

## *In Vitro* $\alpha$ -Amylase and $\alpha$ -Glucosidase Inhibition Activity of *Abutilon indicum* (Linn 1836) Root Extracts

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### ABSTRACT

**Objective:** The study aimed to determine the *in vitro* inhibitory activity of *Abutilon indicum* (Linn 1836) root extract on  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes for the prevention of diabetes, one of the major causes of mortality in the Philippines. **Methods:** This study utilized soxhlation with 95% ethyl alcohol for the extraction method. Three different concentrations (20  $\mu$ g/ml, 40  $\mu$ g/ml and 80  $\mu$ g/ml) of *Abutilon indicum* root extracts were prepared. Root extracts, of varying concentrations, were subjected to inhibitory assay for  $\alpha$ -amylase and  $\alpha$ -glucosidase and quantified using a UV – VIS Spectrophotometer. Absorbance reading was measured at 540 nm and 405 nm for the  $\alpha$ -amylase and the  $\alpha$ -glucosidase inhibitory assay, respectively. **Results:** There was a dose-dependent percent inhibition by the extract against  $\alpha$ -amylase (8.84% - 26.51%) and  $\alpha$ -glucosidase (8.12% - 24.36%). Logarithmic regression analysis revealed the median inhibitory concentration (IC<sub>50</sub>) of  $\alpha$ -amylase (191.64  $\mu$ g/ml) and  $\alpha$ -glucosidase (207.13  $\mu$ g/ml) with a potency and preference for  $\alpha$ -amylase over  $\alpha$ -glucosidase inhibition by the *A. indicum* root extract. **Conclusion:** The findings suggest that *Abutilon indicum* root extracts are inhibitors of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes which possibly help reduce postprandial glucose levels.

**Keywords:** *Abutilon indicum*,  $\alpha$ -amylase inhibitor,  $\alpha$ -glucosidase inhibitor

### INTRODUCTION

Diabetes is one of the major causes of premature death worldwide. In 2012, diabetes was the direct cause of 1.5 million deaths and more than 80% of these diabetes-caused deaths occur in low-income and middle-income countries like the Philippines<sup>1</sup>. Antihyperglycemic agents are used to control blood glucose levels within the normal range. However, these agents are associated with various side effects and limited efficacy. Thus there is a need to search for more effective and safer antihyperglycemic agents from herbs<sup>2-4</sup>. Herbal medicines are known to cause less adverse effects. The increased utilization of herbal medicines for diabetes may be due to the side-effects associated with the conventional antihyperglycemic medicines. The World Health Organization (WHO) has also substantiated the utilization of herbal remedies for the management of diabetes<sup>5,6</sup>. Antihyperglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes<sup>7</sup>. Postprandial hyperglycemia caused by the hydrolysis of carbohydrates by pancreatic  $\alpha$ -amylase and intestinal  $\alpha$ -glucosidase is a serious condition<sup>8</sup>. Pancreatic  $\alpha$ -amylase is a key enzyme in the digestive system and it catalyses the initial step in hydrolysis of starch to a mixture of oligoglucans. These are then acted on by  $\alpha$ -glucosidase and further degraded to glucose which on absorption enters

into blood stream<sup>9</sup>. Inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes is an effective way in the management of diabetes since it can significantly decrease the postprandial increase of blood glucose<sup>10</sup>. In the Philippines, *Abutilon indicum* is found in thickets and waste places in and about towns at low and medium altitudes. The entire plant has medicinal value. It has been reported that various parts of the plant are used as demulcent, aphrodisiac, laxative, diuretic, sedative, astringent, expectorant, tonic, anti-inflammatory, anthelmintic, analgesic and are used to treat leprosy, ulcers, headaches, gonorrhoea, and bladder infection. In Cebu, Philippines, it has been said that the extract obtained from the plant's roots is indicated for 'binat' while the plant's leaves were indicated for goiter. Reports revealed that the leaves exhibited hypoglycemic activity influenced by the presence of phenolic compounds<sup>8</sup> however, the inhibitory  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes activity of its roots have not yet been reported. Thus, the objective of the present study is to investigate the *in vitro* inhibitory activity of the *Abutilon indicum* root extract on  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes.

