In vitro anti-diabetic activity of *Crescentia cujete* (Calabash tree) leaves through alpha-amylase inhibition assay and glucose uptake by yeast cell

Jannah Elmira B. Ronquillo[1*], Mary Grace Aro[1], Reyann Joyce P. Herrera[1], Mercedella I. Lat[1], Mark Kennette H. Ramos[1], Carina R. Magbojos-Magtibay[2], and John Patrick B. Tadiosa[2]
College of Allied Medical Professions,
Lyceum of the Philippines University,
Capitol Site, Batangas City
[1]Student Researcher
[2] Faculty Researcher
*Correspondence: *jannahelmiraronquillo@gmail.com*

Abstract

Diabetes mellitus is a metabolic disease described as presence of hyperglycemia. Primary goal in treating diabetes is to decrease post-prandial hyperglycemia. This is done by, inhibition of enzymes which includes alpha-amylase and effective facilitation of glucose via uptake by cells. *Cresentia cujete* is known to have chemical content like flavonoids, which could contribute to its anti-diabetic activity. Phytochemical screening was done and the extract tested positive for flavonoids, reducing sugar, saponins, and tannins. The activity of the extract was evaluated using alpha-amylase inhibition assay and glucose uptake by the yeast cell, which are both in-vitro assays with Metformin and Metronidazole as standards for the two assays, respectively. The alpha-amylase inhibition assay showed that the percent inhibition of alpha amylase using either Metformin or *C. cujete* extract increases with the increase in concentration. The anti-diabetic activity of *C. cujete* extract was best shown at a concentration of 5 mg/mL wherein a percent inhibition of 42.7% was obtained. This can be compared to Metformin's 55.0% of the same concentration. The glucose uptake by yeast cell showed that even if the concentration of plant extract increases, there will be only minimal effect to the glucose uptake. The extract showed similar glucose uptake at a concentration of 10 mM glucose concentration. It is concluded that *C. cujete* leaf extract has antidiabetic activity based on alpha amylase inhibition assay and Glucose uptake by yeast cell. The antidiabetic activity was found to be dose dependent. In

comparison to the standard drug used in this study, it is observed that Metformin and Metronidazole perform better when compared to the activity of *C. cujete* extract at the same concentration. Researchers recommend further experimentation with regards to *C. cujete* extract's anti-diabetic activity through in vivo methods or using purified plant extract.

Keywords: *Anti-diabetic, Crescentia cujete, Alpha-amylase, glucose uptake, yeast cell*

INTRODUCTION

Diabetes mellitus (DM) is a lifelong metabolic disease cause by failure or loss of tolerance to secret insulin that is responsible in maintenance of blood glucose level in the body. Low insulin secretion may result in high blood glucose level that may later on affect the other body systems (Matsui et al., 2007). Over time, this situation can cause severe damage particularly in the nerves and blood vessels (Espinosa, et al., 2011).

The number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014 according to World Health Organization and expected 5.4% by the end of 2025, with estimated 300 million population particularly the region of Southeast Asia counting for 80 million of the population (Bhatt, Sati, Chandra, Kumar, & Anthwal, 2015). As of 2013, Philippine Center evaluate the antidiabetic activity based on compounds (Roy, et al., 2015).

One beneficial approach for treating diabetes is to lower post-prandial hyperglycemia. This is made by means of suppressing the absorption of glucose through the inhibition of the enzyme specifically a-amylase in the gastrointestinal tract. (Handayani, et al, 2015).

Alpha amylases (a-1,4-glucan-4 glucanohydrolase) are enzymes which are needed for the breakdown of linkages in starch and various other Polysaccharides (Ercan & Sedef, 2016; Heidari, Zareae, & Heidarizadeh, 2005).

Therapeutic control and treatment for diabetes widely involves enzyme inhibitors since they are widely involve in the most important biological energy pathways. Guided utilization is of great advantage in carbohydrate digestion and absorption in order to avoid certain conditions such as diabetes. In this condition, amylase inhibitors are of appropriate importance (Kim & Nho, 2004). Alpha amylase inhibitors slowdown the rate of glucose absorption causing low postprandial plasma glucose rise (Bhandari, et al., 2008).

Most of the commercially available amylase inhibitors are of microbial origin. Diarrhea and flatulence are the common side effects of enzyme inhibitors since they delay the utilization of carbohydrates resulting to fermentation in the colon leaving the non-absorbed sugar.

According to Mahmoud et al. (2010) and Mioko et al. (2001), inhibitors with molecular weight below 250 daltons are widely used since they are absorbed effectively from the gastrointestinal tract into the bloodstream. When compared to the microbial counterparts, amylase inhibitors from medicinal plants are considerably safer and more effective. Only 30% of the 1200 anti-diabetic plants are tested for its effectivity for pharmacological use (Ayyanar, et al., 2008).

Since the early years, plants are already sources of medicine but as of today, they are already well known to both public and scientists as sources of pharmacologically accepted products (Premanath & Lakshmidevi, 2010). The activity and effectivity of the plant depends on the biologically active components that produce therapeutic action on the human body (Aiyelaagbe & Osamudiamen, 2009). Medical plants are only used by 80% of the world population since they are the only available sources of treatment especially in developing countries (EL-Kamali & EL-amir, 2010).

