cumini treated (250 mg/kg) GK rats demonstrated a gradual glucose elevation, which peaked at around 60 min (Fig. 1). However, the treated GK rats had glucose levels that were significantly lower than the untreated rats throughout the oral maltose tolerance test (Fig. 1). This is evidenced by the fact that the glucose AUC (8727 ± 832) was significantly lower ($P < 0.05$) than the control (14910 ± 1857) (Fig. 2) by approximately 41%.

In this report, we show that the extracts of *S. cumini* are potent inhibitors of α-glucosidase, and in addition improves glucose tolerance in GK rats when challenged with maltose. Isolation of the active components is currently being pursued in our laboratory for the purpose of better understanding the mechanism of enzyme inhibition.

1. Experimental

1.1. Materials

α-Glucosidase from baker's yeast (Type I), from *B. stearothermophilus*, *p*-nitrophenyl α-D-glucopyranoside (*p*NPG), rat intestinal acetone powder, sucrose, maltose, and 1-deoxynojirimycin·HCl were purchased from Sigma Chemical Co. (St. Louis, MO). Glucose Autokit was purchased from Wako Pure Chem. Co. (Osaka, Japan). The dried seed kernels of *S. cumini* were obtained and certified by Ganesh Aushadhi Bhandar (Mumbai, India). A voucher specimen has been retained at the Department of Pharmaceutical Sciences at St. John's University. Other reagents were of analytical grade and were used without further purification. Male GK rats were purchased from Taconic Farms. Acarbose was isolated from Precose® 25 mg tablets (Bayer Pharmaceuticals Corporation, West Haven, CT). TrueTrack Smart System glucose meter was purchased from Walgreens pharmacy. Statistical analysis was performed with GraphPad Prism v. 4.

1.2. Preparation of EtOH extract

The powdered seed kernel was extracted in a Soxhlet apparatus with 70% EtOH for a minimum of 8 h. The ethanolic extract was concentrated at 37 °C under reduced pressure and lyophilized to yield a dark resinous material (12.6%).

1.3. Preparation of acetone extract and EtOAc and 1-BuOH fractions

The seed kernel of *S. cumini* was extracted in a manner described previously[10] with some modification. The powdered seed kernel was defatted with petroleum ether in a Soxhlet apparatus for 24 h and then extracted with acetone for 24 h. The acetone extract was evaporated to dryness at 37 °C under reduced pressure to yield a black viscous gum (8%). An aliquot of the acetone extract was suspended in distilled water and extracted with EtOAc in a liquid–liquid continuous extractor for 12 h. The EtOAc extract was evaporated to dryness at 37 °C under reduced pressure to yield a dark green solid (19%). The remaining aqueous layer was then extracted with 1-BuOH in a separatory funnel, and the 1-BuOH was evaporated to dryness at 37 °C under reduced pressure to yield a brown solid (28%).

1.4. Inhibition assay for α-glucosidase activity

1.4.1. With baker's yeast and *B. stearothermophilus* using *p*NPG as substrate. The assay is a modification of the procedure of Pistia-Brueggeman and Hollingsworth.[11] α-Glucosidase (2 μL, 1–2.5 U/mL) was premixed with 20 μL of *S. cumini* extracts at varying concentrations made up in 50 mM phosphate buffer at pH 6.8 and incubated for 5 min at 37 °C. 1 mM *p*NPG (20 μL) in 50 mM of phosphate buffer was added to initiate the reaction, and the mixture was further incubated at 37 °C for 20 min. The reaction was terminated by the addition of 50 μL of 1 M Na_2CO_3, and the final volume was made up to 150 μL. α-Glucosidase activity was determined spectrophotometrically at 405 nm on a Biorad 550 microplate reader (Hercules, CA) by measuring the quantity of *p*-nitrophenol released from *p*NPG. The assay was performed in triplicate. The concentration of the extract required to inhibit 50% of α-glucosidase activity under the assay conditions was defined as the IC_{50} value. The results are reported as $\%I \pm SD$.