### MATERIALS AND METHODS

#### *Preparation of Plant Sample*

The dried roots of *Abutilon indicum* were collected in the month of June from Nasipit, Talamban, Cebu City, Philippines. It was authenticated by the Department of

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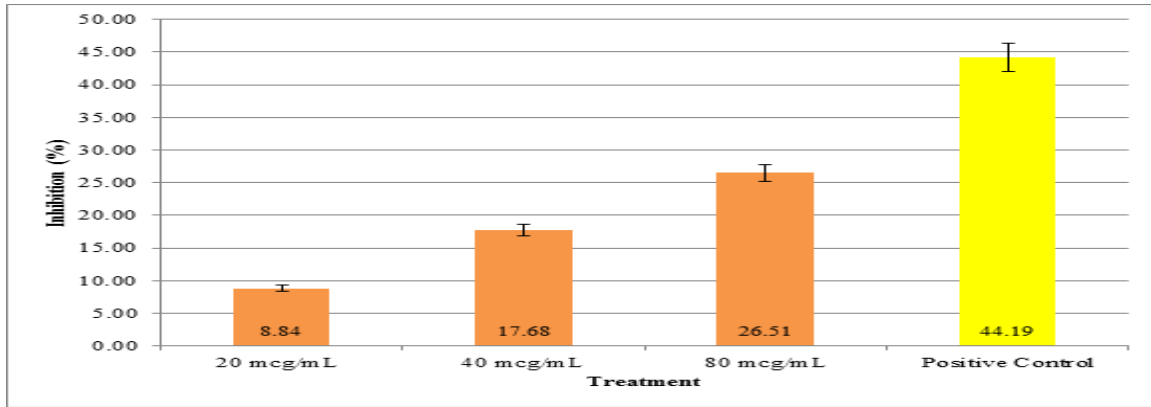


Figure 1: Percent Inhibition of 3-amino-5-nitrosilyclic acid

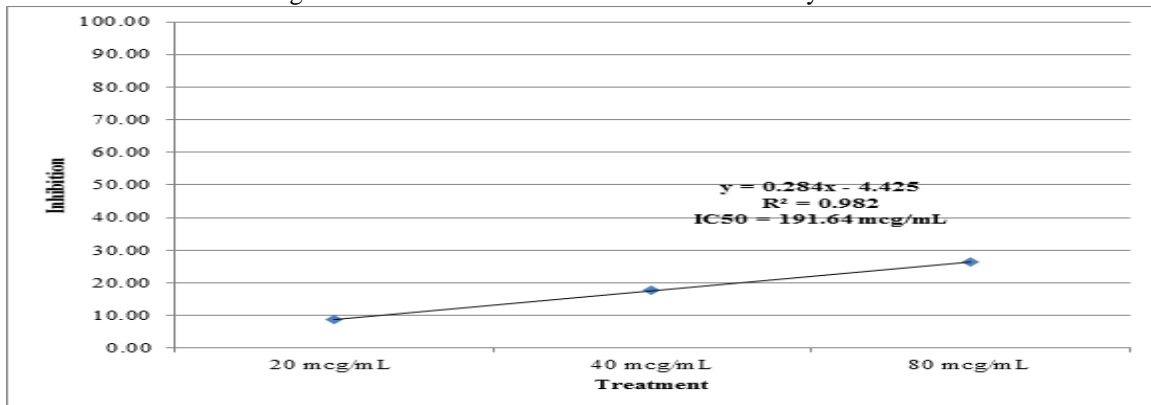


Figure 2: Linear Regression Based on Test Solutions of Ethanol-Free extract on Percent Inhibition in  $\alpha$ -amylase Inhibitory Activity Assay

