

THE EFFECTS OF *LAGERSTROEMIA SPECIOSA* CRUDE TEA EXTRACTS ON THE KINETICS OF PORCINE PANCREATIC ALPHA-AMYLASE

Jann Rochelle Carreon, KonradTomintz, and Nancy Lazaro Llanos Chemistry Department, De La Salle University Manila 2401 Taft Avenue Manila

Abstract: The effects of different concentrations of the crude tea extracts of Lagerstroemia speciosa, on the alpha-amylase activity were determined. Since Banaba or L. speciosa lowers blood sugar, researches were directed towards the effects of the plant to ailments such as obesity, hyperglycemia, and diabetes. Rice is the staple food in the Philippines and is primarily made up of starch, a homopolysaccharide made up of glucose units. In the small intestine, pancreatic amylase then hydrolyzes the partially digested starch into smaller oligosaccharides which is then excreted into the lumen, and The enzyme alpha-amylase or alpha-1, 4 - glucan - 4 - glucanhydrolyzed into glucose. glucanohydrolasescatalyzes the hydrolysis of alpha -1, 4 - D- glycosidic linkages. Alpha-amylase inhibitors have been shown to have an effect in starch digestion and amount of glucose in the bloodstream. The effect of various concentration of crude extracts of L. speciosa on the kinetics of alpha-amylase were determined using the Bernfeld Assay. Starch was used as the substrate whereas porcine pancreatin was the source of alpha-amylase. Various amounts of crude water extracts of Banaba leaves were The Michaelis - Menten constant (K_m) and the maximum velocity (V_{max}) were used. measured. Results show that the alpha-amylase activity with the crude extract at a concentration as low as 0.50-mL demonstrated a significant reduction of the K_m and V_{max} values. With an increase in the amount of the crude water extracts, there was a slight decrease on the kinetics signifying a noncompetitive or an uncompetitive inhibition. The results of the study may provide a basis to design a treatment for diabetes or to lower the blood glucose level of diabetic patients.

Keywords: alpha amylase; Km; Vmax; inhibitors

1. INTRODUCTION

Diabetes mellitus is a disorder in which there is an insulin deficiency or insulin ineffectiveness causing increasing the glucose concentration in the bloodstream. Since Banaba or *L. speciosa* lowers blood sugar, researches were directed towards the effects of the plant to ailments such as obesity, hyperglycemia, and diabetes. Alpha-amylase inhibitors have been shown to have an effect in starch digestion and amount of glucose in the bloodstream. Rice is the staple food in the Philippines and is primarily made up of starch. Starch is a polysaccharide that consists of glucose units linked together by glycosidic bonds. There are two components in starch: amylose; and amylopectin.



Amylose is bonded together by $(1\rightarrow 4)$ - α -D-glucose bonds in a linear formation while amylopectin has a linear formation of $(1\rightarrow 4)$ - α -D-glucose bonds but it is a highly branched in (1->6)- α -D-glucose units that occurs in every 24 to 30 glucose units. In digestion, amylase is the enzyme that breaks down starch into monosaccharides and disaccharides. Alpha-amylase is found in the saliva and pancreatin. In the small intestine, pancreatic amylase hydrolyzes the partially digested starch into smaller oligosaccharides which is then excreted into the lumen, and hydrolyzed into glucose, carried by the bloodstream, is absorbed by the cells. Therefore, inhibition of alpha amylase may have significant effect on the amount of glucose in the bloodstream.

2. METHODOLOGY

Sample Collection

The leaves were washed thoroughly and were dried by using tissue paper. The leaves were weighed to approximately 5.00-g and were placed in a beaker with 100-mL distilled water. The solution was boiled for 5-minutes. The solution was then filtered into a clean beaker, and was set aside to cool to room temperature.

