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Article

Alpha-amylase inhibitors: Cinnamomum cassia and Camellia sinensis extracts against type II diabetes

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SUMMARY

The main enzyme involved in starch digestion is α-amylase. Starch digestion can result in glucose spikes, thus increasing the risk of type II diabetes. Inhibiting starch digestion can help reduce said risk. A common synthetic α-amylase inhibitor is acarbose; however, it may cause gastrointestinal side effects, and it is expensive to purchase. Natural inhibitors can offer safer and more accessible alternatives. Our study evaluates the inhibitory effects of *Cinnamomum cassia* (cassia cinnamon) and *Camellia sinensis* extracts (green tea) on α-amylase and looks at their potential to reduce side effects and possibilities for future pharmaceutical applications. We hypothesized that *C. cassia* and *C. sinensis* will inhibit α-amylase activity. In this study, various concentrations of *C. cassia* and *C. sinensis* solutions were tested using a starch-hydrolysis assay. Our research shows that *C. cassia* and *C. sinensis* extracts effectively inhibit α-amylase as both compounds significantly decrease the degree of color change. This suggests that these compounds may be used for prevention and management of type II diabetes and there is a possibility for dual-therapy approaches aimed at lowering synthetic drug dosages and reducing harmful side effects.

INTRODUCTION

Metabolic disorders are multifactorial. They have several causes, including physical inactivity, excessive calorie intake, genetic factors, and insulin resistance (1,2). An example of a metabolic disorder is Type II diabetes. This condition occurs when the body does not produce enough of the hormone insulin, or produces ineffective insulin which results in insulin resistance (3). Insulin resistance can trigger inflammation and oxidative stress. This reduces the insulin-responsive cells' ability to control blood glucose levels leading to chronic hyperglycemia, which can damage blood vessels, increasing the risk of atherosclerosis, myocardial infarction, and stroke (1).

Blood glucose levels are also dependent on the degree of carbohydrate digestion by enzymes in the digestive system (4). Amylase is a hydrolytic enzyme that catalyses the breakdown of α-1-4-glycosidic bonds in starch into smaller sugars such as maltose, that are then converted into glucose (5). Multiple types of amylases exist, including salivary amylase (AMYI) and pancreatic amylase (AMYII) (6). Salivary amylase alters starch texture by breaking down some glycosidic linkages, shortening the carbon chains. This occurs in the mouth at the start of the digestive process (6). Pancreatic amylase breaks down the rest of the carbon chains in the small intestine (6). This study focused on α-amylase, present mainly in the pancreas and the salivary glands, the main enzyme involved in the hydrolysis of starch (made up of amylose and amylopectin) into glucose, releasing glucose into the bloodstream (7). In individuals with Type II diabetes, blood glucose regulation is impaired. Inhibiting this enzyme can reduce starch digestion, resulting in lowered blood glucose levels.

Amylase activity can be inhibited through two primary mechanisms: competitive and non-competitive inhibition. Competitive inhibitors resemble the enzyme's substrate and compete for the active site, binding only to the free enzyme. Acarbose, a commonly used antidiabetic drug, is a competitive inhibitor that structurally mimics the substrate and binds directly to the active site, preventing starch hydrolysis (8). However, synthetic α-amylase inhibitors such as acarbose, are often associated with harmful side effects, including gastrointestinal disturbances and hepatotoxicity (8).

There are also natural α-amylase inhibitors, common in many plants, that include bioactive compounds such as flavonoids, tannins, and polyphenols (9). Among the most accessible and well-studied sources are *C. cassia* and *C. sinensis*. Both extracts exhibit non-competitive inhibition by binding to alternative sites on the enzyme, thereby reducing catalytic effect without occupying the active site (10). Because of their two different mechanisms of action, acarbose and these two extracts present an opportunity for a potential dual-therapy approach for type II diabetes management.

By exploring ideas such as incorporating natural inhibitors into diabetes management strategies, this research addresses a gap in current literature. Such alternatives would not only reduce treatment side effects, but also improve quality of life and make medication more accessible for low-income populations affected by type II diabetes, which currently impacts over 850 million individuals worldwide (11). The aim of our study is to evaluate the inhibitory effects of *C. cassia* and *C. sinensis* on α-amylase activity, due to their potential to become more accessible treatment options for individuals with Type II diabetes. We hypothesised that natural compounds would exhibit some degree of α-amylase inhibition. To determine if *C. cassia* and *C. sinensis* are able to inhibit α-amylase activity, we used a colorimetric starch-iodine assay. Our results showed that both species exhibited significant α-amylase inhibition with *C. cassia* showing a stronger inhibition than *C. sinensis*, indicated by a darker colour of solution. These results show that *C. cassia* and *C. sinensis* could potentially be employed in type II diabetes treatment and dual-therapy approaches.