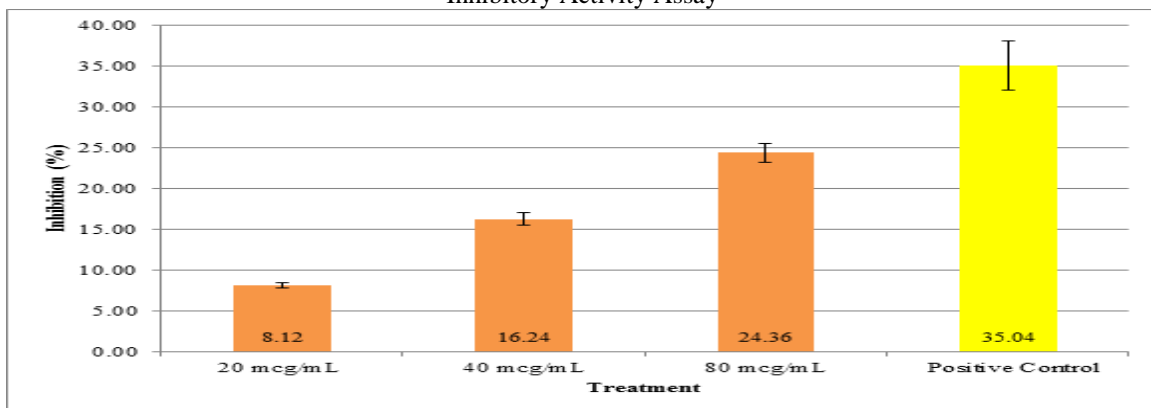


Figure 3: Percent Inhibition of p-nitrophenol

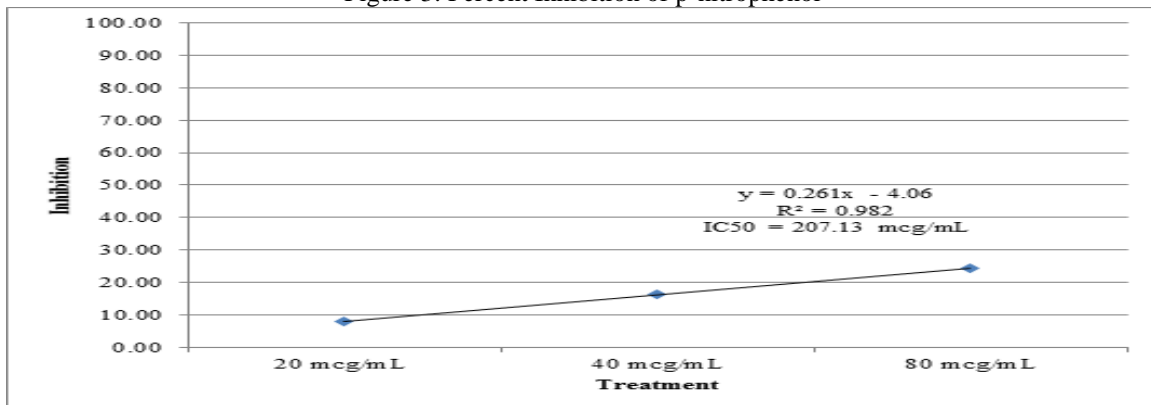


Figure 4: Linear Regression Based on Test Solutions of Ethanol-Free extract on Percent Inhibition in  $\alpha$ -glucosidase Inhibitory Activity Assay

Biology, University of San Carlos. Roots were oven-dried at 50°C for 30 minutes, powdered mechanically using the Wiley mill and sieved through sieve no. 20. It was then stored in an air tight container.

#### *Extraction of Plant Sample*

In a Soxhlet apparatus, 100g of powdered roots were extracted with 500ml of 95% ethanol. Extraction was done repeatedly with the use of the same solvent until a clear colorless solvent was obtained. The obtained extractive was concentrated using the rotary evaporator at 40-50°C and subjected to vacufuge to obtain a solvent – free extract. The extract was stored in cold temperature for further use.