Figure 1. *Crescentia cujete* (Calabash tree)

Crescentia cujete or the gourd tree belongs to the family of Binoniaceae. The tree is 6 to 10m tall with a wide crown and long branches covered with clusters of tripinnate leaves. The leaves are elliptical in shape and are clustered in the anode. Basically, leaves areused as diuretic and also used for treatment of tumor and hematoma and even the case of hypertension (Parente & de Oliveira, 2016). According to Koffi and Solange (2015), C*rescentia cujete*, in a non-experimental validation for

antidiabetic activity study, yielded cyanhidric acid which can stimulate insulin release.

Phytochemical substances isolated from plants have been reported for their hypoglycemic activity either by insulin, enzyme inhibitiors and increased facilitated glucose utilization by the body (Modak, et al., 2007; Li, Zheng, Bukuru, & De Kimpe, 2004).

To date, no scientific report is available to support the anti-diabetic activity of *C. cujete* leaves using in vitro assays; therefore, the study aims to investigate its activity using alpha amylase inhibition and glucose uptake by yeast cell. It also aims to determine the most effective concentration of the extract. If proven to be effective, a naturally available and more affordable alternative agent can be used against diabetes.

MATERIALS AND METHOD

Collection of Plant . The fresh leaves of *C. cujete* were collected locally and were authenticated by University of the Philippines Los Baños Forestry Department.

Preparation of *C. cujete* Extract . Preparation of *C. cujete* leaf extract was based on the protocol of Vijaya et al. (2016) with slight modifications.

The shade dried *C. cujete* leaves were powdered mechanically and stored in an air tight container. Extraction was prepared by adding 80 g of dried powdered *C. cujete* leaves to 800 mL of 95% ethanol. Solution was stored for 48 hours in ambient temperature then filtered using Whatmann filter paper No.1 and was subjected to rotary evaporator at 45°C.

Phytochemical Screening (Kazeem, et al., 2013). For preliminary phytochemical analysis, the freshly prepared *C. cujete* leaf extract was subjected to find the presence or absence of phytoconstituents using standard phytochemical procedures.

Test for Flavonoids (Alkaline Reagent Test). A portion of the C. cujete leaf extract was heated with 10 mL of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. Development of yellow coloration indicates the presence of flavonoids.

Test for Reducing Sugar (Benedict's test). To about 1 g of each *C. cujete* leaf extract in the test tube, 10 mL distilled water was added and the mixture was boiled for 5 minutes. The mixture was filtered while hot and the cooled filtrate made litmus paper alkaline with 20% sodium hydroxide solution. The resulting

solution was boiled with an equal volume of Benedict qualitative solution on a water bath. The formation of a brick-red precipitate depicted the presence of reducing compound.

Test for Saponin (Foam test). Approximately 2 g of *C. cujete* leaf extract was boiled in 20 mL of distilled water in a water bath and filtered. Afterwards, 10 mL of the filtrate was mixed with 5 mL of distilled water and shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously again. The formation of emulsion was observed as an indication of saponin.

Test for Tannins (Ferric Chloride test). For the test for tannins, 0.5 g of *C. cujete* leaf extract was boiled in 20 mL of water in a test tube and filtered. Few drops of 0.1% ferric chloride was added and observed for a brownish green or blue black coloration as an indication of tannins.

Alpha-Amylase Inhibition Assay. The alpha-amylase inhibition was determined by a modified starch iodine protocol by Hossan, et al., (2009). Different concentrations of *C. cujete* leaf extract (5, 4, 3, 2, and 1 mg) per mL of distilled water were taken in pre-labeled test tubes. A volume of 20 µL of alpha-amylase was added to each test tube and was incubated for 10 minutes at 37°C. After incubation, 200 µL of 1% starch solution was added to each test tube and the mixture was re-incubated for 1 hour at 37°C. Then, 200 µL of 1% iodine solution was added to each test tube together with 10 mL distilled water. Metformin was prepared in distilled water at various concentrations and was used as positive control. Absorbance of the mixture was taken at 565 nm. Sample, substrate, and alpha amylase blank were undertaken under the same conditions.

Each experiment was done in triplicate. The percent inhibition was calculated using the following formula.

$$\% \text{ inhibition} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}}$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample.

Glucose Uptake by Yeast Cell (Daksha, Chandrashekar, Lobo, Nayak, & Nilesh, 2012). Yeast cells were prepared by dissolving 1 part commercial baker's yeast to 9 mL distilled water. Mixture was washed by repeated centrifugation (3,000 × g, 5 min) until the supernatant fluids were clear and a 10% (v/v) mixture was prepared. Various concentrations of extracts (1 – 5

mg/mL) were added to 1 mL of glucose solution (5, 10 and 25 mM) and further incubated for 10 min at 37°C. Reaction was started by adding 100 µl of yeast suspension, vortexed and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant.

