In vitro antidiabetic activity of Premna odorata Blanco (Fam. Lamiaceae)

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Deanne Marizthel J. Madrio1, Paola Georgia P. Awatin, Aaron Dell A. Cobeng, Oliver Shane R. Dumaoal, Omar A. Villalobos*
College of Allied Medical Professions, Lyceum of the Philippines University, Capitol Site, Batangas City, Philippines
oavillalobos@lpubatangas.edu.ph

Abstract – *Diabetes mellitus (DM) with its underlying complications, is a multifactorial chronic endocrine disorder which arises from a complex pathogenesis. This study explored the utilization of an endemic plant Premna odorata Blanco aerial parts (leaves, bark, fruits & flower) for in vitro analysis of α-amylase and α-glucosidase inhibition, glucose diffusion inhibition, and glucose uptake in yeast cells enhancement activities. Premna odorata dried aerial parts were sequentially extracted with solvents of increasing polarity (hexane-dichloromethane-methanol). Results for in vitro assays revealed that DCM bark and fruits/flower plant extract have significant α-amylase and α- glucosidase inhibitory activities. The potency of the said DCM extracts was also found to be superior to standard drug with IC50 for α-amylase enzyme from Aspergillus oryzae of (POBDCME 1.02 mg/mL; POFDCME1.163 mg/mL; Acarbose 19.45mg/mL) and porcine pancreas (POBDCME 0.6613mg/mL; POFDCME 1.165mg/mL; Acarbose 39.81mg/mL) and α-glucosidase from Saccharomyces cerevisiae (POBDCME 0.3142mg/mL; POFDCME 0.6738 mg/mL; Acarbose 0.3507mg/mL). POHBE and POFFME also promoted increase (89% and 97%) in glucose uptake in yeast cell and revealed significant glucose diffusion inhibition (73% and 69%) across dialysis membrane respectively, all of which suggest promising potential antidiabetic activity.*

Keywords – *Diabetes mellitu, α-amylase, α-glucosidase, glucose diffusion, glucose uptake, Premna odorata*

INTRODUCTION

Diabetes mellitus (DM) is described as a chronic, progressive, and multifactorial endocrine disorder which was only considered a minor significance to world health, until the 21st century when it became one of the four major non-communicable diseases of the world [1]. It is responsible for over 425 million cases and 5.0 million deaths (9.9% global mortality) among people 20– 99 years of age in 2017 and is expected to escalate to 629 million morbidities in 2045 [2]. In the Philippines, 3,721,900 Filipinos (6.1% of the adult population) is afflicted with diabetes (International Diabetes Federation, 2017). Two major types of diabetes mellitus (DM) are identified, namely Type 1 and Type 2, the latter is more common. Type 1 DM is the representative disorder of the autoimmune demolition of the beta cells located on the pancreatic islet of Langerhans, resulting in insufficient insulin production. Conversely, in Type 2 DM, the pancreas produces reasonable insulin levels, nevertheless, the cells fail to respond to the produced insulin leading to marked decrease in glucose uptake and increased glucose level in the blood [3]. This persistent accumulation of glucose in the blood is the clinical hallmark of diabetes, and when left uncontrolled, may exacerbate to disabling and life-threatening complications affecting the heart, kidneys, nerves and blood vessels. Starch hydrolysis starts with the action of salivary amylase that first degrades polysaccharides into shorter oligosaccharides [4].

Upon reaching the stomach, all reactions by salivary amylase are terminated due to the presence of hydrochloric acid. This is when the pancreas releases another isozyme of alpha amylase and further degrades the partially digested material into smaller oligosaccharides. Subsequent catabolic process of these oligosaccharides by intestinal alpha- glucosidase come about after, and eventually results in simple, absorbable monosaccharide units such as glucose, which will then be absorbed through an identified transport system [5]. Once absorbed, and without optimal uptake and utilization by cells as seen in DM, glucose accumulates in the circulation, and reacts with proteins, generating advanced-glycation end products (AGEs) that increase the formation of reactive oxygen species involve in oxidative stress, which are reported to be precursors of long term diabetic complications [6]. Currently, conventional drugs used in DM, aside from being costly, give rise to intolerable side effects which cause hindrance to patient adherence and treatment success [7]. Thus, antidiabetic potentials of herbal plants are now being explored with the aim of providing cost- effective and more dynamic treatment of diabetes [8]. Among all the plant phytochemicals, flavonoids, alkaloids, lycosides,

polysaccharides, terpenoids, and steroids showed the most promising antidiabetic potentials [9].