Enzyme Assay

A schematic diagram of the enzyme assay is shown in Figure 1:



Figure 1: Preparation of Standard Curve

3.0 RESULTS AND DISCUSSION

FNH-I-007



The Km and Vmax gathered from the Michaelis Menten and Lineweaver Burke plots are shown in Figure 2. Results show a decrease in the Km and Vmax in the presence of various amounts of *L. Speciosa*. This indicates the possible presence of a competitive on non competitive inhibitors in the crude extract. The decrease in the Km values may be attributed to an increase affinity of the substrate for the enzyme while the decrease in Vmax demonstrates a decrease in the rate of hydrolysis of starch. Based on the results, a possible mechanism in maintaining the blood glucose level is shown in the proposed model (Figure 3).



Figure 2: Km and Vmax obtained from the Michaelis-Menten and Lineweaver-Burke Plots



Figure 3: Proposed Model (E= enzyme; S = substrate; I = Inhibitor)

4.0 CONCLUSIONS

The effects of varying concentrations of crude extracts of *Lagerstroemia speciosa* on the activity of alpha amylase were determined using Bernfeld Assay. The alpha

FNH-I-007



amylase activity in the presence of as low as 0.50-mL of the crude extract lowered the

Km and Vmax values both for trial 1 and trial 2, implicating, mixed inhibition: uncompetitive; and noncompetitive inhibition. Results show a minimal inhibitory activity of the leaf extracts against alpha amylase, thus, it is recommended to use higher concentrations of the crude extracts for further studies to give lower values of Km and Vmax. For uncompetitive inhibition, it is recommended to increase the volume of substrate to increase the binding affinity, and lower the value of Km to overcome the binding of the inhibitors

5.0 REFERENCES

- Ali, H., Houghton, P. J., & Soumyanath, A. (2006). α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. *Journal of Ethnopharmacology*, 107, (3) 449-455.
- Bernfeld, P. (1955). Amylases: α and β . *Methods in Enzymology*, 1, 149-158.
- Gibbs, B., Alli I. (1998). Characterization of a purified alpha amylase inhibitor from white kidney beans (*Phaseolus vulgaris*). Food Research and International, 31, 217-225.
- Goto, Y., Yamada, K., Ohyama, T., Matsuo, T., Odaka, H., & Ikeda, H. (1995). An aglucosidase inhibitor, AO-128, retards carbohydrate absorption in rats and humans. *Diabetes Research and Clinical Practice*, 81-87.
- Heidari, R., Zareae, S., & Heiderizadeh, M. (2005). Extraction, purification, and inhibitory effect of alpha amylase inhibitor. *Pakistan Journal of Nutrition*, *4*, 101-105.
- Kakuda, T., Sakane, I., Takihara, T., Ozaki, Y., Takeuchi, H., & Kuroyanagi, M. (1996). Hypoglycemic effect of extracts from *Lagerstroemia speciosa L*. leaves in genetically diabetic KK-AY mice. *Bioscience, Biotechnology, and Biochemistry (Tokyo)*, 60, 204–208.
- Klein, G., Kim, J., Himmeldirk, K., Cao, Y., & Chen, X. (2007). Antidiabetes and Anti-obesity Activity of Lagerstroemia speciosa. *Advance Access Publications*, 401-407.
- Le Berre-Anton, V., Bompard-Gilles, C., Payan, F., & Roque, P. (1998). Characterization and functional properties of alpha amylase inhibitor from kidney beans (*Phaseolus vulgaris*) seeds. *Biochemica et Biophysica Acta*, 1343, (31) 217-225.
- Mcdougall, G., Shpiro, F., Dobson, P., Smith, P., & Blake, A. (2005). Different polyphenolic components of soft fruit inhibits alpha amylase and alpha glucosidase. *J. Agric. Food Chem,* 53, 2760-2766.

FNH-I-007



Tanquilut, N., Tanquilut, M. R., Estacio, M. A., Torres, E. B., Rosario, J. C., & Reyes, B. A. (2009). Hypoglycemic effect of Lagerstroemia speciosa (L.) Pers on alloxan-induced diabetic mice. *Journal of Medicinal Plants Research*, 3, (12) 1066-1071.