

# Evaluation of $\alpha$ -Amylase and $\alpha$ -Glucosidase Inhibitory Activity of Flavonoids

Chunhe Gu, Han Zhang, Clarisa Yosa Putri, Ken Ng\*

Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Australia

\*Corresponding author: Ken Ng, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Royal Parade, Parkville, Melbourne, Victoria, Australia, Tel: 61-3-90353141; E-mail: [ngkf@unimelb.edu.au](mailto:ngkf@unimelb.edu.au)

## Abstract

A number of phenolic acids and flavonoids were screened for inhibitory activity against rat intestinal  $\alpha$ -glucosidase and porcine pancreatic  $\alpha$ -amylase. The phenolic acids were not inhibitory to both enzymes at 1 mM. Inhibition of  $\alpha$ -amylase by flavonoids mirrored the inhibition of  $\alpha$ -glucosidase in that both baicalein and myricetin inhibited the enzyme most strongly, but differs in that spectrum of flavonoid inhibition for  $\alpha$ -amylase only restricted to these two flavonoids while it was more wide spread for the inhibition of  $\alpha$ -glucosidase. Baicalein inhibited  $\alpha$ -amylase and  $\alpha$ -glucosidase activity by  $41.1\pm 2.6\%$  and  $56.9\pm 1.7\%$  at 1 mM, respectively. Myricetin inhibited  $\alpha$ -amylase and  $\alpha$ -glucosidase activity by  $35.9\pm 3.8\%$  and  $47.7\pm 2.4\%$  at 1 mM, respectively. The IC<sub>50</sub> (concentration required for 50% reduction activity) of baicalein ( $0.713\pm 0.034$  mM) and myricetin ( $1.030\pm 0.026$  mM) confirmed that baicalein is the most potent flavonoid inhibitor against  $\alpha$ -glucosidase in the flavonoid series studied.

**Keywords:** Diabetes;  $\alpha$ -Amylase inhibitors;  $\alpha$ -Glucosidase inhibitors; Baicalein; Myricetin; Flavonoids

Received Date: December 10, 2015  
Accepted Date: December 24, 2015  
Published Date: December 28, 2015

**Citation:** Ng, K., et al. Evaluation of A-Amylase and A-Glucosidase Inhibitory Activity of Flavonoids. (2015) Int J Food Nutr Sci 2(2): 174-179.

DOI: 10.15436/2377-0619.15.042



## Introduction

Controlling postprandial hyperglycaemia at the early pre-diabetes stage is an important preventative strategy against developing full blown diabetes mellitus and in glycemic control for diabetics (DM)<sup>[1]</sup>. The WHO advises a controlled and balanced diet with low calorie intake and also active lifestyle with certain degree of exercise to lower the risk of developing DM<sup>[2]</sup>. For long term treatment and management of DM, medical approaches usually involve drugs that can be used together with lifestyle interventions for glycemic control and delay the onset of diabetic complications<sup>[3]</sup>. Several pharmaceutical drugs are currently available to treat DM and are clinically prescribed in monotherapy or in combination therapy that usually included  $\alpha$ -glucosidase inhibitors (AGIs), to increase efficacy in glycemic control and modulation of postprandial hyperglycaemia<sup>[4-6]</sup>.

Synthetic AGIs such as acarbose, voglibose, and miglitol have been available as oral anti-diabetic drugs since early 1990s, and acarbose has become the most commonly prescribed AGI<sup>[7]</sup>. Unlike the other oral anti-diabetic drugs, AGIs act locally in the gastrointestinal tract by inhibiting intestinal starch digestion<sup>[8]</sup>. However, treatment with AGIs is costly which is made worst by the long-term requirement for the drugs in diabetes<sup>[6]</sup>. Furthermore, the very strong inhibitory action of acarbose against pancreatic  $\alpha$ -amylase limits starch digestion that promotes undigested starch fermentation in the colon, which resulted in unpleasant gastrointestinal side effects such as increases production of gases, flatulence, abdominal pain, and diarrhoea. These side effects are responsible for significant discontinuation of AGIs therapy by patients<sup>[6,7]</sup>.

Flavonoids are a class of plant secondary metabolites with a basic structure of a 15-carbon skeleton consisting a benzene ring (ring B) and a heterocyclic ring (ring A and C). Over 9000 flavonoid species have been identified to date and the number is still growing<sup>[8]</sup>. Flavonoids are specially enriched in foods like parsley, blueberries, citrus, cocoa, peanut, black and green tea, and are also found in medicinal herbs<sup>[9-11]</sup>. Several plant products have been shown to have potential for use in DM treatment. For instance,

grape skin extract has been shown to inhibit mammalian intestinal α-glucosidase activity and suppresses postprandial glycemic response in streptozocin-treated mice<sup>[12]</sup>. Green tea extract has been shown to strongly inhibit rat intestinal α-glucosidase<sup>[9]</sup>, and the *in vitro* effects of the combined treatments of green tea flavonoid extracts and acarbose have been shown to produce synergistic effect in inhibiting α-amylase and α-glucosidase at low enzyme concentrations<sup>[13]</sup>. All these evidence indicate that flavonoids have great potential in DM prevention and management by controlling postprandial hyperglycaemia.