Samples were measured using spectrophotometer at 540nm. Metronidazole was used as standard drug. The percentage in glucose uptake by yeast cells was calculated using the following formula:

$$\% \text{ uptake} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}}$$

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample.

Statistical Analysis

All the measurements were done in triplicate and results are expressed in terms of mean ± standard deviation and IC_{50} values. ANOVA (One Way Analysis of Variants) was used to determine whether there are any statistically significant differences between the means of Metformin and *C. cujete* leaf extract in alpha amylase inhibition assay. ANOVA was also used to compare the means of Metronidazole and *C. cujete* leaf extract on glucose uptake by yeast cell. Post-Hoc Test (Tukey Honest Significant Difference) was also used to compare the mean of two or more concentration level to obtain multiple comparison. The value of $p<0.05$ from the results were considered significant for each group.

RESULTS AND DISCUSSION

Extraction of *C. cujete* leaves

From 80 grams of crushed dried leaves, 10.150 grams of sticky dark green color of *C. cujete* leaf extract was obtained after evaporation with a percentage yield of 12.7% as shown in Figure 2. The appearance of the extract is the same with the study of Anwuchaepe, et al., (2017). However, they used methanol for extraction and was able to produce a yield of 4.90% from 2000 g of powdered leaves, lesser than what was obtained.

Phytochemical Screening

Qualitative phytochemical analysis of *C. cujete* leaf extract was done for screening several phytochemicals as shown in Table 1.

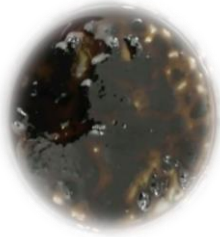

Figure 2. *C. cujete* leaf extract

Analysis of *C. cujete* leaf extract revealed the presence of flavonoids as manifested by yellow coloration while the presence of reducing sugar was shown by the positive result of brick red precipitate. *C. cujete* leaf extract was also tested positive for tannins and was indicated by formation of emulsion while presence of saponin was also observed by brownish green coloration. Similar findings of positive flavonoids, reducing sugar, saponins and tannins were obtained from the phytochemical screening of *C. cujete* leaf extract by Olaniyi et al., (2018). The phytochemical screening results are also shown in Figure 3.

Table 1
Results of phytochemical screening of *C. cujete* leaf extract

Phytochemical Components	Result
Flavonoids	+
Reducing Sugar	+
Saponin	+
Tannins	+

Legend: (+) present

These phytochemicals have hypoglycemic action. This is supported by the fact that methanolic extract of *Cajanus cajan* that has also hypoglycemic effect contains similar phytochemicals like saponins and tannins (Ezike, et al., 2010).

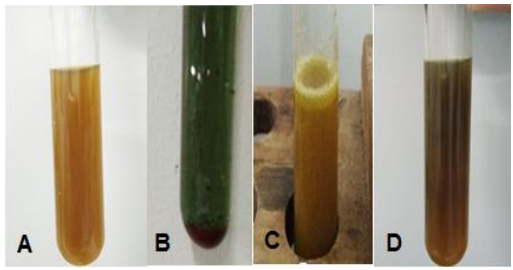

Figure 3. Phytochemical Screening of (A) Flavonoids (B) Reducing sugar (C) Saponin (D) Tannins

Alpha-Amylase Inhibition Assay

Table 2 shows the percent inhibition of the alpha-amylase due to the addition of Metformin and *C. cujete* leaf extract. It reveals that 1 mg/ml of Metformin inhibited 28.3% of alpha amylase as compared to 1 mg/ml of *C. cujete* leaf extract that inhibited 20.3%. For 2 mg/ml, it inhibited 31.3% while *C. cujete* leaf extract inhibited 24.7% of alpha amylase. For concentrations 3 mg/ml, 4 mg/ml and 5 mg/ml of metformin, they inhibited 35.0%, 41.3% and 55.0% alpha amylase, respectively in comparison with *C. cujete* extract that inhibited 32.7 %, 35.7%, 42.7%, respectively. Based from the results, inhibition using Metformin and *C. cujete* leaf extract showed an increase in number of percent inhibition as the concentration increases. The varying effects of the different concentrations may be caused by the phytochemicals present in the extract such as flavonoids, saponins and tannins.

Table 2
Percent Inhibition of Alpha Amylase

	Percent Inhibition of Alpha Amylase*	
	Metformin (Standard)	*C. cujete* extract
1 mg/ml	28.3%	20.3%
2 mg/ml	31.3%	24.7%
3 mg/ml	35.0%	32.7%
4 mg/ml	41.3%	35.7%
5 mg/ml	55.0%	42.7%

**Test were performed in triplicate*

Flavonoids, which are widely distributed in the plant kingdom and present in considerable quantities in common food products have been used since ancient times by physician to treat a great variety of human diseases such as diabetes, coronary heart

disease, and cancers. It is demonstrated that hydroxylation in ring C and methylation of the hydroxyl group in ring B of flavonoids may weaken the binding affinities to alpha-amylase (Yuan et al., 2014). Saponins displays a competitive mode of inhibition on α-amylase and as an indication that saponin competed with the substrate at the active site of the enzyme. Thus, there is modification of the enzyme structure, rendering inhibitor-binding position accessible (Adugna et al., 2004). Tannins could bind to the digestive enzymes α-amylase and glucoamylase, thereby inhibiting starch hydrolysis (Barrett et al., 2013).