#### *Preparation of Test Solution and Positive Control*

*Abutilon indicum* L. root extract stock solution (10,000 µg/ml) was prepared. From the stock solution, different concentrations 20µg/ml (0.002%), 40µg/ml (0.004%) and 80µg/ml (0.008%) were prepared. Moreover, acarbose was used as the positive control. Fifty mg of acarbose tablet was pulverized using a mortar and pestle. The 50 mg powdered acarbose was dissolved in 50 ml of phosphate buffer and was diluted appropriately using phosphate buffer to obtain a concentration of 2.5 g/mL<sup>11</sup>.

#### *Phytochemical Testing*

The extract was tested for the presence of alkaloid, glycosides, tannins, steroids, reducing sugars, proteins and amino acids, phenolic compounds and flavonoids.

#### *$\alpha$ -Amylase Inhibition Assay*

The  $\alpha$ -amylase inhibitory activity was determined using the method of Bernfield<sup>12</sup>. *Abutilon indicum* ethanolic root extract (500 µL) and 0.02mol/L sodium phosphate buffer (pH 6.9 with 0.006mol/L NaCl) containing porcine pancreatic  $\alpha$ -amylase (0.5mg/mL) was incubated at 25°C for 10 minutes. This was followed by the addition of 500µL of starch solution (1%) in 0.02mol/L sodium phosphate buffer to the reacting mixture and was incubated at 25°C for 10 min. The reaction was stopped by the addition of 1mL of dinitrosalicylic acid followed by incubation in a boiling water bath for 5 min, and then it was cooled to room temperature. The reddish brown-colored reaction mixture, 3-amino-5-nitrosalicylic acid (ANS), was diluted by adding 10ml of distilled water. The absorbance of the orange-colored diluted 3-amino-5-nitrosalicylic acid was then measured at 540 nm using UV-Vis spectrophotometer.

#### *$\alpha$ -Glucosidase Inhibition assay*

The  $\alpha$ -glucosidase inhibitory activity was determined according to the method described by Apostolidis 2007<sup>13</sup>. *Abutilon indicum* ethanolic root extract (50µL) and yeast  $\alpha$ -glucosidase solution (100µL) were incubated at 25°C for 10 min followed by the addition of 50µL of 5mM/L p-nitrophenyl- $\alpha$ -D-glucoopyranoside solution in 0.1mol/L phosphate buffer (pH 6.9). The yellow-colored reaction mixture, 4-nitrophenol, was then incubated at 25°C for 5 min and its absorbance was measured at 405nm using UV – VIS spectrophotometer. Acarbose was used as a positive control and the inhibitory activity of  $\alpha$ -amylase and  $\alpha$ -glucosidase was calculated using the following formula, % Inhibition = [(Abs Control - Abs Sample) / Abs Control] x 100.

#### *Calculation of 50% Inhibitory Concentration (IC<sub>50</sub>)*

The IC<sub>50</sub> values, defined as the concentration of the extract that inhibited 50% of the enzyme activity, was determined from plots of percent inhibition versus log inhibitor concentration and was calculated by logarithmic regression analysis from the mean inhibitory values.

## RESULTS

### *Alpha Amylase Inhibition Assay*

#### *Percent Inhibitory Activity of 3-amino-5-nitrosalicylic acid.*

As the concentration of the test solution increases, its absorbance reading decreases which indicates lower activity of  $\alpha$ -amylase on its substrate (3-amino-5-nitrosalicylic acid) resulting to higher inhibitory activity. Among the test solutions, the 80 µg/mL test solution, being the highest concentration, gave the highest percent inhibitory activity (26.51% ± 1.31) (See Figure 1).

#### *Statistical Analysis: One-Way ANOVA*

In the  $\alpha$ -amylase inhibitory activity assay, one-way ANOVA revealed a p-value of 6.59x10<sup>-09</sup> for ethanol-free extract. The p-value was observed to be lesser than that of the standard p-value of 0.01. This shows that there is a significant difference among the test groups (20 µg/mL, 40 µg/mL, 80 µg/mL and positive control); thus, the results vary and are comparable from one test group to another (See Table 1).