In this paper we compare the inhibition of mammalian α-amylase and α-glucosidase by different classes of flavonoids so as to get a better understanding of their contribution to inhibitory activities in plant foods or herbs. We also raised the issue of using starch/3,5-dinitrosalicylic acid reagent in accessing the inhibition of α-amylase by flavonoids.

## Experimental Section

### Chemicals

Acarbose (A8980), 3,5-dinitrosalicylic acid (DNS; 128848), p-nitrophenyl-α-D-glucopyranoside (pNPG; N7014), p-nitrophenyl-α-D-maltopentaoside (pNPG5; N1519), porcine pancreatic α-amylase (A4262), rat intestinal acetone powder (I1630) were from Sigma-Aldrich (Castle Hills, NSW).

The following phenolic acids and flavonoids were also from Sigma-Aldrich (Castle Hills, NSW). Phenolic acids: p-Coumaric acid (C9008), 3, 4-dihydroxybenzoic acid (protocatechuic acid; 37580), ferulic acid (128708), 4-hydroxybenzoic acid (240141), sinapic acid (D7927), and 3,4,5-trihydroxybenzoic acid (gallic acid; 398225). Flavonoids: Apigenin (A3145), baicalein (465119), baicalin (572667), chrysin (C80105), daidzein (D7802), (±)-catechin (C1788), (-)-epigallocatechin gallate (E4143), (-)-epigallocatechin (E3768), flavone (F20003), galangin (282200), genistein (G6649), 5-hydroxyflavone (H4405), 6-hydroxyflavone (411035), 7-hydroxyflavone (H4530), isocitricitrin (quercetin 3-O-β-D-glucoside; 17793), kaempferol (K0133), oroxylin A (O0641), myricetin (70050), naringenin (N5893), pinocembrin (P5239), quercetin (Q0125), rutin (quercetin 3-O-β-D-rutinoside; R5143).

All other chemicals and organic solvents used were of analytical-grade or better. Deionised water (18 mΩ) used was produced using a Synergy UV Millipore System (Millipore).

### Preparation of rat intestinal α-glucosidase

Mixture of rat intestinal α-glucosidase was prepared by suspending 1 g of freeze-dried rat intestinal acetone powder with 25 mL of PBS. The mixture was maintained chilled in an ice/water bath and sonicated using a tip sonicator with  $f = 50$  Hz (Unisonics, Sydney, Australia) for 8 min. The sonicated mixture was then centrifuged for 30 minutes at 14,000 rpm using a bench top centrifuge and the supernatant containing the α-glucosidase was recovered, pooled and kept at -20°C until use. Protein content was determined to be 0.21 mg protein/mL using the Bradford method and bovine serum albumin as the protein standard (Sigma-Aldrich).

### Assay of α-glucosidase activity

Rat intestinal α-glucosidase activity was assayed using pNPG substrate and spectroscopic determination of the liberat-

ed p-nitrophenol by absorbance measurement as previously described<sup>[9]</sup>. 100 μL of phenolic acid or flavonoid inhibitor (10 mM in DMSO) was mixed with 100 μL of rat intestinal α-glucosidase (0.21 mg protein/mL) and 700 μL of buffer (0.12 M potassium phosphate pH 6.8 + 1 % w:w NaCl) in an Eppendorf tube. The mixture was pre-incubated at 37 °C for 5 min after which 100 μL of pNPG (2.5 mM in water) was added and the reaction mixture further incubated at 37°C for 60 min. Termination of reaction was achieved by chilling the tubes in ice/water bath, rather than using NaOH or Tris alkaline buffer to avoid changes in absorbance of mixture due to instability of flavonoids in alkaline conditions. The absorbance of the liberated p-nitrophenol was promptly measured at λ 410 nm using a spectrophotometer (Novaspec II, USA). Reaction tubes without added pNPG substrate and with or without inhibitor were used as blank to correct for α-glucosidase extract colour and inhibitor absorbance. The percentage Inhibition of α-glucosidase activity was calculated by using the formula:

$$\left[ 1 - \left( \frac{\text{Absorbance}_{\text{corrected for enzyme color}}^{\text{control incubation}} - \text{Absorbance}_{\text{corrected for extract + enzyme color}}^{\text{Extract incubation}}}{\text{Absorbance}_{\text{Corrected for enzyme color}}^{\text{Control incubation}}} \right) \right] \times 100\%$$

### Determination of IC50 for α-glucosidase activity

The IC50s (concentration of inhibitor required to reduce 50% of enzyme activity) for α-glucosidase activity were determined for strong inhibitory compounds by measuring % inhibition of activity at a range of inhibitor concentrations. Enzyme activity versus inhibitor concentration plot was then produced for each inhibitor and data points were fitted by a non-linear regression method (sigmoidal) to fit the non-linear concentration dependent of enzyme-inhibitor interaction at low and high concentrations<sup>[14]</sup>. All measurements were done in triplicate and the mean IC50±SD were obtained.