Premna odorata Blanco (Lamiaceae)

P. odorata is an endemic plant locally known as 'Alagau' or 'Agbau' [10]. At present, it is incorporated into the seven segments of the local preparation 'Pito-Pito' [11]. Previous studies showed that P.odorata Blanco possesses anti- tubercular activity [12], cytotoxic activity in cancer cell lines, antimicrobial, anti- inflammatory and chemopreventive properties [11]. Phytochemical review of written works also revealed the sort Premna to be an abundant source of flavonoids, diterpenoids, and iridoid glycosides wherein the latter is known to have significant hypoglycemic activity [13].

P. odorata Blanco, specifically, is a source of several iridoid glycosides, however the current studies did not describe the potential pharmacologic activity of the isolated compounds [10]. No study exploring the antidiabetic potential of this plant has been made, but relative species under the same genus were proven to possess the target activity.

Figure 1. ***Premna odorata*** **Blanco tree from Naujan, Oriental Mindoro**

Objective of the Study

Generally, this study aims to determine the potential antidiabetic activities of the different aerial parts of Premna odorata. Specifically, (1) to screen the aerial part and extract of Premna odorata thru in vitro α- amylase inhibition assay using Aspergillus oryzae amylase enzyme, (2) evaluate and compute the IC50 of the most active plant part and extract thru in vitro α-amylase inhibition assay using Aspergillus oryzae and Porcine pancreatic amylase enzyme, (3) evaluate and compute the IC50 of the most active plant part and extract thru in vitro α-glucosidase inhibition assay, (4) determine the free radical scavenging activity of the most active extract and (5) quantify the total phenolic and total flavonoid present in the plant extracts with the highest α-glucosidase inhibition activity. Moreover, the study also aims to explore other antidiabetic mechanisms other than enzyme inhibition. Specifically, (6) to assess the ability of P.odorata extracts to entrap and retard glucose transport to external solution thru glucose diffusion inhibition assay and (7) to evaluate the ability of the extracts to enhance glucose uptake and utilization by cells thru glucose uptake in yeast cell assay. However, the identification, isolation and purification of the active compounds are beyond the scope of this study.

MATERIALS AND METHODS

Chemicals and Reagents. Analytical grade Dimethylsulfoxide (DMSO), n-Hexane and ethanol were purchased from RCL Labscan. Technical grade dichloromethane and methanol were purchased from RTC Laboratories. Quercetin, gallic acid, p-nitrophenyl-a-D-glucopyranoside and Folin-Ciocalteaue's phenol reagent were procured from Sigma Aldrich. Ascorbic acid and 3,5- dinitrosalicylic acid (DNSA) analytical reagents were purchased from Loba Chemie Laboratories. Acarbose was purchased from Bayer Laboratories. Enzyme. α-Amylase enzyme from Aspergillus oryzae (1.5U/mg) and Porcine pancreas (5U/mg), and α-Glucosidase from Saccharomyces cerevisiae (10U/mg) were purchased from Sigma aldrich.

Plant collection, authentication and preparation

Aerial parts of Premna odorata (leaves, fruits/flowers and bark) were collected from Naujan, Oriental Mindoro and authenticated at the University of Santo Tomas-Research Center for the Natural and Applied Sciences-Plant Herbarium. The collected parts were washed and subjected to air drying at room temperature for 3 weeks. Dried P. odorata were loaded into a mechanical blender until coarse.

Plant extraction

Exhaustive sequential extraction thru maceration using solvents of increasing polarity was performed. Dried aerial parts of P. odorata were loaded into a 1000-mL flask and repeatedly extracted with hexane until the solvent became colorless. Afterwards, residual hexane was evaporated at room temperature. Similar extraction techniques were employed using dichloromethane and methanol using the same plant sample. Maceration was done for 24 hours.

Pooled extracts were concentrated in vacuo and stored in a sealed amber bottle and coded as follows; POHE (P. odorata Hexane Extract) PODCME (P. odorata Dichloromethane Extract) and POME (P.

odorata Methanol Extract). Coded extracts were stored in -20°C until further use.