#### *Post Hoc Analysis (Percent Inhibitory Activity)*

In order to determine which of the test groups differ significantly among each other, Tukey post hoc analysis was done. In this analysis, each of the test groups in the alpha-amylase inhibition assay was compared against each other in order to determine the specific test group which is most effective in the inhibition of the alpha-amylase enzyme based on the percent inhibition activity (See Table 2).

### *Alpha Glucosidase Inhibition Assay*

#### *Percent Inhibitory Activity of p-nitrophenol.*

The higher the concentration of the test solution; the lower the absorbance reading which also means the lower the activity level of  $\alpha$ -glucosidase on its substrate (p-nitrophenol) resulting to higher inhibitory activity. Among the test solutions, the 80mcg/mL test solution having the highest concentration also has the highest percent inhibitory activity (24.36% ± 1.11) (See Figure 3).

#### *Statistical Analysis: One-Way ANOVA*

In the  $\alpha$ -glucosidase inhibitory activity assays, one-way ANOVA showed a p-value of 2.36x10<sup>-07</sup> for the ethanol-free extract. The p-value was observed to be lesser than that of the standard p-value of 0.01. This shows that there is a significant difference among the test groups (20 µg/mL, 40 µg/mL, 80 µg/mL and positive control); thus, the results vary and are comparable from one test group to another (See Table 3).

#### *Post Hoc Analysis (Percent Inhibitory Activity)*

In order to determine which of the test groups differ significantly between each other, the post hoc analysis by

Tukey was done. In this analysis, each of the test groups in the alpha-glucosidase inhibition assay was compared

Table 1: One-way ANOVA Results for  $\alpha$ -amylase Inhibitory Activity Assays

Source	SS	df	MS	F	P-value
Treatment	2,050.4823	3	683.49410	367.82	6.59x10 <sup>-09</sup>
Error	14.8660	8	1.85825		
Total	2,065.3483	11			

Table 2: Results of Post hoc Analysis of Ethanol-Free Extract  $\alpha$ -amylase Inhibitory Activity Assays

Groups	p level	Interpretation
20 $\mu$ g/mL v 40 $\mu$ g/mL	p<0.01	Significant
20 $\mu$ g/mL v 80 $\mu$ g/mL	p<0.01	Significant
20 $\mu$ g/mL v Positive	p<0.01	Significant
40 $\mu$ g/mL v 80 $\mu$ g/mL	p<0.01	Significant
40 $\mu$ g/mL v Positive	p<0.01	Significant
80 $\mu$ g/mL v Positive	p<0.01	Significant

Table 3: One-way ANOVA Results for  $\alpha$ -glucosidase Inhibitory Activity Assays

Source	SS	df	MS	F	P-value
Treatment	1,191.1028	3	397.03428	148.53	2.36x10 <sup>-07</sup>
Error	21.3847	8	2.67308		
Total	1,212.4875	11			

Table 4: Results of Post Hoc Analysis of Ethanol-Free Extract on  $\alpha$ -glucosidase Inhibitory Activity Assays

Groups	p level	Interpretation
20 $\mu$ g/mL v 40 $\mu$ g/mL	p<0.01	Significant
20 $\mu$ g/mL v 80 $\mu$ g/mL	p<0.01	Significant
20 $\mu$ g/mL v Positive	p<0.01	Significant
40 $\mu$ g/mL v 80 $\mu$ g/mL	p<0.01	Significant
40 $\mu$ g/mL v Positive	p<0.01	Significant
80 $\mu$ g/mL v Positive	p<0.01	Significant

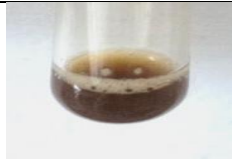
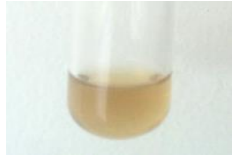




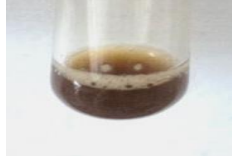
against each other in order to determine what test group is most effective in the inhibition of the alpha-glucosidase enzyme based on the percent inhibition activity (See Table 4).