### Assay of α-amylase activity

α-Amylase activity was assayed using the chromogenic substrate p-nitrophenyl-α-D-maltopentaoside (PNPG5) as described by Funke and Melzig<sup>[15]</sup> with modifications. The assay was adapted to microplate platform using 96 wells microplate. Porcine pancreas α-amylase was diluted to 50 units/mL in buffer (20 mM sodium phosphate buffer pH 6.8 + 7 mM NaCl + 1 mM CaCl<sub>2</sub>). PNPG5 was made up to 25 mM in the same buffer. 50 μL of PNPG5, 10 μL of flavonoids (in DMSO), and 190 μL buffer were added to each well, and 50 μL of enzyme was added to initiate the reaction. Absorbance was measured at λ 405 nm using a Multiscan Spectrum microplate reader (Multiskan GO, Thermo Scientific, Australia) at 4 minute intervals for up to 120 minutes. Absorbance for control enzyme activity without inhibitor and for inhibitor background without added enzyme was also acquired. The percentage inhibition of α-amylase activity was calculated by the ratio of the linear gradients of control and extract incubation.

$$\% \text{ Inhibition } (\alpha\text{-amylase}) = \left[ 1 - \frac{\text{Slope of reaction vs time trend line of inhibitor}}{\text{Slope of reaction vs time trend line of control}} \right] \times 100\%$$

### Statistical analysis

Percentage Inhibition and IC50 values were expressed as mean±SD from triplicate determinations. Uncertainty of the mean was reported to 2 significant figures according to the Euro-

pean Analytical Chemist Guidelines<sup>[16]</sup>. Statistical comparisons of means were performed using one-way analysis of variance (ANOVA) followed by the Fisher's protected Least Significant Difference Test using SAS software version 9.2. Analyses with  $p < 0.05$  were considered to be statistically different.

## Results and Discussion

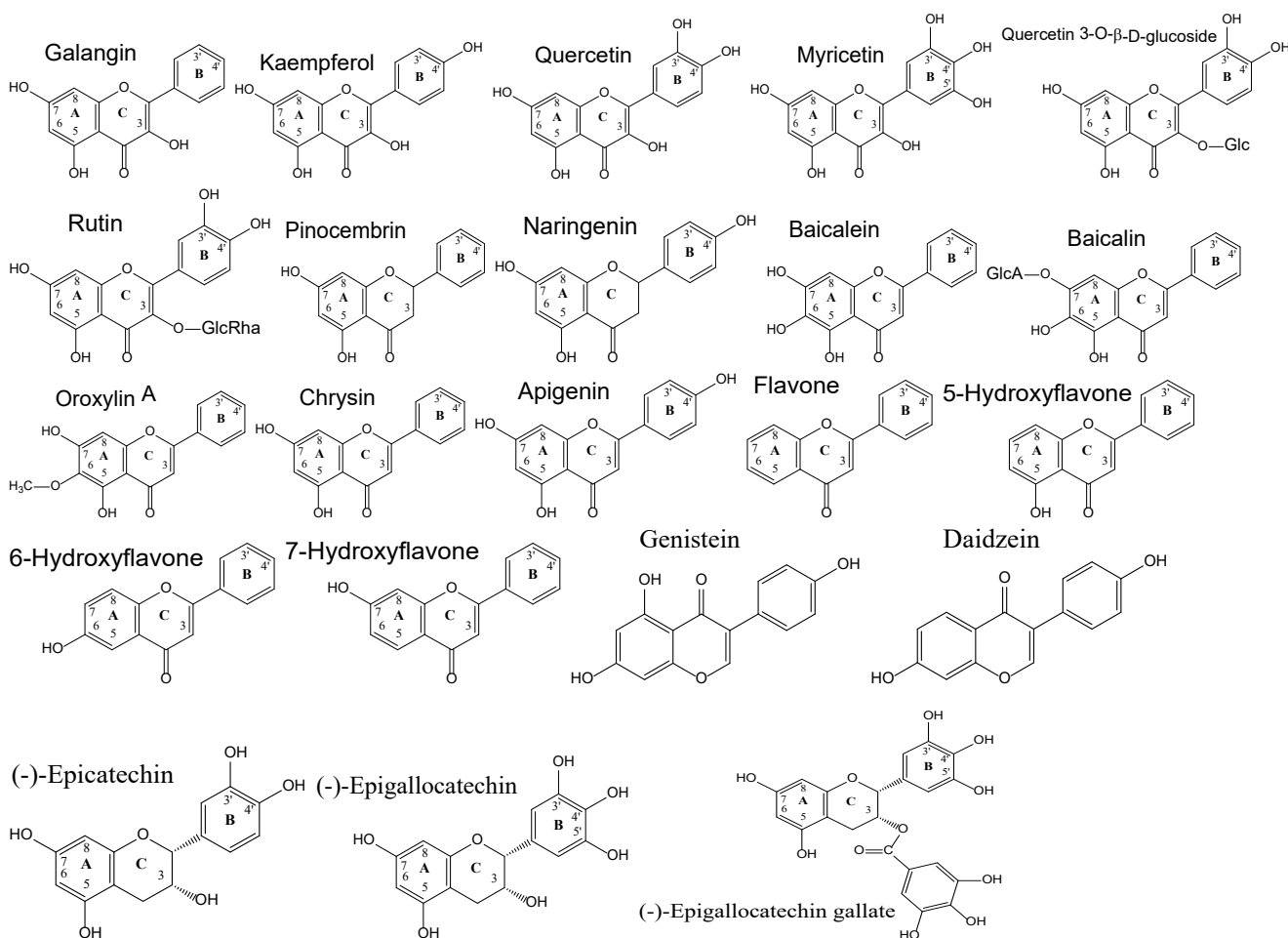
### Inhibition of rat intestinal $\alpha$ -glucosidase by flavonoids