α-Amylase inhibition assay

To screen the alpha amylase inhibitory activity of the extract, the method devised by Xiao et al., [14] using microplate-starch iodine based assay was carried out with slight modifications. In a microcentrifuge tube, 100 uL of the plant extract and 20 uL of alpha-amylase solution was incubated at 37°C for 5 minutes. The substrate solution (100 uL of hydrolyzed starch solution) was added to the reaction mixture and incubated for 30 mins at 37°C. Twenty (20) uL of 1M HCl was added to halt the enzymatic reaction followed by the addition of the colorimetric indicator, 50 uL iodine reagent (5mM I2 and 5mM KI). The iodine treated sample was diluted with PBS to obtain a total reaction mixture volume of 1 mL. Then, an aliquot of 200 uL from the reaction mixture was transferred into a transparent flat- bottomed 96-well microplate and the absorbance at 580 nm (A580) was measured using a microplate reader. A control sample without the plant extract was prepared and blank reaction was carried out. The result was compared to the test samples with the plant extract. Acarbose was used as the standard inhibitor.

α-Glucosidase inhibition assay

The α-glucosidase inhibitory assay was performed according to the methods of Dehghan, Salehi, & Amiri, (2018). Briefly, 50 µL α-glucosidase solution (10 U/mL in PBS) was added to mixtures of 100 µL aliquots of sample solution in DMSO (1000, 500, 250,125, 62.5 µg/ml) and 620 µL phosphate buffered saline (0.1 M, pH 7.4) and incubated at 37 °C. After 20 min, 100 µL of substrate (10 mM p-nitrophenyl α-D-glucopyranoside in PBS) was added to the reaction mixture and incubated at 37 °C for 30 min. Then, the reaction was terminated by adding 150 µL of 1M Na2CO3. The enzymatic hydrolysis of substrate was measured at 405 nm wherein the amount p-nitrophenol released during the reaction was noted. Individual blanks were prepared for correcting the background absorbance, where the enzymes were replaced with water. Controls were conducted in an identical manner replacing the plant extracts with dimethylsulfoxide. Acarbose was used as a positive control. All experiments were carried out in triplicate.

DPPH Free radical scavenging assay

The free radical scavenging activity of the extracts were examined in vitro following the protocol set by Sailaja, Bharathi, & Kvsrg [15]. Solutions of the extracts were added to 100 µL of DPPH in ethanol in a 96-well plate. Then, the plate was kept for 20 minutes at ambient temperature and the absorbance was measured at 517 nm. The difference in absorbance between the control and the sample extracts were calculated and expressed as % scavenging of DPPH by the extracts. Ascorbic acid was used as the positive control. Results were expressed in triplicate and IC50 of the extracts were generated.

Total Phenolic Content

To test for the total phenolic content of the plant extracts with the highest inhibitory activity, the protocol devised by Ghafar et al., [16] was followed with minor modifications in process, wherein 200 µl of the plant extract was mixed with 800 µl of 2N Folin-Ciocalteaue reagent. The reaction mixture was incubated at room temperature for 5 minutes to initiate the reaction and subsequently, 20% Na2CO3 was added to increase the sensitivity of the Folin-Ciocalteaue reagent. Final reaction mixture was incubated at a dark place for 2 hours. Reaction mixture was read at 590nm. Gallic acid was used for the preparation of standard curve and control was conducted in an identical manner replacing the plant extracts with water.

Total Flavonoid Content

For the quantification of total flavonoid content of P. odorata, the method by Ghafar et al., [16] was followed. A reaction mixture composed of 100 µl of the plant extract and 100 µl of 1% aluminum chloride in methanol was prepared. Then, the reaction mixture was incubated at room temperature for 1 hour and the absorbance was read at 590nm. Quercetin was used for the preparation of standard curve and controls were conducted in an identical manner replacing the plant extracts with methanol.

Glucose uptake by yeast cell assay

Yeast cells were prepared according to method described by Cirillo, [17]. Commercial baker's yeast was repeatedly washed with distilled water with the aid of centrifugation until a clear supernatant liquid was achieved. A 10% (v/v) yeast suspension was prepared using distilled water. Glucose solution (5mM) in distilled water was prepared. A 1 ml glucose solution was added to 100 µl aliquots of varying concentration of the extracts and incubated for 10 minutes at 37°C. 100 µl of yeast suspension was added to the mixture for the reaction to proceed. Mixture was vortexed and

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further incubated at 37°C for 60 mins. Afterwards, the tubes were centrifuged for 5 mins and glucose present in the supernatant liquid was estimated using DNSA method. Percent glucose uptake was measured using the formula:

$$\%\ uptake\ in\ yeast\ cell = \frac{(Abs.\ of\ control - Abs.\ of\ sample)}{Abs.\ of\ control}\ x\ 100$$

All experiments were carried out in triplicate.