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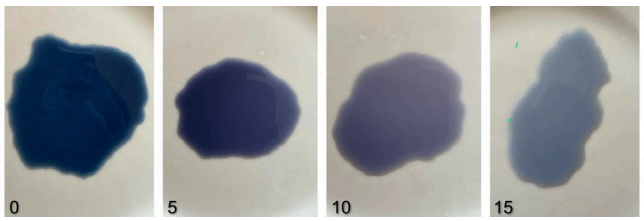

Figure 1: Color change of 0.25% Camellia sinensis extract, measured by a starch-iodine assay. The color change of 0.25% C. sinensis extract 0, 5, 10, and 15 minutes after the addition of 4mL of 1% starch solution. The color change indicates the level of starch digestion by α-amylase, a darker color indicating lower starch digestion and a lighter color indicating greater starch digestion.

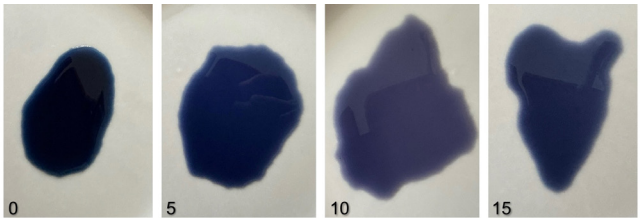

Figure 2: Color change of 0.25% Cinnamomum cassia extract, measured by a starch-iodine assay. The color change of 0.25% C. cassia extract 0, 5, 10, and 15 minutes after the addition of 4mL of 1% starch solution. The color change indicates the level of starch digestion by α-amylase, a darker color indicating lower starch digestion and a lighter color indicating greater starch digestion.

RESULTS

To evaluate the inhibitory power of *C. sinensis* extracts and *C. cassia* on α-amylase, we performed a starch iodine test. We inferred the inhibitory power from the brightness of the solution. When in contact with starch, iodine solution will go from a yellow-brown colour to a blue-black colour. A darker solution indicates a larger amount of starch present. The brighter the solution the more starch has been broken down by the α-amylase; therefore the amount of starch has decreased, and the inhibition was less effective.

We looked at three concentrations of each species, 0.25%, 0.125%, and 0.0625% (w/v), as well as a control of 0% concentration. The colour change was recorded at the start and every 5 minutes for 15 minutes (**Figures 1 and 2**). There was a significant decrease in solution brightness in 0.25% and 0.0625% extracts of *C. sinensis* extracts compared to the control (**Figure 3**). The 0.25% extract showed the lowest increase in brightness, reflecting the strongest inhibition (linear regression analysis, p=0.0163, R^2= 0.87). The 0.0625% extract also showed significant inhibition (p=0.03, R^2= 0.86). The results with 0.125% concentration were not statistically significant (p=0.2458, R^2= 0.55).

C. cassia also demonstrated a significant decrease in solution brightness (**Figure 4**). The greatest inhibitory power was shown in 0.25% concentration of C. cassia, presented by the lowest brightness increase (p<0.0001, R^2= 0.76) followed by 0.125% concentration (p=0.0001, R^2= 0.75). However, the results with 0.0625% concentration were not statistically significant (p=0.1338, R^2= 0.75).

Taken together the data suggests that *C. cassia* and *C. sinensis* extracts significantly inhibit α-amylase. When the 0.25% concentration solutions of *C. cassia and C. sinensis* were compared, *C. cassia* showed a significantly greater decrease in brightness (p=0.0082, R^2= 0.76).