Figure 1 shows the structures of the flavonoids studied, which are flavones (flavone, 5-hydroxyflavone, 6-hydroxyflavone, 7-hydroxyflavone, chrysin, baicalein, baicalin, oroxylin A), isoflavones (genistein, daidzein), flavonols (galangan, kaempferol, quercetin, myricetin), flavanones (pinocembrin, naringenin), and flavanols ( $(\pm)$ -catechin, (-)-epigallocatechin, (-)-epigallocatechin gallate). Initial screening of flavonoid inhibitory activity against rat intestinal  $\alpha$ -glucosidase as an indication of its anti-hyperglycemic potential as inhibitor of starch digestion was carried out at a fixed concentration of 1 mM. This is so that comparisons to be made between and within the five subclasses of flavonoids (Table 1). Some hydroxybenzoic acids (4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, 3,4,5-trihydroxybenzoic acid) and hydroxycinnamic acids (*p*-coumaric acid, ferulic acid, sinapic acid) were also screen for inhibitory activity.

None of the hydroxybenzoic and hydroxycinnamic ac-

ids was inhibitory (data not shown). Of the flavone series, baicalein was the most potent inhibitor. Apigenin, chrysin, baicalin and oroxylin A were weak inhibitors, and flavone, 5-hydroxyflavone, 6-hydroxyflavone and 7-hydroxyflavone were not inhibitory. Of the flavonols series, myricetin was the strongest inhibitor while galangan, kaempferol and quercetin were weak inhibitors. Both isoquercitrin and rutin which have sugar attached to the C-ring C3-OH were not inhibitory. Of the flavanols series, epigallocatechin gallate was the strongest inhibitors while epigallocatechin was weak and catechin not inhibitory. The isoflavones genistein and daidzein, and the flavanones pinocembrin and naringenin were weak inhibitors.

Clearly, the flavonoid structure and the position and number of hydroxyl groups are determining factors for  $\alpha$ -glucosidase inhibition. The primary importance of the A-ring C5-OH, C6-OH and C7-OH group was cleared as shown by the inhibitory effect of the flavones series, which were in accord with previous reports<sup>[17-20]</sup>. Modification of any one of the hydroxyl groups in the A-ring diminished inhibitory activity as seen with oroxylin A (methylated C6-OH) and baicalin (glucuronidated C7-OH). Likewise, reduced number of hydroxyl groups in the A-ring also diminished inhibitory activity as seen with flavone, 5-hydroxyflavone, 6-hydroxyflavone, 7-hydroxyflavone, and chrysin and apigenin (both lacking C6-OH). The three B-ring OH groups in myricetin mitigated for the lack of A-ring C6-OH in the structure for its observed inhibitory activity that was comparable to baicalein.



**Figure 1:** Structures of flavonoids studied

**Table 1:** Inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase activity

Compounds	Porcine pan-creas $\alpha$ -amy-lase	rat intestinal	$\alpha$ -glucosi-dase
	% Inhibition (1 mM)*	% Inhibition (1 mM)*	IC <sub>50</sub> (mM)**
<b>Flavones</b>			
Flavone	0	0	-
5-Hydroxyflavone	0	0	-
6-Hydroxyflavone	4.5±1.1 <sup>c</sup>	0	-
7-Hydroxyflavone	4.8±3.6 <sup>c</sup>	0	-
Chrysin (5,7-dihy-droxyflavone)	5.8±1.1 <sup>c</sup>	12.8±1.4 <sup>f</sup>	-
Apigenin	5.7±1.7 <sup>c</sup>	14.4±1.5 <sup>c</sup>	-
Baicalein (5,6,7-tri-hydroxyflavone)	41.1±2.6 <sup>b</sup>	56.9±1.7 <sup>b</sup>	0.713±0.034 <sup>a</sup>
Baicalin	13.2±2.4 <sup>d</sup>	23.4±2.7 <sup>d</sup>	4.62±0.38 <sup>d</sup>
Oroxylin A	3.3±2.8 <sup>c</sup>	11.1±2.3 <sup>f</sup>	-
<b>Isoflavones</b>			
Genistein	0	2.9±1.1 <sup>h</sup>	-
Daidzein	0	7.6±1.7 <sup>g</sup>	-
<b>Flavonols</b>			
Galangan	0	12.8±3.0 <sup>f</sup>	-
Kaempferol	0	11.2±1.3 <sup>f</sup>	-
Quercetin	0	16.1±1.6 <sup>c</sup>	-
Myricetin	35.9±3.8 <sup>c</sup>	47.7±2.4 <sup>c</sup>	1.030±.026 <sup>b</sup>
Isoquercitrin	0	0	-
Rutin	0	0	-
<b>Flavanones</b>			
Pinocembrin	0	3.4±1.7 <sup>h</sup>	-
Naringenin	0	6.0±1.6 <sup>g</sup>	-
<b>Flavanols</b>			
(±)-Catechin	0	7.2±1.8 <sup>g</sup>	-
(-)-Epigallocatechin	0	13.6±3.5 <sup>c,f</sup>	-
(-)-Epigallo-cat-echingallate	0	22.7±1.1 <sup>d</sup>	2.208±0.038 <sup>c</sup>
<b>Control</b>			
Acarbose	81.3±1.6 <sup>a</sup>	77.2±2.6 <sup>a</sup>	0.541±0.036 <sup>c</sup>

\* Inhibitory effect of compounds was measured at 1 mM and percentage inhibition was relative to control reaction without added inhibitor.