Glucose diffusion inhibition assay

The potential of P. odorata to inhibit the diffusion of glucose into the external solution were also explored according to the method devised by Sattar et al., (2012), with minor modification in process. In a dialysis tube (6cm x 15 mm), 15 ml of known concentration of glucose (2.5mM) and NaCl (0.15M) was introduced. The sealed dialysis tube was placed in a 50-ml centrifuge tube containing 0.15 M NaCl and placed on an orbital shaker at room temperature and was mixed in constant motion for 24 hours. The diffusion of glucose into the external solution was then measured and glucose concentration was measured using DNSA method. Percent inhibitions were calculated. All experiments were carried out in triplicate.

Statistical Analysis

The corresponding percentage inhibitions of samples were computed using Microsoft Excel and IC50 were generated using GraphPad Prism 7.

RESULTS AND DISCUSSION

Plant Extraction

Concentrated extracts were weighed and percentage yield was computed using the formula:

$$\%yield = \left[\frac{\text{Wt. of concentrated extract}}{\text{Wt. of dried raw material}}\right] x100$$

Table 1. Calculated percentage yield of extracts

Plant Part	Hexane	DCM	Methanol
Leaves	3.54%	1.89%	8.11%
Stem Bark	0.19%	2.17%	0.79%
Fruits & Flower	1.64%	0.76%	1.94%

α-Amylase inhibition screening assay

In a normal physiological process, polysaccharides are degraded by alpha amylase enzyme into smaller oligosaccharides. In the presence of an inhibitor, the activity of *α*-amylase is reduced, inhibiting the hydrolysis of polysaccharides into absorbable glucose units [5]. This was revealed upon the addition of a colorimetric indicator (iodine reagent), forming a blue-violet coloration indicating the presence of starch-iodo complex in the reaction mixture. Based on the result of the screening assay, Acarbose with a 50mg/mL concentration showed 100% inhibition of a 2U alpha amylase enzyme from *A.oryzae*. As shown on Figure 3, 500 µg/mL of *P. odorata*. Bark DCM extract and *P. odorata* fruits and flower DCM extract was also able to inhibit the said enzyme with 100.00 and 88.79 percentage inhibition respectively. *P. odorata* DCM bark extract and *P. odorata* DCM fruits and flower extract were used to further check the inhibitory control 50 (IC50) for the said enzyme. These two extracts were chosen based on the analysis of variance wherein post hoc analysis resulted in >0.05 p value indicating that there was no significant difference between the inhibitory activity the standard drug and the said extracts against alpha amylase enzyme from A. oryzae.

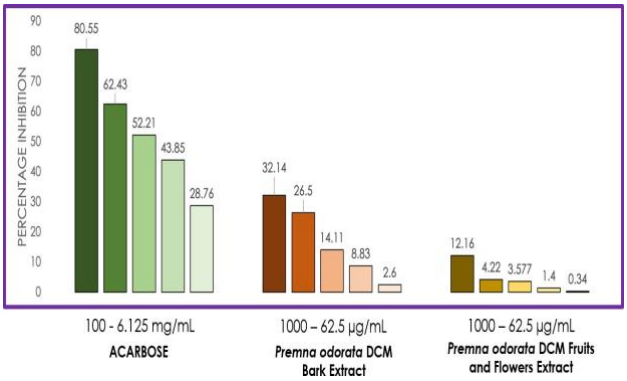


Figure 3. Graph showing percentage inhibition of *P. odorata* extracts and acarbose for alpha amylase screening assay

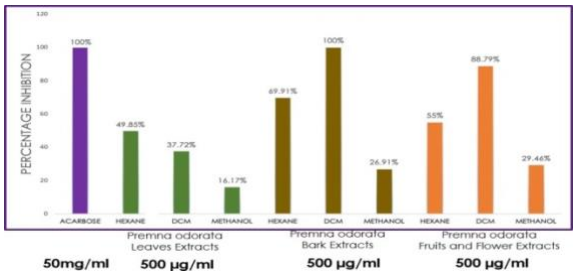


Figure 4. Graph showing percentage inhibition of *P. odorata* DCM bark and fruits and flower extracts and acarbose for alpha amylase inhibition assay

α-Amylase Inhibition Assay (*Aspergillus oryzae*)

For the inhibition assay, *α*-amylase enzyme from *A.*

oryzae was increased from 2U for the screening assay into 5U. Results showed that acarbose with the highest concentration 100 mg/mL, revealed 80.55% inhibition of the enzyme. For *P. odorata* DCM bark extract, 32.14% of the *α*-amylase enzyme were inhibited during the reaction, while the highest concentration (1000 µg/mL) of *P. odorata* DCM fruits and flower extract inhibited 12.16 % of the enzyme.