1.4.2. With rat intestine using either sucrose or maltose as substrate. Rat intestinal acetone powder (200 mg) was hand homogenized using 10 mL of ice-cold 50 mM phosphate buffer. The contents were centrifuged at 8000 rpm for 25 min. The supernatant was applied to a Sephadex G-100 column and eluted with ice-cold 50 mM phosphate buffer to isolate the α-glucosidase

used for the assay. The reaction mixture consisted of crude enzyme solution (as sucrase, 0.2 mL; as maltase 0.05 mL), substrate (sucrose, 56 mM, 0.2 mL; maltose, 5 mM, 0.3 mL). Test samples and substrates were made up in 50 mM phosphate buffer (pH 6.7). The amount of liberated glucose was measured by the glucoseoxidase method using a commercially available Autokit from Wako.

1.5. In vivo GK rat studies

All animal experimental protocols were approved by the Animal Care Committee of St. John's University. In all tolerance tests, ten 5–6-week-old male GK rats, fasted for 16 h, were used. Control rats were orally administered saline 1 h prior to a maltose challenge (2.0 g/kg). Treated GK rats were orally administered the acetone extract of *S. cumini* (250 mg/kg) 1 h prior to the maltose or sucrose challenge (2.0 g/kg). Tail blood glucose levels were determined every 30 min until resting levels were achieved. Data are expressed as the mean ± standard error of the mean (SEM). Significance in AUC between treated and untreated animals was determined using Student's *t*-test.

1.6. Scanning electron microscopy

The methods for sample preparation and spectrum acquisition were followed according to a protocol published by Goldstein and Newbury.[12] Briefly, the crude herb was mounted on a carbon stub, carbon-coated to conduct electricity by means of carbon fiber evaporation, and subjected to X-ray microanalysis. Elemental composition of crude *S. cumini* was determined under vacuum with an acceleration voltage of 20 kV.

References

1. DeFronzo, R. A. *Diabetes Rev.* **1997**, *5*, 177–269.
2. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. *Diabetes Care* **2004**, *27*, 1047–1053.
3. Bell, D. S. H. *Am. J. Med.* **2004**, *116*, 23S–29S.
4. Schmidit, D. D.; Frommer, W.; Junge, B.; Muller, L.; Wingender, W.; Truscheit, E.; Schafer, D. *Naturwissenschaften* **1977**, *64*, 535–536.
5. Asano, N.; Tomioka, E.; Kizu, H.; Matsui, K. *Carbohydr. Res.* **1994**, *253*, 235–245.
6. Lee, D.-S.; Lee, S.-H. *FEBS Lett.* **2001**, *501*, 84–86.
7. Ross, I. A. *Syzygium cumini* (Linn.) Skeels. In *Medicinal Plants of the World*. In *Chemical Constituents, Traditional and Modern Medicinal Uses*, 2nd ed.; Humana Press: Totowa, NJ, 2003; Vol. 1, pp 445–454.
8. Kim, Y.-M.; Wang, M.-H.; Rhee, H.-I. *Carbohydr. Res.* **2004**, *339*, 715–717.
9. Obi, E.; Akunyili, D.; Ekpo, B.; Orisakwe, O. *Sci. Total Environ.* **2006**, *369*, 35–41.
10. Bhatia, I. S.; Bajaj, K. L. *Planta Med.* **1975**, *28*, 346–352.
11. Pistia-Brueggeman, G.; Hollingsworth, R. I. *Tetrahedron* **2001**, *57*, 8773–8778.
12. Goldstein, J.; Newbury, D. E.; Echlin, P. In *Scanning Electron Microscopy and X-Ray Microanalysis: A Text for Biologists, Materials Scientists, and Geologists*; Plenum Press: New York, 1981; pp 453, 461.