

**Phytochemical Characterization and *In-vitro*
 α -glucosidase Inhibitory Effects of the Methanolic
Extract of *Musa errans* var. *botoan* (Musaceae)**

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Abstract: Alpha-glucosidase inhibitors are used to control blood glucose levels in type 2 diabetes. *Musa errans*, locally known as botoan, is an endemic form of banana that grows in the Philippines. A study reported on the hypoglycemic potential of *M. errans in vivo* (Feliciano, Galecio, Labutong, Reyes & Serrano, 2014) but there are no current studies on the *in-vitro* inhibitory potential of this plant. The study aims to investigate the phytochemical characteristics and the antidiabetic activity of the methanolic extract of *M. errans* through α -glucosidase inhibition. Thin layer chromatography detected the presence of phenols, tannins, flavonoids, anthraquinones, essential oils, and indoles. Percent inhibition of the *M. errans* extract is at 97 ± 0.27 using concentrations of 500, 250, 125, and 62.5 $\mu\text{g/mL}$. The α -glucosidase IC_{50} of *M. errans* was found to be 0.0002634 (95% CI: 0.00009154 to 0.001178) while the IC_{50} of Acarbose is 78.3 $\mu\text{g/mL}$ (95% CI: 73.89 to 83.25). The results of the study indicate the antidiabetic potential of *M. errans* and will serve as additional knowledge for developing antidiabetic drugs from natural sources.

Keywords: *Musa errans*; *Alpha-glucosidase*; *in-vitro*; *Type 2 diabetes*

1. INTRODUCTION

Diabetes Mellitus is a chronic disease characterized by hyperglycemia either resulting when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. The hormone responsible for maintaining blood sugar levels is insulin. The body's ineffective use of insulin is called Type 2 diabetes (World Health Organization (WHO), 2018). Hyperglycemia is a sign of uncontrolled diabetes. It is responsible for long term damage affecting various organs such as eyes, kidneys, nerves, heart, and blood vessels (American Diabetes Association [ADA], 2004).

The most common type of diabetes in the Philippines is Type 2 diabetes and around 1.7 million Filipinos remain undiagnosed (Tan, 2015). In 2018, the International Diabetes Federation (IDF) estimated over 3.7 million cases of Type 2 diabetes in the Philippines with a 6.2% prevalence rate in adults.

Alterations in the composition of caloric content of diet have profound influences on the development of type 2 diabetes mellitus. Drugs that act on the gastrointestinal tract to interfere with carbohydrate digestion might be useful agents for the treatment of diabetes (Lebovitz, 2016). Alpha-glucosidase inhibitors, also known as untapped diamonds of diabetology are unique class of anti-diabetic drug that works to delay carbohydrate absorption in the gastrointestinal tract (Karla, 2014).

Health performance and economic performance are interlinked resulting in many Filipinos relying in “out-of-pocket” expenses for health maintenance including treatment and laboratory regimens. This burden may lead to complications due to poor medication therapy compliance (Tan, 2015).

Musa errans from the family Musaceae, is an endemic form of wild banana that grows spontaneously in most parts of the Philippines (Ragasa, Martinez, Chua, & Rideout, 2007). A study conducted by Feliciano, Galecio, Labutong, Reyes, and Serrano (2014) reported that the unripe fruit methanolic extract of *Musa errans* has a potential hypoglycemic property however, there are no studies focusing on the *in-vitro* inhibitory potential of the extract. Therefore, the main objective of this study is to investigate the phytochemical characteristics and the antidiabetic activity of the methanolic extract of *M. errans* through α -glucosidase inhibition.

2. METHODOLOGY

2.1 Plant Material

Fresh unripe botoan fruits were collected from Siniloan, Laguna. A sample was submitted to the National Museum-Botany Division and was authenticated as *M. errans* var. botoan. The fruit was separated from the peel and was cut and oven-dried in an oven (Heratherm OGS60) at 50°C. The samples were periodically weighed until constant weight was obtained. After

drying, the samples were ground to a moderately coarse powder (Sieve no.40) using a wiley-mill (Thermos Scientific 3383-L30). The sample was macerated in 95% methanol for 48 hours and filtered using Whatman filter paper no. 40. The filtrate was concentrated using a rotary evaporator (Eyela SB-1100) and was subjected in a water bath until reaching a semi-solid consistency. The extract was kept in a tight, light resistant container and stored in a cool place.

2.2 Phytochemical Screening by Thin Layer Chromatography

Phytochemical analysis of the extract was conducted using Thin Layer Chromatography (TLC) as adapted from Guevara (2005). Two hundred milligrams of the methanolic extract was dissolved in 4 mL of methanol. Glass jars with tightly fitted covers were used as developing chambers and were lined with filter paper. Enough solvent was poured in the jar to give a height of about 10 mm. A capillary tube was used as an applicator and was dipped into the extract allowing it to be filled to a height of about 20 mm. The capillary was used to spot the TLC plate with the extract, about 15 mm from the lower edge of the plate. In between applications, spots were air dried. The intended distance (the solvent front) where the solvent will travel was marked lightly.