After generating the IC50 of each inhibitor, results revealed that the IC50 *of P. odorata* DCM bark extract (1.02 mg/mL) and *P. odorata* DCM fruits and flower extract (1.163 mg/mL) were significantly lower than that of acarbose (19.45mg/mL). This would mean that 50% of the α-amylase enzyme from A. oryzae present in the reaction were inhibited using only 1mg of the two DCM extracts of P. odorata compared to 19.45 mg requirement for acarbose.

α-Amylase Inhibition Assay (Porcine pancreatic enzyme)

Since normal physiologic process of glucose metabolism entails the action of pancreatic α-amylase after the termination of the action of salivary amylase in the stomach [8], acarbose, P. odorata DCM bark extract and P. odorata DCM fruits and flower extracts were further subjected to α- amylase inhibition assay using 5U of porcine pancreatic enzyme. Results shown in Figure 5 revealed that the highest concentration (100mg/mL) of acarbose was able to inhibit 74.23% of enzyme present in the reaction mixture, while P. odorata DCM bark extract and P. odorata DCM fruits and flower extract with concentrations of 1000 µg/mL inhibited 50.71% and 22.25% of the enzyme respectively. The IC50 of P. odorata DCM bark extracts was significantly lower (0.6613mg/mL) than that of the standard drug acarbose (39.81mg/mL). From these results, it can be inferred that the plant extract is more potent than acarbose in inhibiting 50% of α- amylase enzyme from porcine pancreas present in the reaction mixture. This is also true for the results revealed by P. odorata DCM fruits and flower extract (1.165mg/mL IC50).

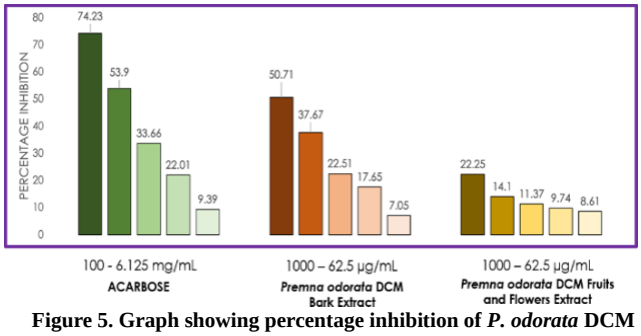
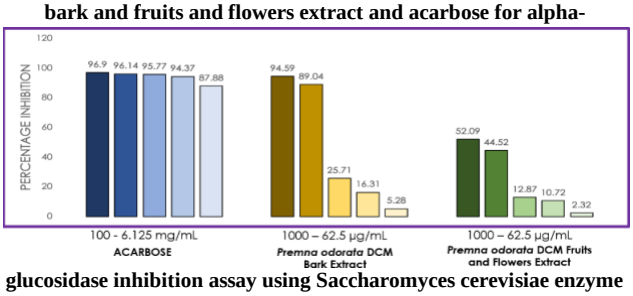


Figure 5. Graph showing percentage inhibition of *P. odorata* DCM bark and fruits and flowers extract and acarbose for alpha- amylase inhibition assay using porcine pancreatic amylase enzyme

α-Glucosidase inhibition assay

For the α-glucosidase inhibition assay, results showed that acarbose (100mg/mL) was able to inhibit 96.9% of 1U/mL α-glucosidase enzyme from Saccharomyces cerevisiae. The percentage inhibition of P. odorata bark DCM extracts (94.59%) was almost similar with that of acarbose even at a concentration of 1000µg/mL. Results also showed that P. odorata DCM fruits and flower extract was also able to inhibit α-glucosidase enzyme with 52.09 % inhibition. The results revealed by acarbose and P. odorata DCM bark extracts to inhibit 50% of the enzyme present on the reaction were almost similar yielding an IC50 of 0.3507 and 0.3142 respectively. Significant inhibition of α-glucosidase enzyme that were revealed by P. odorata DCM extracts indicated that the final stage for glucose metabolism was also retarded and the availability of absorbable glucose units should potentially decrease [5]. Results are summarized in Figure 6.