### DISCUSSION

The inhibition of the enzymes was determined through the difference in absorbance of test solutions and positive control from the blank. Degrees of absorbance of 3-amino-5-nitrosalicylic acid (ANS), for the assay of  $\alpha$ -amylase, and *p*-nitrophenol, for the assay of  $\alpha$ -glucosidase, are inversely proportional to the percent inhibition of the enzymes, thus, increase in absorbance will result to decrease in percent inhibition and vice-versa. Based on the calculated percent inhibition, there is an  $\alpha$ -amylase and an  $\alpha$ -glucosidase inhibitory activities of *Abutilon indicum* ethanolic root extract. In both  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition assays, the 80  $\mu$ g/mL test solution exhibited the highest percent inhibition. In  $\alpha$ -amylase inhibition assay, the test solution with 80  $\mu$ g/mL concentration had the highest percent inhibition (26.51%  $\pm$  1.31) among the plant extracts followed by 40  $\mu$ g/mL and 20  $\mu$ g/mL concentrations. The lowest concentration showed the least percent inhibition (8.84%  $\pm$  0.44). The half maximal inhibitory concentration (IC<sub>50</sub>) of the ethanol-free extract in the  $\alpha$ -amylase assay and  $\alpha$ -glucosidase assay was determined to be 191.64mcg/mL and 207.13mcg/mL, respectively. This study is parallel

with *Pants* showing a dose-dependent percent inhibition by the extract against  $\alpha$ -amylase and  $\alpha$ -glucosidase. Also, the logarithmic regression analysis revealing the IC<sub>50</sub> in both studies showed a potency and preference for  $\alpha$ -amylase over  $\alpha$ -glucosidase inhibition by *Abutilon indicum*<sup>8</sup>.  $\alpha$ -amylase is an enzyme which hydrolyzes complex starch to oligosaccharides, on the other hand,  $\alpha$ -glucosidase hydrolyses oligosaccharides, trisaccharides, and disaccharides into monosaccharides, like glucose. Acarbose like drugs inhibits  $\alpha$ -glucosidase and are responsible for reducing post-prandial hyperglycemia, such medications are important for patients recently diagnosed with type 2 diabetes. Such medications also prove to be useful for individuals taking sulfonylureas and metformin, which help to maintain their blood-glucose levels within a safe limit. Reducing sugars, proteins and amino acids found in the *A. indicum* root extract are plausible phytoconstituents for the resulting activity. Reducing sugars, and proteins could be useful in controlling blood glucose levels. Preliminary in vitro data showed that *A. indicum* root extract exhibited inhibition activity on both enzymes however, in vivo assays have to be conducted to extrapolate its use in humans<sup>15,16</sup>. In both  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibition assay, as the concentration increases; the absorbance reading decreases while the percent inhibition increases. This means that the higher the concentration of the test solution; the lower the activity levels of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes on

Table 5: Phytochemical Screening of *Abutilon indicum* ethanolic root extract

Phytoconstituents	Test	Positive Results	<i>Abutilon indicum</i> Ethanolic Root Extract*	Actual Results
Alkaloids	Mayer's Test	Turbidity	-	
Glycosides	Keller-Kiliani Test	A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides.	-	
Reducing Sugars/ Carbohydrates	Molisch's Test	Violet ring at the interphase	+	
Phenolic compounds & Tannins	Ferric chloride & Test	Green or blue-black color	-	
Steroids	Liebermann- Burchard's Test	Green color	-	
Proteins and amino acids	Ninhydrin Test	Violet color	+	
Flavonoids	Ammonia Test	Yellow solution	-	

\*(+) present; (-) absent

their corresponding substrates resulting to a higher inhibitory activity of each test solution. This could be an implication that *Abutilon indicum* ethanolic root extract is a potential compound for the management of diabetes.

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