The spotted plates were placed in the equilibrated chamber ensuring that the points of application are above the surface of the solvent. The solvent was allowed to travel until it reached the mark. The developed chromatogram was removed from the chamber. The position of the solvent front was marked and allowed to air-dry. The developed chromatogram was visualized under ultraviolet (UV) light, short wave (240nm) and long wave UV (365nm). Different visualizing agents were sprayed on the developed chromatogram. Appearance of spots indicate the presence of phytochemicals in the extract. The chromatogram was traced on a piece of tracing paper indicating the spots, shape and point of greatest concentration; the solvent front and the sample origin or point of application. A ruler was used to measure the distance moved by the solvent and distance moved by spot, from which the retention factor (R_F) values of the various spots were calculated. The R_F of each spot was recorded and its respective color compared against different visualizing agents used.

2.3 Inhibition Assay for Alpha-glucosidase Activity

The reagents used in this assay were purchased from Sigma-Aldrich (Singapore). Inhibitory α -glucosidase activity was determined spectrophotometrically. Twenty microliter of enzyme solution and 120 μ l of extract in 0.5% dimethyl sulfoxide (DMSO) of 0.01 M potassium phosphate buffer were mixed and pre-incubated at 37°C. After 15 minutes of pre-incubation, p-nitrophenyl- α -D-glucopyranoside (PNPG) (20 μ l) was added and incubated together at 37°C. Eighty microliter of 0.2 M Na_2CO_3 in 0.1 M potassium phosphate buffer was added to the test tube after 15 minutes to stop the reaction. The amount of PNP released was quantified using a 96-

well UV/VIS spectrophotometer (Thermo Scientific MultiSkan GO 51119200) at 405 nm. Acarbose was used as the positive control (Feng, Yang, & Wang, 2011). The α -glucosidase inhibitory activity was expressed as percentage inhibition. Percent inhibition was calculated based on the formula of Sheng et al. (2014):

$$\text{Inhibition (\%)} = \{1 - (A_{\text{sample}}/A_{\text{control}})\} \times 100$$

The IC₅₀ values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha glucosidase inhibitor. All tests were performed in triplicate.

3. RESULTS AND DISCUSSION

The thin layer chromatographic plates were developed using chloroform and methanol (5:1) as the solvent system. Vanillin-sulfuric acid, methanolic potassium hydroxide, and potassium ferricyanide–ferric chloride spray reagents detected the presence of phenols, tannins, flavonoids, anthraquinones and essential oil in in the *M. errans* extract while the presence of indoles were identified using the Van-urk Salkowski reagent. The results for Thin Layer Chromatography are summarized in Table 1.

The α -glucosidase inhibitory activity of the methanolic extract of *Musa errans* was determined using a series of concentration. As presented in Table 2, the percent inhibition at 62.5, 125, 250, and 500 $\mu\text{g/mL}$ concentrations showed a percentage inhibition of 97 ± 0.27 . The maximum inhibition of 98.02% was seen at the highest concentration of 500 $\mu\text{g/mL}$. *M. errans* extract showed an IC₅₀ value of 0.0002634 $\mu\text{g/mL}$ (95% C.I.) while the IC₅₀ value of the standard acarbose is $78.3 \pm 1.33 \mu\text{g/mL}$.

TLC is a chromatographic technique for separating mixtures. Specifically, this technique can be used to identify the presence of compounds in a given substance. Results of the phytochemical analysis of *M. errans* methanolic extract are consistent with the results obtained by Feliciano et al. (2014). Indoles, phenols, tannins, flavonoids, and anthraquinones were detected using a qualitative phytochemical analysis (test tube method) of the methanolic extract. Akinlolu, Salau, Ekor and Otulana (2012) used the methanolic extract of *M. sapientum* sucker. Presence of phenols, tannins, flavonoids, antraquinones and indoles were obtained from the phytochemical screening. Three *Musa* species (*Musa acuminata x balbisiana*, *Musa paradisiaca*, and *Musa sapientum*) were analyzed by Ogbonna, Izundu, Okoye, and Ikeyi (2016). Phenols, alkaloids, tannins, flavonoids were found to be present in the methanolic extracts.