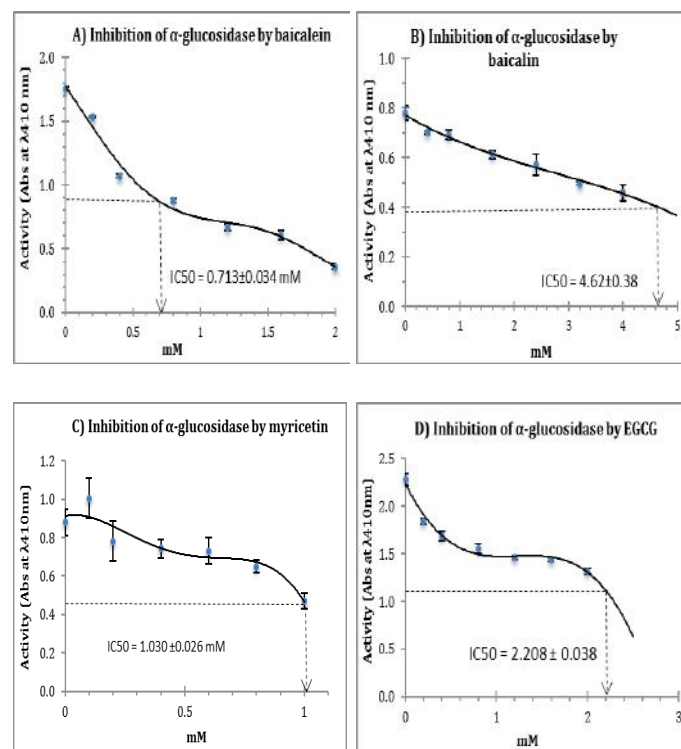
\*\* Concentration of compound that produced 50% inhibition of activity derived from non-linear Percentage Inhibition versus Inhibitor Concentration plots.

- Not determined.

<sup>a,b,c</sup> Values in the same column followed by different superscript were significantly different (P<0.05) in ANOVA and LSD test.

In order to get a better evaluation of the potency of the inhibitory flavonoids, the IC<sub>50</sub>, which is the concentration of inhibitor required for 50% reduction in activity, were determined (Table 1). Baicalein was the most potent inhibitor with an IC<sub>50</sub> of 0.713 mM, followed closely by myricetin at 0.908 mM and (-)-epigallocatechin gallate at 2.208 mM. The most notable feature of the IC<sub>50</sub> results was that none of the inhibitory flavonoids were able to completely inhibit  $\alpha$ -glucosidase activity at

high concentration (Figure 2). Mammalian  $\alpha$ -glucosidase such as those from rat intestine contains more than one enzyme activity from maltase-glucoamylase (MGAM) and sucrase-isomaltase (SI) complexes<sup>[21,22]</sup>. MGAM and SI have been shown to utilise the  $\rho$ NPG substrate with different Km and the inhibitors were suggested to be selective towards one or more of these enzymes, which would explain why complete inhibition of enzyme activity was not observed at higher flavonoid concentration. Thus, it is not possible to obtain meaningful inhibition constants (K<sub>i</sub>) from double reciprocal plots of substrate-inhibitor concentrations using such crude extracts. Nonetheless, comparison of IC<sub>50</sub> showed that the inhibitory potency of baicalein was closed to that of acarbose, a pharmaceutically prescribed  $\alpha$ -glucosidase inhibitor, which has an IC<sub>50</sub> of 0.541 mM measured in the same system.

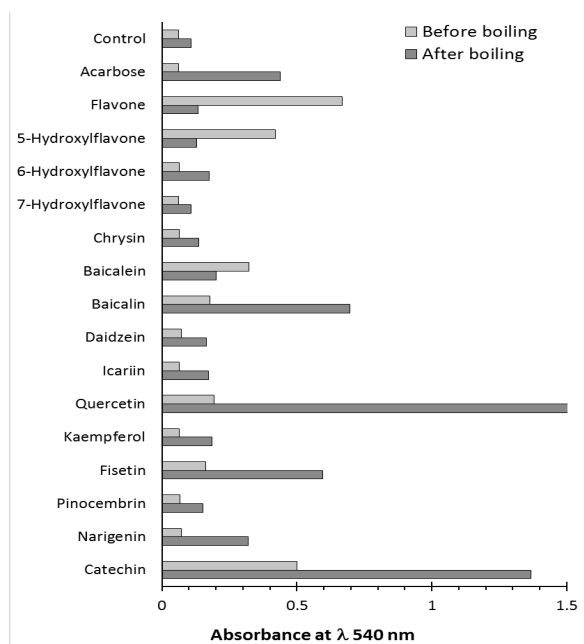


**Figure 2:** IC<sub>50</sub> of flavonoids against rat intestinal  $\alpha$ -glucosidase Activity data points (absorbance) were plotted as mean±SD (n=3).