DISCUSSION

Although both species exhibited significant α-amylase inhibition, *C. cassia* had a greater inhibitory power than *C. sinensis*. This is likely due to differences in the chemical nature and potency of their bioactive compounds, such as polyphenols. Polyphenols in *C. cassia* are thought to contribute strongly to α-amylase inhibition (12). However, *C. sinensis* is rich in catechins, a subgroup of polyphenols, which have shown to inhibit the enzyme, but may interact differently with α-amylase, resulting in a different inhibitory power (13,14). These findings show promise because compared to synthetic inhibitors like acarbose, both *C. cassia* (cassia cinnamon) and *C. sinensis* (green tea) are low-cost and widely available in supermarkets and herb stores worldwide and are generally free from side effects (15,16). This makes them viable options for communities with limited healthcare access, particularly low and middle income countries where 81% of adults with Type II diabetes reside (17). In these settings, prescription medications may be unaffordable or unavailable, therefore, natural inhibitors could serve as a cost-effective alternative. In such contexts, even partial glycemic control through dietary means can substantially improve long-term health outcomes (18,19). Individuals with high blood glucose levels could benefit from regularly including these natural products in their diet. When *C. cassia* is added to meals or *C. sinensis* extracts are consumed, the breakdown of starch into glucose slows, leading to reduced glucose absorption (20). This helps lower glucose spikes after meals, preventing insulin resistance (20). In addition, both *C. cassia* and *C. sinensis* have been associated with improved metabolic health and decreased oxidative stress (21). This supports overall glycemic stability.

However, while promising, natural inhibitors are not yet sustainable replacements for pharmaceutical treatments. One important limitation is dosage consistency. Unlike prescription medications, which provide exact dosages tailored to individual needs, the concentration of bioactive compounds in natural sources can vary significantly. For example, one cup of green tea can contain anywhere between 20-80 mg of Epigallocatechin gallate (EGCG), a catechin present in *C. sinensis* that has been shown to inhibit α-amylase (13, 22). This variation makes it difficult to predict physiological effects with precision, which is essential for clinical use. However, with further research these inconsistencies can be overcome. This would require isolating and stabilizing the active compounds from natural sources, which would allow for the production of supplements with reliable dosages. This could pave the way for several practical applications: early dietary intervention in prediabetic individuals, combination therapies, and even the development of new drug classes incorporating both synthetic and plant-derived inhibitors.

It is important to acknowledge that our study has certain limitations. The extractions of *C. cassia* and *C. sinensis* may not have captured consistent concentrations of bioactive compounds. Catechins and polyphenols in *C. sinensis* are water-soluble and therefore were likely extracted by the soaking method used. However, *C. cassia* contains water and fat-soluble compounds, and while water-soluble polyphenols

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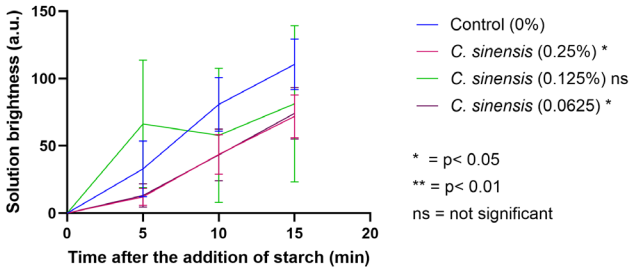

Figure 3: Camellia sinensis extract solution brightness over time. Data contained within this figure represents an analysis of the brightness of solution as displayed in Figure 1. The y-axis represents the relative solution brightness (a.u.), with increasing values indicating a color change from dark to light, indicating the amount of starch present over a 15 minute period. Significance determined relative to control.

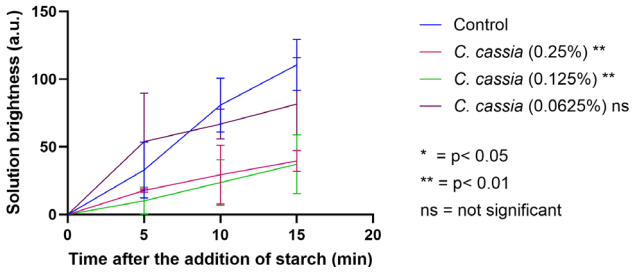

Figure 4: Cinnamomum cassia extract solution brightness over time. Data contained within this figure represents an analysis of the brightness of solution as displayed in Figure 2. The y-axis represents the relative solution brightness (a.u.), with increasing values indicating a color change from dark to light, reflecting how much starch is left in the solution across a 15 minute period. Significance determined relative to control.

were extracted, fat-soluble compounds likely were not. This may have influenced the inhibitory power observed.This study relied on a colorimetric starch-iodine assay, using brightness as an indicator of enzyme activity, which was measured using the digital program ImageJ. As a home-based investigation, the experiment was subject to potential inaccuracies: external lighting, camera sensitivity, and ambient conditions may have affected brightness reading. Furthermore, the lack of kinetic analysis such as Michaelis-Menten modelling or inhibitor constant (Ki) calculations limit the depth of insight into inhibition mechanisms. While accessible, the starch-iodine method is less precise than direct glucose quantification techniques or spectrophotometric measurements. Therefore, while the results are promising and suggest potential inhibitory effects of cinnamon and green tea, further investigation using standardised methods and precise instrumentation is needed to validate these observations and determine their pharmacological significance. Techniques such as HPLC could be used to accurately quantify the concentrations of bioactive compounds present.