Table 1
Results of TLC using Chloroform and Methanol (5:1) as Solvent System

R _f (cm)	Visual	Short (240nm)	Long (365nm)	Spray 1	Spray 2	Spray 3	Spray 4	Interpretation
0.1688			Light Blue Spot			Blue Spot		(+) Phenols, Tannins, Flavonoids
0.2412	Light Yellow Spot		Light Blue Spot			Blue Spot		(+) Phenols, Tannins, Flavonoids
0.2667		Dark Spot					Red Violet Spot	(+) Indoles
0.3099		Dark Spot	Light Blue Spot		Light Yellow Spot			(+) Anthraquinones
0.3859	Light Yellow Spot	Dark Spot				Blue Spot		(+) Phenols, Tannins, Flavonoids
0.3867	Light Yellow Spot	Dark Spot	Light Blue Spot				Red Violet Spot	(+) Indoles
0.425	Light Yellow Spot	Dark Spot					Red Violet Spot	(+) Indoles
0.4489					Light Yellow Spot			(+) Anthraquinones
0.462						Blue Spot		(+) Phenols, Tannins, Flavonoids
0.577		Dark Spot				Blue Spot		(+) Phenols, Tannins, Flavonoids
0.5946	Light Yellow Spot		Light Blue Spot		Light Yellow Spot			(+) Anthraquinones
0.67		Dark Spot					Red Violet Spot	(+) Indoles
0.7035			Light Blue Spot		Light Yellow Spot			(+) Anthraquinones
0.722	Light Yellow Spot	Dark Spot		Light Orange Spot				(+) Phenols, Essential Oil
0.7621	Light Yellow Spot	Dark Spot			Light Orange Spot			(+) Anthraquinones
0.8074		Dark Spot		Light Orange Spot				(+) Phenols, Essential Oil
0.8543					Light Orange Spot			(+) Anthraquinones
0.8704		Dark Spot		Light Orange Spot				(+) Phenols, Essential Oil

Note:
Spray 1- Vanillin Sulfuric Acid
Spray 2- Methanolic Potassium Hydroxide

Spray 3- Potassium Ferricyanide Ferric Chloride
Spray 4- Van-Urk Salkowski

Table 2
Percent Inhibitions of *M. errans* at Various Concentrations

Concentrations	500 µg/ml	250 µg/ml	125 µg/ml	62.5 µg/ml
	97.91804	97.885	97.62062	97.38929
	97.98414	97.75281	97.65367	97.35625
	98.01718	97.68672	97.65367	97.25711
Sum	293.9194	293.3245	292.928	292.0027
Mean	97.97312	97.77484	97.64265	97.33422

Majority of the studies conducted on the species of *Musa* showed the presence of polyphenols. Phenolics represent a large group of phytochemicals that offer a number of benefits to health (Kennedy, 2014). Included in this group are tannins, glycosides, flavones, and anthocyanidins. They are the most abundant antioxidants in the human diet (Pandey & Rizvi, 2009). Several animal models and a limited number of human studies have revealed that polyphenols decrease hyperglycemia and improve acute insulin secretion and insulin sensitivity. The reported possible mechanisms include a decrease in glucose absorption in the intestine and suppression of carbohydrate digestion, stimulation of insulin secretion from the pancreatic β -cells, modulation of glucose release from the liver, activation of insulin receptors and glucose uptake in the insulin-sensitive tissues, modulation of intracellular signaling pathways, and gene expression (Aryaeian, Sedehi, & Arablou, 2017).

Diabetes mellitus is a metabolic disorder characterized by an abnormal increase in blood glucose levels after eating (postprandial hyperglycemia). Controlling postprandial hyperglycemia is important in the management the disease (Li et al., 2008). Certain enzymes in the digestive tract are known to delay glucose absorption. α -glucosidase inhibitors can slow down postprandial hyperglycemia and help to avoid the onset of late diabetic complications (Yin, Zhang, W., Feng, Zhang, Y., & Kang, 2014). In α -glucosidase inhibition, a slowdown of digestion occurs by blocking enzymes in the small intestine that breakdown carbohydrates. The glucose from food enters the bloodstream more slowly, thus reducing blood glucose levels elevation after eating. Acarbose, an example of oral hypoglycemic drug, inhibits the α -glucosidase enzyme, and is used as a standard inhibitor (Ghosh & Konishi, 2007). Many α -glucosidase inhibitors from plant sources are flavonoids, alkaloids, terpenoids, anthocyanins, glycosides, and phenolic compounds (Kumar, Narwal, Kumar, & Prakash, 2011).

Half-maximal inhibitory concentration (IC_{50}) is defined as the concentration of a drug *in-vitro* that is required for 50% inhibition. It is a quantitative measure that indicates how much a substance is needed to inhibit a biological process such as an enzyme by half. IC_{50} is an extensively used method to measure a drug's efficacy (Aykul & Martinez-Hackert, 2016). The *M. errans* methanolic extract showed a stronger inhibitory activity with an IC_{50} of 0.0002634 $\mu\text{g/mL}$ (95% CI: 0.00009154 to 0.001178) than that of the standard, Acarbose ($IC_{50} = 78.30$; 95% CI: 73.89 to 83.25). The result signifies that the extract is better in inhibiting α -glucosidase than Acarbose.

4. CONCLUSION

The study indicates that the methanolic extract of *M. errans* possessed an *in-vitro* antidiabetic activity. The α -glucosidase inhibition activity of *M. errans* is more pronounced than Acarbose, an alpha glucosidase inhibitor for treatment of diabetes. The phytochemicals

responsible for the inhibitory activity could be polyphenols as literature shows an association of polyphenols and the antidiabetic properties of plants.

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