Figure 6. Graph showing percentage inhibition of P. odorata DCM bark and fruits and flowers extract and acarbose for alpha-



glucosidase inhibition assay using Saccharomyces cerevisiae enzyme

DPPH radical scavenging assay

Several studies revealed that antioxidants also play a role in preventing diabetic complications by preventing oxidative stress [19]. Hence, P. odorata DCM bark and fruits/flower extracts were tested for their antioxidant properties. The principle behind this test is dependent upon the electron-giving capacity of antioxidant compounds present on the extracts and standard drug which would turn DPPH from violet to yellow as a positive result [13]. Upon measuring the absorbance, results revealed that the extracts in 1000ug/mL concentration possess some antioxidant activities against DPPH radicals, generating 32.85% and 52. 32% inhibition, respectively. However, compared to ascorbic acid (125mg/mL) with 71.27% inhibition, which has already recognized antioxidant properties, the results of extracts summarized in Fig. 7 were less effective, and the potency of the standard drug is far superior, with IC50 of 5.67mg/mL which is lower than the samples with IC50 of 993mg/mL and 615.9 mg/mL respectively for bark and fruits/flower. These findings indicate that the antioxidant activity of P. odorata Blanco cannot be considered as a mechanism of the antidiabetic claim of the plant.

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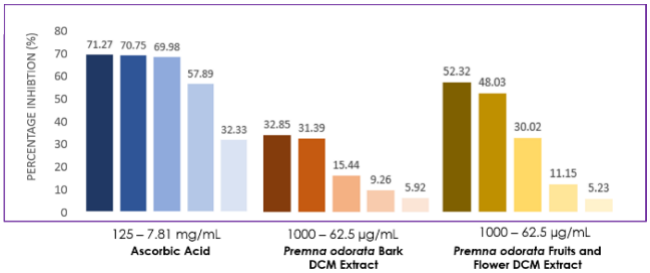

Figure 7. Graph showing percentage inhibition of *P. odorata* DCM bark and fruits and flowers extract and ascorbic acid for DPPH free radicals scavenging assay

Total Phenolic Content

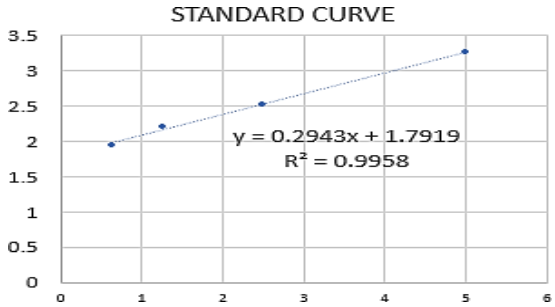

Figure 8. Standard calibration curve of gallic acid for total phenol determination

Based on the results obtained from the previous assays, it can be observed that the majority of activities were found on extracts which used dichloromethane (DCM) as the extracting solvent. DCM is classified as a semi- polar solvent and most semi-polar phytochemicals from the plant are expected to be extracted by it. Phenolic compounds are known to be semi-polar, hence, the total phenolic content of P. odorata DCM extracts were also calculated. The principle behind the test was based on Folin-Ciocalteaue reagent being an oxidizing agent that may oxidize the gallic acid present in the reaction mixture. As the concentration of gallic acid decreases, the absorbance also decreases forming a linear regression which can be seen on the graph Figure 8. Gallic acid was used to prepare the standard curve and results generated an equation of the line y = 0.0066x + 0.2487 and R^2 of 0.9965. The total phenolic content of P. odorata DCM bark extracts and P. odorata DCM fruits and flower extracts were computed and revealed 55.65 ± 3.43 µg and 48.33 ± 2.06 µg gallic acid equivalent per 500 µg/mL of the extracts respectively.

Total Flavonoid Content

A linear equation was generated y = 0.0091x + 0.1952; R^2 = 0.9892 as seen from the graph in Figure 9, from the standard curve of quercetin which is an example of a flavonoid compound. Based on the linear equation obtained, the quercetin equivalent values were computed and results showed P. odorata DCM bark extract and P. odorata DCM fruits and flower extracts having 18.47 ± 3.04 µg and 17.85 ± 1.76 µg per 500 µg/mL of the extracts, respectively.