**Inhibition of porcine pancreatic  $\alpha$ -amylase by flavonoids**

Xu, et al.<sup>[23]</sup> raised the issue of reducing activity of some polyphenols with 3,5-dinitrosalicylic acid (DNS) that is commonly employed in the assay of  $\alpha$ -amylase activity with starch as substrate. This is because the activity assay depends on the reduction of DNS by liberated glucose in generating the chromogen measured by its absorbance at  $\lambda_{max}$  540 nm. Furthermore, evidence showed significant higher *in vivo* digestion rate of high amylose corn starch compared to normal and waxy corn starch in the presence of tea polyphenols, indicating that interactions between polyphenols and amylose which might have enhanced substrate binding to  $\alpha$ -amylase<sup>[24]</sup>. Another compounding factor of the method unsuitability with polyphenols is the required boiling step in NaOH solution to generate the chromogen, condition in which some polyphenols would be unstable. Flavonoid that contains the catechol or gallate structural element such as flavonols and catechin are known to undergo auto-oxidation and conjugation reactions in alkaline solution<sup>[8]</sup>. Indeed, the

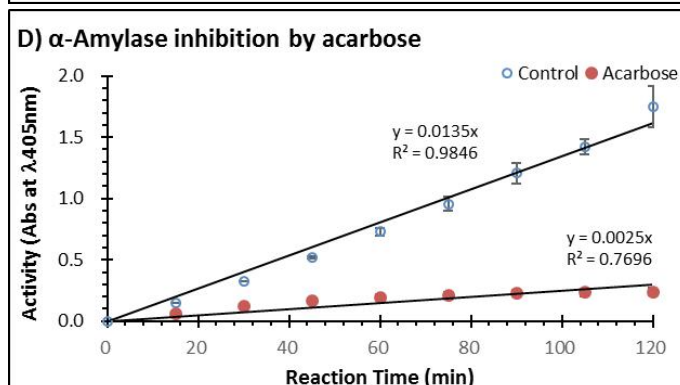
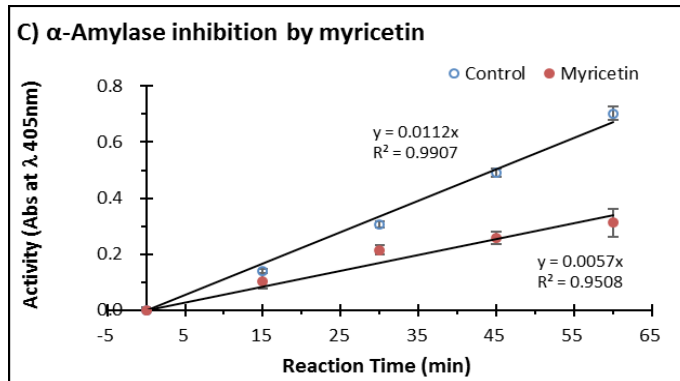
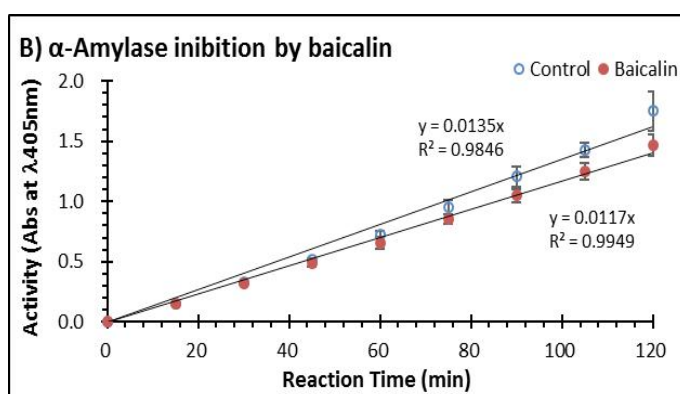
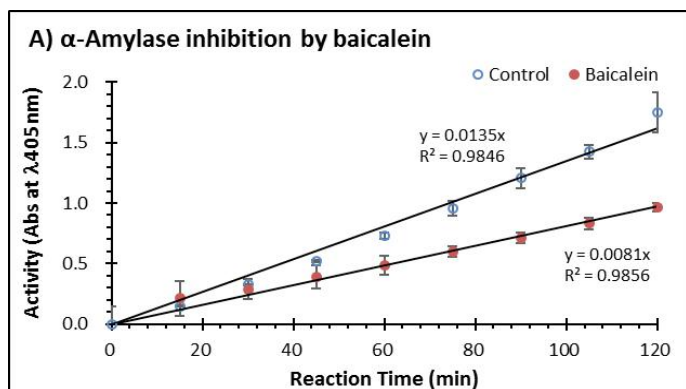
absorbance of the boiling mixture used in the starch-DNS assay of  $\alpha$ -amylase activity in the absence of starch and enzyme is enhanced at varying degree for the different flavonoids (Figure 3), indicating secondary chemical reactions have occurred. The absorbance changes with baicalein, quercetin and catechin were most prominent. Even acarbose produced significant absorbance changes with boiling. The instability of flavonoids in the boiling procedure rendered this method not suitable for measuring  $\alpha$ -amylase activity with flavonoids. It raised questions about the validity of reports that used starch-DNS method in evaluating  $\alpha$ -amylase inhibitory potential of phenolic acids and flavonoids as documented in some reviews<sup>[5,11,25]</sup>.