Shown in Figure 4 is the percent inhibition of Metformin versus *C. cujete* leaf extract. It is noticeable that as the concentration of either Metformin or *C. cujete* leaf extract increases, inhibition also increases showing a concentration dependent antidiabetic activity.

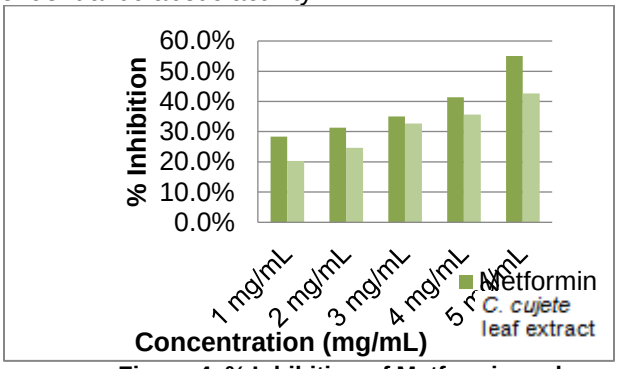

Figure 4. % Inhibition of Metformin and *C. cujete* leaf extract

Statistical comparison as shown in Table 3 for *C. cujete* leaf extract against the positive control (Metformin) was done to know if there is really a significant difference between the effects of the concentrations after allowing *C. cujete* leaf extract react with alpha amylase. It was found out that there was a significant difference between the concentration of *C. cujete* leaf extract and the positive control because the obtained p-value (p=0.044) was less than 0.05 level of significance. The mean percent inhibition of Metfromin is 38.2% compared while *C. cujete* leaf extract had 31.2%. This confirms that Metformin shows better inhibitory effect against alpha amylase than *C. cujete* leaf extract as one of the standard drugs that delays carbohydrate digestion, it causes a reduction in the rate of glucose absorption and; thus, lower the glucose levels (Lalitha, 2016).

Table 3
Comparison on the effect of *C. cujete* leaf extract to Metformin (positive control)

	Mean Percent Inhibition	p-value	Interpretation
Metformin	38.2	0.044	Significant
C. cujete leaf extract	31.2		

Legend: Significant at p-value < 0.05

Some studies of different ethanolic plant extract agree with the findings. When the ethanolic extract of the bark of *Elaeocarpus ganitrus* was tested for in vitro alpha amylase inhibitory effect, the sample showed a concentration dependent inhibitory effect up to 82% (Talukdar, et al., 2017). As a result of this, the antidiabetic activity of *Elaeocarpus ganitrus* can be attributed to the presence of various phytochemical such as flavonoids, tannins and saponins which also tested positive for *C. cujete* leaf extract.

Table 4 shows the multiple comparisons between Metformin and *C. cujete* leaf extract.

For Metformin, when 1 mg/ml concentration was compared to varying concentrations of 2 mg/ml, 3 mg/ml, 4 mg/ml and 5 mg/ml, all the p-values obtained were below 0.05 level of significance which shows that there is a significant difference in inhibitory activity of Metformin from 1 mg/ml compared to 5 mg/ml concentration. This implies that 5 mg/ml of Metformin favor the most inhibitory activity against alpha amylase correlated from the inhibition seen from Figure 4. However, in *C. cujete* leaf extract, when 1 mg/ml was compared to varying concentrations of 2 mg/ml, 3 mg/ml, 4 mg/ml and 5 mg/ml, all concentrations have significant differences since the obtained p-values were below 0.05 except between 3 mg/ml and 4 mg/ ml concentrations since the obtained p-value was 0.108. This implies that 5 mg/ml of *C. cujete* leaf extract has the highest inhibitory activity among the concentration. However, 3 mg/ml and 4 mg/ml has the same effect on the inhibition of alpha amylase, this can be due to the presence of more phytochemicals extracted. This is supported by the study of Tamil, et al., (2010) in which phytochemical constituents particularly flavonoids, tannins and saponins may be responsible for its anti-diabetic activity.