In conclusion, the findings of our investigation highlight the potential role of plant-derived inhibitors in type II diabetes treatment strategies. Looking forward, it is possible that future treatments may include hybrid pharmaceuticals combining established drugs with purified natural compounds, which could minimize the side-effects of synthetic α-amylase inhibitors. With further research and more precise dosing, natural inhibitors could become useful complementary agents in managing type II diabetes especially as part of lifestyle or dual therapies alongside conventional medication.

MATERIALS AND METHODS

Solution preparation

Separate solutions of 1% starch and α-amylase were prepared by dissolving 1 gram of cornstarch (Maizena) and 1 gram of α-amylase powder (Gozdawa) into 100 mL of pre-boiled purified water in separate beakers at approximately 60°C. The mixtures were stirred with a glass rod until fully dissolved and translucent, then cooled to room temperature. Inhibitor solutions were prepared using a similar method. *C. sinensis* extract was prepared by steeping 1 g of green tea (Messmer) in 100 mL of purified water at 80°C for 10 minutes with gentle stirring. This was done to ensure that as many polyphenols and catechins as possible are extracted. The solution was filtered and cooled to room temperature. Concentrations of 0.5% and 0.25% were obtained using serial dilutions.

C. cassia extract was prepared by boiling a 1 g *C. cassia* stick (Fuchs) in 100 mL of purified water at 100°C for 30 minutes with occasional stirring. The solution was filtered and cooled to room temperature (25°C). Serial dilutions were performed to obtain 0.5% and 0.25% concentrations.
The control solution was 100 mL of pre-boiled water at room temperature. The iodine solution was prepared by adding 10 drops of iodine solution (Reig Jofre) to 5 mL of pre-boiled water at room temperature (25°C).

Experimental Procedure

A water bath was maintained 37-40°C using a hot plate to simulate human body temperature. In separate test tubes, 2 mL of 1% α-amylase solution was combined with 2 mL of inhibitor solution at concentrations of 0% (control), 0.25%, 0.5%, and 1%. Test tubes were incubated in the water bath for 5 minutes, with occasional gentle stirring to allow sufficient enzyme-inhibitor interaction. Subsequently, 4 mL of 1% starch solution was added to each test tube and mixed gently. Therefore, the final tested solutions were 0.25%, 0.125%, and 0.0625%.

Color change analysis

Immediately, 1 mL was withdrawn from each mixture and mixed with 1 mL of dilute iodine solution on a white ceramic surface. The resulting color change was recorded using an iPad (A16) camera (Apple) as an indication of starch presence. This test was repeated every 5 minutes for a total duration of 15 minutes. A digital program, ImageJ.js, was used to quantify brightness levels. The image was uploaded to the program and the region with the solution was outlined to isolate it from the background, brightness values were only measured in the selected area. ImageJ provided a single value for the outlined region. This was the value used to estimate enzyme inhibition.

Statistical analysis

The experiment was conducted with three technical replicates. The mean increase in solution brightness for each inhibitor concentration at each time point was calculated. A

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linear regression analysis was performed using the Graphpad Prism (version 10.6.1) application. The calculation of the goodness of fit value (R^2) was done with the WPS Office (version 2025 Update 12.2.0.23196 32-bit).