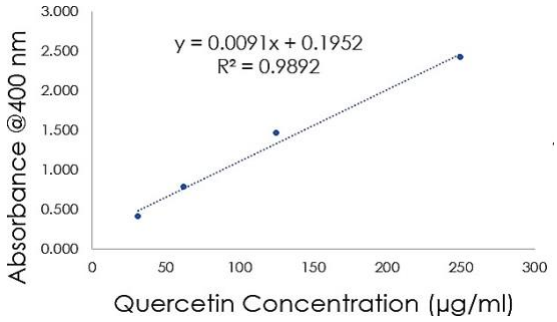

Figure 9. Standard calibration curve of quercetin for total flavonoid determination

Glucose uptake by yeast cell assay

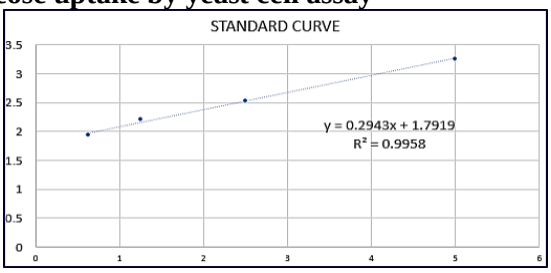

Figure 10. Standard calibration curve of glucose for glucose uptake by yeast cell assay

Recent studies have already utilized the mechanism of glucose uptake in yeast cell for the determination of hypoglycemic activities. This method was originally based on the principle of the study of Cirillo [17], suggesting that the movement of glucose in yeast cell is mediated by stereospecific membrane carriers, and can be used for in- vitro illustration of sugar transport on higher organisms [18]. Based on the equation of the line y = 0.2943x + 1.7919 generated from the linear regression of glucose, percent glucose uptake in yeast cell by P. odorata extracts were calculated and summarized in Figure 11.

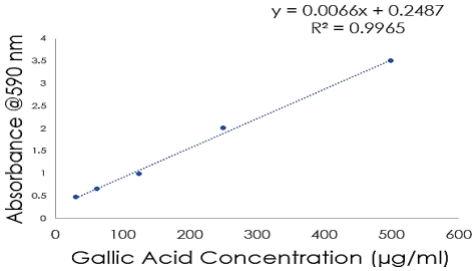

Figure 11. Standard calibration curve of glucose for glucose diffusion inhibition assay

Results revealed that P. odorata hexane bark (POHBE) and methanol fruits/flower extracts (POMFFE) exhibited the greatest activities and were able to promote the uptake of glucose in 5mM concentration across yeast membrane by 91.3% and 96.8% respectively. The assay was repeated using the extracts which generated the highest percentage uptake on the screening assay using increasing concentration of extracts to determine the correlation between the dose of extracts and glucose uptake capacity. Results revealed that with 1mg/mL of POMFFE, the glucose uptake capacity in yeast cell is at 33% and has reached almost 98% when 5mg/mL of extract was used (Figure 12), suggesting directly proportional relationship between the number of carriers that would bind glucose and transport it across membranes, and the increase in uptake capacity.

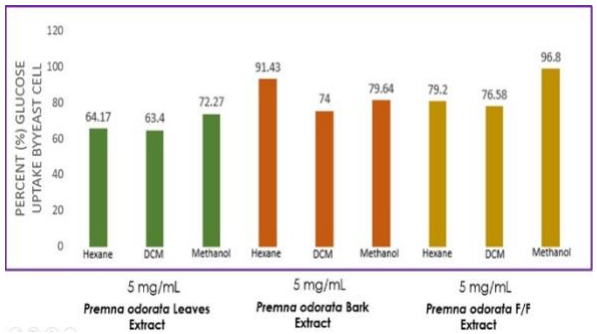

Figure 12. Effect of *P. odorata* extracts on glucose uptake by yeast cell

The results are also true for POHBE. However, the glucose uptake in yeast cells is influenced by several variables, one of which is the intracellular concentration of glucose and the rate of its metabolism by yeast cell that can affect the concentration gradient and thus may naturally enhance glucose uptake and alter the results. Hence, we recommend for further studies to explore the activity of POMFFE and POHBE to bind glucose and transport it across cell membranes in vivo using adipose or muscle tissues.