Table 4
Multiple Comparisons between Concentrations of Metformin and *C. cujete* Extract

Varying Concentration		Metformin		*C. cujete* leaf extract	
		p-value	Interpretation	p-value	Interpretation
1 mg/mL	2 mg/mL	0.045	Significant	0.016	Significant
	3 mg/mL	0.000	Significant	0.000	Significant
	4 mg/mL	0.000	Significant	0.000	Significant
	5 mg/mL	0.000	Significant	0.000	Significant
2 mg/mL	1 mg/mL	0.045	Significant	0.016	Significant
	3 mg/mL	0.014	Significant	0.000	Significant
	4 mg/mL	0.000	Significant	0.000	Significant
	5 mg/mL	0.000	Significant	0.000	Significant
3 mg/mL	1 mg/mL	0.000	Significant	0.000	Significant
	2 mg/mL	0.014	Significant	0.000	Significant
	4 mg/mL	0.000	Significant	0.108	Not Significant
	5 mg/mL	0.000	Significant	0.000	Significant
4 mg/mL	1 mg/mL	0.000	Significant	0.000	Significant
	2 mg/mL	0.000	Significant	0.000	Significant
	3 mg/mL	0.000	Significant	0.108	Not Significant
	5 mg/mL	0.000	Significant	0.001	Significant
5 mg/mL	1 mg/mL	0.000	Significant	0.000	Significant
	2 mg/mL	0.000	Significant	0.000	Significant
	3 mg/mL	0.000	Significant	0.000	Significant
	4 mg/mL	0.000	Significant	0.001	Significant

Glucose uptake by yeast cell

Table 5 shows percent glucose uptake by yeast cell using Metronidazole and *C. cujete* leaf extract. Effect of Metronidazole on glucose uptake by the yeast cells at 5 mM glucose concentration for 1mg/ml, 2 mg/ml, 3 mg/ml, 4mg/ml and 5 mg/ml were 72.1%, 74.8%, 76.1%, 77.8% and 81.6%, respectively, while 24.0%, 61.1%, 77.9%, 78.8% and 80.1%, respectively, were the results for *C. cujete* leaf extract. At 10 mM glucose concentration for 1 mg/ml – 5 mg/ml of Metronidazole results were 50.2%, 60.1%, 61.8%, 74.9% and 85.8%, respectively, while for *C. cujete* leaf extract 48.9%, 68.9%, 72.8%, 76.1% and 74.7%, respectively.were.obtained. For 25 mM glucose concentration, Metronidazole effect for 1 mg/ml – 5 mg/ml were 44.0%, 47.5%, 51.2%, 53.0% of banaba aqueous extract (*Lagerstroemia speciosa*) are the compounds responsible for glucose transport inside the cell, thereby lowering extracellular glucose and the highest capacity of 65.4%. While

for C. cujete leaf extract for 25 mM glucose concentration, 21.8%, 23.5%, 26.8%, 48.6% and 67.6% were obtained.

Table 5
% Glucose Uptake by Yeast Cell

Conc.	5 mM		10 mM		25 mM	
	Metronidazole	*C. cujete* leaf extract	Metronidazole	*C. cujete* leaf extract	Metronidazole	*C. cujete* leaf extract
1 mg/mL	72.1%	24.0%	50.2%	48.9%	44.0%	21.8%
2 mg/mL	74.8%	61.1%	60.1%	68.9%	47.5%	23.5%
3 mg/mL	76.1%	77.9%	61.8%	72.8%	51.2%	26.8%
4 mg/mL	77.8%	78.8%	74.9%	76.1%	53.0%	48.6%
5 mg/mL	81.6%	80.1%	85.8%	74.7%	65.4%	67.6%

**Test were performed in triplicate*

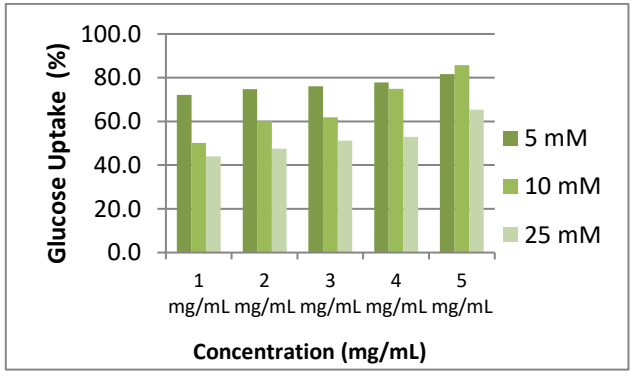

Figure 5. Glucose Uptake Using Metronidazole for Glucose Concentrations

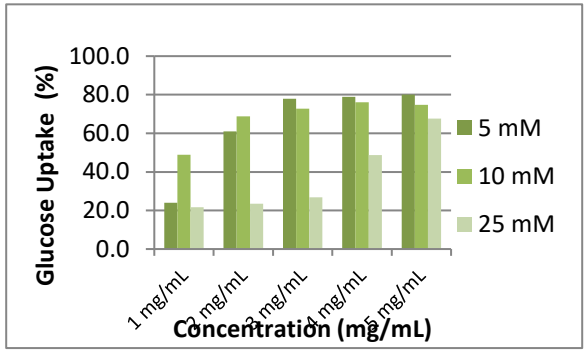

Figure 6. Glucose Uptake Using *C. cujete* leaf extract for Different Glucose Concentrations

Shown in Figures 5 and 6 are the glucose uptakes using both Metronidazole and *C. cujete* leaf extract. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. It is noticeable that increasing the concentration of both Metronidazole and *C. cujete* leaf extract leads to increase in the glucose uptake, while increasing the molar concentration of the glucose resulted to decrease in the glucose uptake. This can be attributed to phytochemicals that have hypoglycemic effect.