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REFERENCES

1. "About Insulin Resistance and Type 2 Diabetes." *Centers for Disease Control and Prevention.* www.cdc.gov/diabetes/about/insulin-resistance-type-2-diabetes.html. Accessed 14 Apr. 2025
2. "Obesity." *Mayo Clinic.* www.mayoclinic.org/diseases-conditions/obesity/symptoms-causes/syc-20375742. Accessed 15 Apr. 2025
3. "What Is Type 2 Diabetes?" *NHS*, 10 Feb. 2025, www.nhs.uk/conditions/type-2-diabetes/what-is-type-2-diabetes/. Accessed 1 Feb. 2026
4. Holesh, Julie E, et al. "Physiology, Carbohydrates." *StatPearls*, StatPearls Publishing, 2023.
5. "PDB101: Molecule of the Month: Alpha-Amylase." *RCSB: PDB-101*, 2019, https://pdb101.rcsb.org/motm/74. Accessed 15 Apr. 2025.
6. Akinfemiwa, Ololade, et al. "Amylase." *StatPearls,* StatPearls Publishing, 2023.
7. Date, Kimie, et al. "α-Amylase Expressed in Human Small Intestinal Epithelial Cells Is Essential for Cell Proliferation and Differentiation." *Journal of Cellular Biochemistry*, vol. 121, no. 2, 1 Feb. 2020, https://doi.org/10.1002/jcb.29357.
8. "Acarbose." *LiverTox: Clinical and Research Information on Drug-Induced Liver Injury,* National Institute of Diabetes and Digestive and Kidney Diseases, 2012.
9. Kashtoh, Hamdy, and Kwang-Hyun Baek. "New Insights into the Latest Advancement in α-Amylase Inhibitors of Plant Origin with Anti-Diabetic Effects." *Plants*, vol. 12, no. 16, 14 Aug. 2023, https://doi.org/10.3390/plants12162944.
10. Forester, Sarah C., et al. "Inhibition of Starch Digestion by the Green Tea Polyphenol, (−)-Epigallocatechin-3-Gallate." *Molecular Nutrition & Food Research*, vol. 56, no. 11, 1 Nov. 2012, https://doi.org/10.1002/mnfr.201200206.
11. "Diabetes Facts & Figures." *International Diabetes Federation*. https://idf.org/about-diabetes/diabetes-facts-figures/. Accessed 15 Apr. 2025.
12. Alzeina, Oula, and Lina Soubh. "Evaluation of Polyphenols' Inhibitory Activity of α-Amylase in Cinnamon." *Research Journal of Science and Technology*, vol. 11, no. 3, 2019, https://doi.org/10.5958/2349-2988.2019.00026.3.
13. Ćorković, Ina, et al. "Dietary Polyphenols as Natural Inhibitors of α-Amylase and α-Glucosidase." *Life*, vol. 12, no. 11, 25 Oct. 2022, https://doi.org/10.3390/life12111692.
14. Gonçalves Bortolini, Débora, et al. "Processing, Chemical Signature and Food Industry Applications of Camellia Sinensis Teas: An Overview." Food Chemistry: X, vol. 12, no. 100160, 30 Dec. 2021, https://doi.org/10.1016/j.fochx.2021.100160
15. Gu, Dan-Tong, et al. "Safety of Cinnamon: An Umbrella Review of Meta-Analyses and Systematic Reviews of Randomized Clinical Trials." *Frontiers in Pharmacology*, vol. 12, no. 790901, 18 Jan. 2022, https://doi.org/10.3389/fphar.2021.790901.
16. Younes, Maged, et al. "Scientific Opinion on the Safety of Green Tea Catechins." *EFSA Journal*, vol. 16, no. 4, Apr. 2018, https://doi.org/10.2903/j.efsa.2018.5239.
17. International Diabetes Federation. "Global." *Diabetes Atlas*, 4 Apr. 2025, diabetesatlas.org/data-by-location/global/. Accessed 1 Feb. 2026.
18. del Bosque-Plata, Laura, and Claudia Gragnoli. "Cinnamon Treatment Shows Promise for Glycemic Control but May Cause Adverse Effects in Some People." *Clinical Nutrition Open Science*, vol. 59, 10 Jan. 2025, pp. 184–188, https://doi.org/10.1016/j.nutos.2025.01.003.
19. Tolmie, M., et al. "The Potential Antidiabetic Properties of Green and Purple Tea [*Camellia sinensis* (L.) O Kuntze], Purple Tea Ellagitannins, and Urolithins." *Journal of Ethnopharmacology*, vol. 309, 12 June 2023, https://doi.org/10.1016/j.jep.2023.116377.
20. Magistrelli, Ashley, and Jo Carol Chezem. "Effect of Ground Cinnamon on Postprandial Blood Glucose Concentration in Normal-Weight and Obese Adults." *Journal of the Academy of Nutrition and Dietetics*, vol. 112, no. 11, 1 Nov. 2012, https://doi.org/10.1016/j.jand.2012.07.037.
21. Prasanth, Mani, et al. "A Review of the Role of Green Tea (*Camellia Sinensis*) in Antiphotoaging, Stress Resistance, Neuroprotection, and Autophagy." *Nutrients*, vol. 11, no. 2, 23 Feb. 2019, https://doi.org/10.3390/nu11020474.
22. ConsumerLab.com. "Big Differences Found among Green Teas." *ConsumerLab.com*, 2018, www.consumerlab.com/news/big-differences-among-green-teas/05-10-2018/. Accessed 15 Apr. 2025.

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