**Figure 3:** Effect of boiling on the stability of flavonoids in the starch-DNS assay.

Absorbance of mixtures containing buffer (20 mM sodium phosphate buffer pH 6.8 + 7 mM NaCl + 1 mM CaCl<sub>2</sub>) and DNS reagent (1% DNS [w:v] + 12% Na-K-tartrate [w:v] in 0.4 M NaOH in 2:1 ratio [v:v]) before and after boiling (water bath, 20 min). Flavonoid or acarbose was added as DMSO solution to a final concentration of 1mM and 10% DMSO (v:v).

Hence, an alternative method that employed a chromogenic malto-pentaoside substrate was employed which is similar to the  $\alpha$ -glucosidase assay in that liberated p-nitrophenol is measured by absorbance to indicate enzyme activity.



**Figure 4:** Inhibition of porcine pancreatic  $\alpha$ -amylase. Enzyme activity was measured in the absence (control) or present of inhibitor (at 1 mM reaction concentration). Activity data points were plotted as mean $\pm$ SD.

Figure 4 shows the linear enzyme kinetics with time for porcine pancreatic  $\alpha$ -amylase in the absence or presence of baicalein, baicalin or acarbose that can be obtained by this method. The inhibition can be accurately determined by the ratio of the slope gradients of inhibitor over that of control (without inhibitor). The results (Table 1) showed that inhibition of  $\alpha$ -amylase by flavonoids mirrored the inhibition of  $\alpha$ -glucosidase in that both baicalein and myricetin inhibited the enzyme strongly, but differs in that spectrum of flavonoid inhibition for  $\alpha$ -amylase only restricted to these two flavonoids.

None of the hydroxybenzoic and hydroxycinnamic acids inhibited  $\alpha$ -amylase activity measured at 1 mM (data not shown). Baicalein was the most potent inhibitor with 41.1% inhibition and that loss of or modification of the A-ring OH groups resulted in diminished or loss of inhibitory activity, as can be seen with the inhibition effect in the flavones series. The primary importance of the A-ring C6-O was highlighted by the fact that apigenin, chrysin and oroxylin A has little or no inhibitory activity while baicalin retained some. Myricetin is the only

compound outside of the flavones series that exhibited strong inhibitory activity with 35.9% inhibition, and again this can be attributed to its B-ring hydroxylation feature but its inhibition was weaker than baicalein.

The proposed structural requirements of flavonoids for inhibiting  $\alpha$ -amylase have been raised by Piparo, et al.<sup>[26]</sup> based on molecular modelling. Similar to acarbose, the inhibitory activity of flavonoids was attributed to two interactions: the hydroxyl groups in the flavonoid molecular structure can form hydrogen bonds with the OH groups in active side chains of functional amino acids of the enzyme, and conjugated  $\pi$ -system is likely to be formed between the AC ring system and the indole Trp<sup>59</sup> in the enzyme. Both the interactions can hinder the reaction between  $\alpha$ -amylase and starch and thus inhibit the starch digestion.

## Conclusion

The structural requirements of phenolic compounds for the inhibition of mammalian pancreatic  $\alpha$ -amylase and intestinal  $\alpha$ -glucosidase appear similar. The flavonoid structure and the C5, C6 and C7 OH groups of the A-ring are essential elements for inhibitory activity. Baicalein is the most potent bioactive flavonoid that inhibited both  $\alpha$ -amylase and  $\alpha$ -glucosidase. Baicalein is not a common plant flavonoid but is a major flavonoid from the root of *Scutellaria baicalensis* Georgi, which is a medicinal plant that has been used in Traditional Chinese Medicine<sup>[27]</sup>. The common food flavonol, myricetin, is also a strong flavonoid inhibitor of  $\alpha$ -amylase and  $\alpha$ -glucosidase activities. Delivery of these two bioflavonoids in concentrated form, for example as capsules, might have the potential to reduce postprandial hyperglycaemia with food intake by inhibiting starch digestion.