Table 6
Comparison between Mean Glucose Uptake

Glucose Concentration	Extract	Mean Glucose Uptake	p-value	Interpretation
5 mM	Metronidazole	76.4800	0.044	Significant
	C. cujete leaf extract	64.3800		
10 mM	Metronidazole	66.5600	0.463	Not significant
	C. cujete leaf extract	68.2800		
25 mM	Metronidazole	52.2200	0.034	Significant
	C. cujete leaf extract	37.6400		

Statistical comparison as shown in Table 6 for *C. cujete* leaf extract to the positive control is done to know if there is really a significant difference between the effects of the concentrations

against glucose uptake after addition of *C. cujete* leaf extract and Metronidazole.

The table reveals that there is a significant difference between the Metronidazole and *C. cujete* leaf extract in 5 mM and 25 mM glucose concentration with p-values of 0.044 and 0.034, respectively. This implies that there was a major difference seen when using both lowest Metronidazole and *C. cujete* leaf extract at the and highest glucose concentration used in the assay.

However, in 10 mM glucose concentration using *C. cujete* leaf extract, the results were similar to the positive control in effectiveness as evidenced by the p-value of 0.463. In relation to this, Cirillo (1962), pointed out that the rate at which the internal sugar approaches equilibrium with the external medium is greater at the lower concentration such as 10 mM glucose concentration; thus, explains the same effectiveness of Metronidazole and *C. cujete* leaf extract.

Some studies of different ethanolic plant extract are in contrary with the findings. For example, after the treatment of the yeast cells with the ethanolic extract of *Bruguiera cylindrica*, the glucose uptake did not show same expected results according to the research of Pitchaipillai and Ponniah (2017) even though it contains phytochemical like tannins that is believed to be involved in glucose uptake.

Table 7 is the multiple comparisons of Metronidazole and *C. cujete* extract. The 1 mg/mL incremental increase in the substances concentration resulted to significant differences for Metronidazole except for the glucose uptake between 2 and 3 mg/mL for 5 mM glucose and, 3 to 4 mg/mL for 25 mM glucose. On the other hand, significant differences were obtained for *C. cujete* leaf extract except between 3 to 4 mg/ml and 4 to 5 mg/ml in 5 mM glucose, 2 to 3 mg/ml, 3 to 4 mg/ml and 3 to 5 mg/ml for 10mM glucose and 1 to 2 mg/ml for 25 mM glucose. Based from the results, it is noticeable that as the concentration of both Metronidazole and *C. cujete* leaf extract increase, glucose uptake also increases.

The present data suggests that the plant extract is capable of enhancing glucose uptake effectively, which in turn suggests that it is capable of enhancing effective glucose utilization at 5 mg/ml concentration, thereby controlling blood glucose level as also suggested by other reports (Abirami & Natarajan, 2014; Ahmed, et al., 2009). However, Metronidazole still has higher hypoglycemic effect since it diffuses by selective absorption.

Table 7
Multiple Comparison of Glucose Uptake per Concentration

		Metronidazole			*C. cujete* leaf extract		
	Conc 2	**5 mM**	**10 mM**	**25 mM**	**5 mM**	**10 mM**	**25 mM**
1 mg/mL	2 mg/mL	0.001*	0.000*	0.001*	0.000*	0.000*	0.174 (NS)
	3 mg/mL	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	4 mg/mL	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	5 mg/mL	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
2 mg/mL	1 mg/mL	0.001*	0.000*	0.001*	0.000*	0.000*	0.174 (NS)
	3 mg/mL	0.109(NS)	0.005*	0.000*	0.000*	0.165(NS)	0.009*
	4 mg/mL	0.001*	0.000*	0.000*	0.000*	0.007*	0.000*
	5 mg/mL	0.000*	0.000*	0.000*	0.000*	0.026*	0.000*
3 mg/mL	1 mg/mL	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	2 mg/mL	0.109(NS)	0.005*	0.000*	0.000*	0.165(NS)	0.009*
	4 mg/mL	0.032*	0.000*	0.072(NS)	0.202(NS)	0.298(NS)	0.000*
	5 mg/mL	0.000*	0.000*	0.000*	0.002*	0.752(NS)	0.000*
4 mg/mL	1 mg/mL	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	2 mg/mL	0.001*	0.000*	0.000*	0.000*	0.007*	0.000*
	3 mg/mL	0.032*	0.000*	0.072(NS)	0.202(NS)	0.298(NS)	0.000*
	5 mg/mL	0.000*	0.000*	0.000*	0.076(NS)	0.896(NS)	0.000*
5 mg/mL	1 mg/mL	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
	2 mg/mL	0.000*	0.000*	0.000*	0.000*	0.026*	0.000*
	3 mg/mL	0.000*	0.000*	0.000*	0.002*	0.752(NS)	0.000*
	4 mg/mL	0.000*	0.000*	0.000*	0.076(NS)	0.896	0.000*

*Legend: NS- Not significant *The mean difference is significant at the 0.05 level*

This discrepancy can be attributed to phytochemicals that have hypoglycemic effect. It is believed in recent studies that glucose can transport across the cell membrane thru specific membrane carriers. Carriers may transport solutes down the concentration gradient, meaning the transport is only attained if intracellular glucose is removed. Hence, if the glucose inside the cell is reduced or utilized then therefore the transport of glucose outside the cell can possibly occur causing lower glucose outside the cell. The data obtained shows that it is capable of glucose

utilization, thereby controlling blood glucose level. (Sudha and Asna,.2009).