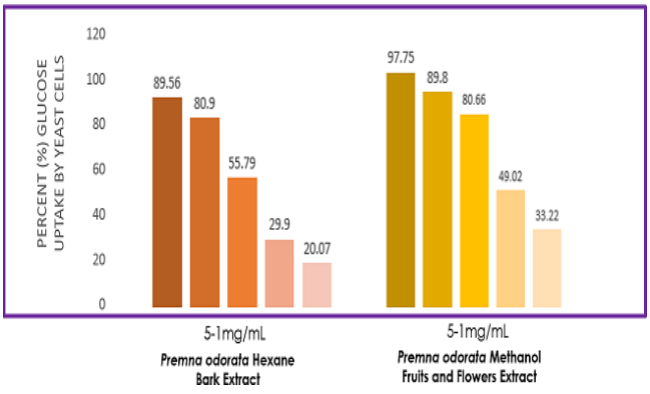

Figure 13. Percent glucose uptake by yeast cell of *P. odorata* bark hexane and fruits and flowers methanol

Glucose diffusion inhibition assay

Recent studies demonstrated that dietary fibers from plants modulate postprandial rise of glucose levels and is the reason behind their use in the diet of type 2 diabetic patients. For this assay, the dialysis membrane simulated the intestinal mucosa and the movement of glucose from the internal to the external solution was measured, simulating the transport of glucose from the intestine to the blood. The principle behind this test is that dietary fiber from the extracts, if there is any, would entrap glucose by rendering a more viscous environment than normal, retarding its absorption [18].

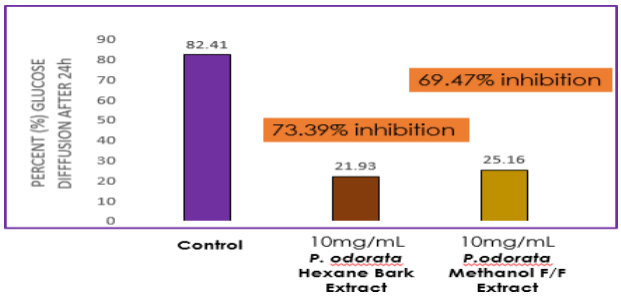

Figure 14. Percent glucose diffusion inhibition of *P. odorata* bark hexane and fruits and flowers methanol

The effect of the POMFFE and POHBE in inhibiting glucose diffusion across dialysis membrane are summarized in Figure 14. After generating the standard curve, the diffusion of glucose in 2.5 mM concentration to the external solution after 24 hours was calculated and percent inhibition of POMFFE and POHBE in 10mg/mL concentration was computed. Results revealed that there was a significant decrease in glucose diffusion on the solution with extracts (POHBE = 21.93% diffusion; POMFFE=25.16% diffusion) compared to that of control without extracts, wherein 82.41% of glucose diffused to the external solution. POHBE revealed greater inhibition of 73.39% than POMFFE with 69.47% inhibition. Reports from recent studies showed that water soluble dietary fibers have more activity than water insoluble fibers in inhibiting glucose diffusion due to their more viscous nature. However, the result of this study revealed more inhibition coming from the non- polar hexane extract compared to the polar methanolic extract. This can be explained by the factors that were reported to affect glucose diffusion: the adsorptive capacity and viscosity of dietary fibers [21]. This assay only explored the effect of viscosity factor, and maybe POHBE has a greater adsorptive capacity than POMFFE, hence the results. Thus, we further recommend testing for the glucose adsorption capacity of POHBE and POMFFE.

CONCLUSION AND RECOMMENDATION

This study is the first to demonstrate the antidiabetic activity of Premna odorata Blanco in vitro using a multi-mechanistic approach. Among all the plant aerial part extracts, DCM extracts of the bark and fruits and flower of P. odorata exhibited significant inhibition of α-amylase upon screening and exhibited superior IC50 results for α-amylase and α-glucosidase enzymes against standard drug acarbose.

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Furthermore, the antioxidant properties of the above extracts were also tested and showed inferior activity than ascorbic acid. This is also the first study to quantify the phenolic and flavonoid content of the bark and fruits and flower DCM extracts of the said plant and results suggest that mostly of the phenolic compounds present on the DCM extracts of P. odorata were flavonoid in nature. Results also revealed that POHBE and POMFFE can potentially inhibit glucose diffusion in vivo, thus further inhibiting the absorption of glucose. The ability of P. odorata aerial parts extracts to enhance glucose uptake in yeast cell were also tested and revealed that hexane bark and methanol fruits and flower extracts promoted increase in glucose uptake capacity in yeast cell which can be used as initial reference for further studies in vivo. Based on these current findings, we can suggest that P.odorata Blanco can be a potential source of novel compounds that may provide more cost effective and dynamic treatment of diabetes. For further studies, it is recommended for identification, isolation, and purification of the active compound and test the activity of the active extracts using animal model. Other in vitro assay involving the other mechanisms or control of hyperglycemia can be done to further assess the antidiabetic claim of Premna odorata bark and fruits and flower plant parts

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