**Conflicts of Interest:** Author declare no conflict of interest.

## References

1. Smyth, S., Heron, A. Diabetes and obesity: the twin epidemics. (2006) *Nature Medicine* 12(1): 75-80.
2. Diabetes. (2014). World Health Organization.
3. Cheng, A.Y.Y., Fantus, I.G. Oral antihyperglycemic therapy for type 2 diabetes mellitus. (2005) *CMAJ* 172(2): 213-226.
4. Mizuno, C.S., Chittiboyina, A.G., Kurtz, T.W., et al. Type 2 Diabetes and Oral Antihyperglycemic Drugs. (2008) *Curr Med Chem* 15(1): 61-74.
5. Hung, H.Y., Qian, K., Morris-Natschke, S.L., et al. Recent discovery of plant-derived anti-diabetic natural products. (2012) *Nat Prod Rep* 29(5): 580-606.
6. Agarwal, A.A., Jadhav, P.R., Deshmukh, Y.A. Prescribing pattern and efficacy of anti-diabetic drugs in maintaining optimal glycemic levels in diabetic patients. (2014) *J Basic Clin Pharm* 5(3): 79-83.
7. Standl, E., Schnell, O. Alpha-glucosidase inhibitors 2012-cardiovascular considerations and trial evaluation. (2012) *DiabVasc Dis Res* 9(3): 163-169.
8. Crozier, A., Jaganath, I.B., Clifford, M. N. Dietary phenolics: chemistry, bioavailability and effects on health. (2009) *Nat Prod Rep* 26(8): 1001-1043.
9. Kee, K.T., Koh, M., Oong, L.X., et al. Screening culinary herbs for antioxidant and alpha-glucosidase inhibitory activities. (2013) *International Journal of Food Science and Technology* 48(9): 1884-1891.
10. Phan, M.A.T., Wang, J., Tang, J., et al. Evaluation of  $\alpha$ -glucosidase inhibition potential of some flavonoids from *Epimediumbrevivornum*. (2013) *Food Science & Technology* 53(2): 492-498.
11. Lacroix, I.M., Li-Chan, E.C. Overview of food products and dietary constituents with antidiabetic properties and their putative mechanisms of action: a natural approach to complement pharmacotherapy in the management of diabetes. (2014) *Mol Nutr Food Res* 58(1): 61-78.
12. Zhang, L., Hogan, S., Li, J., et al. Grape skin extract inhibits mammalian intestinal  $\alpha$ -glucosidase activity and suppresses postprandial glycemic response in streptozocin-treated mice. (2011) *Food Chemistry* 126(2): 466-471.
13. Gao, J., Xu, P., Wang, Y., Wang, Y., et al. Combined effects of green tea extracts, green tea polyphenols or epigallocatechingallate with acarbose on inhibition against  $\alpha$ -amylase and  $\alpha$ -glucosidase in vitro. (2013) *Molecules* 18(9): 11614-11623.
14. Burlingham, B.T., Widlanski, T.S. An Intuitive Look at the Relationship of Ki and IC50: A More General Use for the Dixon Plot. (2003) *J Chem Educ* 80(2): 214-218.
15. Funke, I., Melzig, M.F. Effect of different phenolic compounds on alpha-amylase activity: screening by microplate-reader based kinetic assay. (2005) *Pharmazie* 60(10): 796-797.
16. Quantifying uncertainty in analytical measurement 3<sup>rd</sup>Edn. (2012) Eurachem/CITAC guide.
17. Gao, H., Kawabata, J. Importance of the B Ring and Its Substitution on the  $\alpha$ -Glucosidase Inhibitory Activity of Baicalein, 5,6,7-Trihydroxyflavone. (2004) *Biosci Biotechnol Biochem* 68(9): 1858-1864.
18. Gao, H., Nishioka, T., Kawabata, J., et al. Structure-activity Relationships for  $\alpha$ -Glucosidase Inhibition of Baicalein, 5,6,7-Trihydroxyflavone: the Effect of A-Ring Substitution. (2004) *Biosci Biotechnol Biochem* 68(2): 369-375.
19. Gao, H., Kawabata, J. alpha-Glucosidase inhibition of 6-hydroxyflavones. Part 3: Synthesis and evaluation of 2,3,4-trihydroxybenzoyl-containing flavonoid analogs and 6-aminoflavones as alpha-glucosidase inhibitors. (2005) *Bioorg Med Chem* 13(5): 1661-1671.
20. Babu, H., Rao, V., Tiwari, A., et al. Synthesis and biological evaluation of novel 8-aminomethylated oroxylin A analogues as  $\alpha$ -glucosidase inhibitors. (2008) *Bioorg Med Chem Lett* 18(5): 1659-1662.
21. Jones, K., Sim, L., Mohan, S., et al. Mapping the intestinal alpha-glucogenic enzyme specificities of starch digesting maltase-glucoamylase and sucrase-isomaltase. (2011) *Bioorg Med Chem* 19(13): 3929-3934.
22. Dhital, S., Lin, A.H., Hamaker, B.R., et al. Mammalian mucosal alpha-glucosidase coordinate with alpha-amylase in the initial starch hydrolysis stage to have a role in starch digestion beyond glucogenesis. (2013) *PLoS One* 8(4): e62546.
23. Xu, H., Leng, X., Wang, M., et al. Glucose measurement in the presence of tea polyphenols. (2012) *Food Analytical Methods* 5(5): 1027-1032.
24. Liu, J., Wang, M., Peng, S., et al. Effect of green tea catechins on the postprandial glycemic response to starches differing in amylose content. (2011) *J Agric Food Chem* 59(9): 4582-4588.
25. Xiao, J., Ni, X., Kai G., et al. A review on structure-activity relationship of dietary polyphenols inhibiting  $\alpha$ -amylase. (2013) *Critical Review in Food Science and Nutrition* 53(5): 497-506.
26. Piparo, E.I., Scheib, H., Frei, N., et al. Flavonoids for controlling starch digestion: structural requirements for inhibiting human  $\alpha$ -amylase. (2008) *J Med Chem* 51(12): 3555-3561.
27. Li, W.L., Zheng, H.C., Bukuru, J., et al. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. (2004) *J Ethnopharmacol* 92(1): 1-21.