CONCLUSION

It is concluded that *C.cujete* leaf extract has antidiabetic activity based on alpha amylase inhibition assay and Glucose uptake by yeast cell. The antidiabetic activity was found to be dose dependent. In comparison to the standard drug used in this study, it is observed that Metformin and Metronidazole perform better when compared to the activity of *C. cujete* extract at the same concentration.

RECOMMENDATION

It is recommended that future researchers look for other ways to evaluate the anti-diabetic activity of *C. cujete* leaves, particularly in vivo procedures. Furthermore, purification of extract particulary for flavonoids, tannins and, saponins must be prepared to identify the specific active phytochemical present responsible for the antidiabetic activity.

REFERENCES

Abirami N, and Natarajan B., (2014). Isolation and Characterization of (4Z, 12Z)-Cyclopentadeca-4, 12-Dienone from Indian Medicinal Plant Grewia hirsuta and its Hyperglycemic Effect on 3 T3 and L6 Cell Lines. Int J Pharm Biol Sci. 2014;6:393-8.

Adugna, S., Alemu, L.A., Kelemu, T., Tekola, H., Kibret, B., Genet, S.(2004). Medical Biochemistry (Lecture Notes For Health Science Students) Gondar University, Jimma University, Debub University in Collaboration with the Ethiopia Public Health Training Initiative, The Carter Center, the Ethiopia Ministry of Health, and the Ethiopia Ministry of Education..

Ahmed, F., Asna, U., Sudha, S.(2009). Effect of various Ayurvedic formulations and medicinal plants on carbohydrate hydrolyzing enzymes and glucose uptake by yeast cells. ISSN: 0974-6943

Aiyelaagbe, O.O., Osamudiamen, P.M. (2009). Phytochemical screening of active compounds in Mangifera indica Leaves from Ibadan, Oyo State. *Plant Science Research.*2(1):11-13.

Anwuchaepe, A., Onyegbule, F., Ajaghaku, D., Nwafor, F. and Okoye, F. (2017). Evaluation of the in vivo antioxidant, toxicological and chromatographical profil. [online] Apjhs.com.

Ayyanar, M., Sankarasivaraman, K. & Ignacimuthu S. (2008).Traditional herbal medicines used for the treatment of diabetes among two major groups in south Tamilnadu India. *Ethnobotanical leaflets*, 12, 276-280.

Barrett, A., Ndou, T., Hughey, C., Straut, C., Howell, A., Dai, Z. and Kaletunc, G. (2013). Inhibition of α-Amylase and Glucoamylase by Tannins Extracted from Cocoa, Pomegranates, Cranberries, and Grapes. Journal of Agricultural and Food Chemistry, 61(7), pp.1477-1486.

Bhandari, M.R., N. Jong-Anurakkun, G. Hong & J. Kawabata,(2008). α-Glucosidase and α-amylase inhibitory activities of nepalese medicinal herb. *Food Chem*., 106: 247-252.

Bhatt U.P., S.C. Sati, Subhash Chandra, Sudhir Kumar, Amit Anthwal.(2015). Evaluation of In Vivo and In Vitro Anti-diabetic Activity of Roylea cinerea. *Int. J. Pharm. Sci*. Rev. Res., 32(2),; Article No. 33, Pages: 210-213

Cirillo, V. (1962). Mechanism of Glucose Transport Across the yeast cell http://jb.asm.org/content/84/3/485.full.pd

Daksha, G., Chandrashekar, Lobo, R., Nayak, Y., & Nilesh, G. (2012). In-vitroAntidiabetic activity of stem bark of Bauhinia purpurea Linn. *Der Pharmacia Lettre*, *4*(2), 614-619.

Department of Health. (2017). DOH leads World diabetes observance in the Philippines. Retrieved from htps://www.doh.gov.ph/node/11786

EL-Kamali, H.H., and EL-amir, M.Y., (2010) Antibacterial activity and Phytochemical screening of ethanol extracts obtained from selected Sudanese medicinal plants. Current research Journal of Biological Sciences; 2(2):143-146.

Ercan P., Sedef Nehir El.(2016) Inhibitory effects of chickpea and Tribulus terrestris onlipase, α-amylase and α-glucosidase. *Food Chemistry*.

Espinosa J.J, Rios M.A., Martínez,S.L.(2011). Antidiabetic activity of some pentacyclic acid triterpenoids, role of PTPe1B: In vitro, in silico, and in vivo approaches. *European Journal of Medicinal Chemistry* 46 2243-2251

Ezike, A. C., Akah, P. A., Okoli, C. C., & Okpala, C. B. (2010). Experimental evidence for the antidiabetic activity of *cajanus cajan*leaves in rats. *Journal of Basic and Clinical Pharmacy*, *1*(2), 81–84.

Handayani, R., Azizahwati, R. S., Hasyyati, U.S., (2015). Antidiabetic Activity and Phytochemical Screening of Extracts from Indonesian Plants by Inhibition of Alpha Amylase, Alpha

Glucosidase and Dipeptidyl Peptidase IV. *Pakistan Journal of Biological Sciences* 18 (6): 279-284.

Hossan SJ, El-Sayed M, Aoshima H. (2009).Antioxidative and anti α-amylase activities of four wild plants consumed by nomads in Egypt. Orient Pharm Exp Med.;9(3):217–224.

Kazeem M.I., J. O. Adamson, and I. A. Ogunwande.(2013).Modes of Inhibition of -Amylase and -Glucosidase by Aqueous Extract of Morinda lucida Benth Leaf.

Kim, S.D., & Nho, H.J, (2004). Isolation and characterization of α-Glucosidase inhibitor from the fungus Ganoderma lucidum. Journal of Microbiology, 42, 223-227.

Koffi, N., Solange T. M.,(2015). Ethnobotanical Study of Plants Used to Treat Arterial Hypertension, in Traditional Medicine, by Abbey and Krobou Populations of Agboville (Côte-d'Ivoire). European Journal of Scientific Research, ISSN 1450-216X Vol.35 No.1 pp 85-98

Liu, I., J. Kim, Y. Li, X. Liu, J. Li, X. Chen, An. (2001). Extract of Lagerstroemia speciosa L. has insulin-like glucose uptake–stimulatory and adipocyte differentiation–inhibitory activities in 3T3-L1 cells, J. Nutr. 131 2242–2247

Mahmoud, N., Ebrahim, H, A., Hezareh, A., Yaghmaei, P., Parivar, K., Larijani, B. (2010). Trans- chalcone: a novel small molecule inhibitor of mammalian alpha-amylase. *Mol Biol Rep*, 38(3):1617-20.

Matsui, T., Tanaka, T., Tamura, S., Toshima, A., Miyata, Y., Tanaka, K., et al. (2007). Alpha glucosidase inhibitory profile of catechins and theaflavins. *Journal of Agricultural and Food Chemistry*

Mioko K.N., Masami Ki, Emiko,K., Masao, S., IkuoI., Toshihiro, M. and Katsumi,I. (2001) Intestinal Absorbability of WheatAllergens,Subunits of a Wheat alpha-Amaylase Inhibitor, Expressed by Bacteria. *Bioscience, Biotechnology, and Biochemistry*. 65:2448-2455.

Modak, M., Dixit, P., Londhe, J., Ghaskadbi, S.,Devasagayam T.P.A. (2007). Indian Herbs and Herbal Drugs Used for the Treatment of Diabetes, *Journal of Clinical Biochemistry and Nutrition.* 40: 163-173.

Nam S., Smith D., Q. Dou P.,(2001) Tannic acid potently inhibits tumor cell proteasome activity, increases p27 and Bax expression, and induces G1 arrest and apoptosis, Cancer Epidemiol. Biomarkers Prev. 10 .1083–1088.

Olaniyi, M., Lawal, I., & Olaniyi, A., (2018). Proximate, phytochemical screening and mineral analysis of Crescentia

cujete L. leaves. Journal of Medicinal Plants for Economic Development. 2. 7. 10.4102/jomped.v2i1.28.

Parente, F. G, Oliveira, A. P.(2016). Phytochemical and antioxidant activity of methanolic fraction from the leaves of Crescentia cujete L. (Bignoniaceae). *Journal of Chemical and Pharmaceutical Research*, , 8(2):231-236

Roy A., Dement A.D., Cho K.H., and Kim J.H., (2015) Assessing glucose uptake through the yeast hexose transporter 1 (Hxt1). PLoS One.10:e012198510.1371/journal.pone. 0121985

Talukdar, N., Dutta, A., Chakraborty, R. and Das, K. (2017). Screening of phytochemicals, antioxidant and inhibitory effect on alpha-amylase by ethanolic extract of elaeocarpus ganitrus (bark) | *international journal of pharmaceutical sciences and research*

Tamil, I., Dineshkumar, B., Nandhakumar M., Senthilkumar, M., and Mitra A. (2010). In vitro study on α-amylase inhibitory activity of an Indian medicinal plant, Phyllanthus amarus. *Indian Journal of Pharmacology*. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2959209/https ://www.

Vijaya, A., Manikandan., Vijaya K.,, Sampath K., and Agaath, H.,(2016). “Phytopharmacological overview of Psidium guajava Linn.,” Pharmacognosy Journal, vol. 8, no. 4, pp. 314–320,

Yuan, E., Liu, B., Wei, Q., Yang, J., Chen, L. andLi, Q. (2014). Structure activity relationships of flavonoids as potent alpha-amylase inhibitors. - PubMed - NCBI. [online] Ncbi.nlm.nih.gov.