# Screening of the Mammalian Alpha-Glucosidase Inhibitory Activity of Selected *Ficus* Species from Mount Makiling, Laguna, Philippines

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**ABSTRACT**

Herbal medicine is gaining acceptance in the medical and scientific sectors in the Philippines. With a variety of potential species, *Ficus* species, an endemic family found on Mount Makiling, have been believed to have folkloric medicinal uses for diabetes, which are growing popular among Filipino diabetics. Diabetes mellitus is the sixth leading cause of death in the Philippines, accounting for over 6 million cases. Thus, this study aimed to screen the mammalian alpha-glucosidase inhibitory activity of selected *Ficus* species leaves collected from Mount Makiling, Laguna, Philippines. The aqueous and ethanolic crude extracts of selected *Ficus* species were subjected to two methods: phytochemical analysis and in vitro mammalian alpha-glucosidase inhibitory activity. IC50 was determined from a generated non-linear regression extrapolated from the concentration-% inhibition plot. The ethanolic crude extract of *F. nota* leaves has the most significant inhibitory activity against mammalian alpha-glucosidase due to its lowest IC50 value of 8.866 (µg/mL). *F. nota* leaves extracts showed the presence of carbohydrates, flavonoids, saponins, tannins, and glycosides, all of which were found to inhibit alpha-glucosidase and have anti-hyperglycemic activity. Further investigation into the ethanolic crude extract of *F. nota* leaves must be conducted to identify and isolate the active compound that inhibits alpha-glucosidase and elucidate its structure.

***Keywords****: Diabetes Mellitus; Ficus species; IC50; Mammalian alpha-glucosidase inhibitory activity; Mount Makiling.*

## INTRODUCTION

Drug discovery and development takes an average of 10 - 15 years before it reaches the market (Whalen et al., 2015). One of the first steps to discover potential new drugs is screening new pharmacologically active plant compounds that can be discovered (Atanasov et al., 2015). This concept motivates the Institute of Herbal Medicine (IHM) of the Philippines to collect folkloric data while scientific tests validate the traditional healers' claims. Up to this day, natural products have a profound role in improving human health (Kingston, 2011). As a route to drug discovery, natural products contribute to drug development with diverse sources, especially in countries like the Philippines (De Corte, 2016). The Philippines is one of the 17 mega diversity countries that host more than 52,177 species, of which more than half is found nowhere else in the world (Ong et al., 2002). With a lot of different potential species, the field of herbal medicine in the Philippines is finding its rightful place in the medical and scientific community (Villamor et al., 2017).

One of the most diverse ecosystems with species diversity in the Philippines is Mount Makiling (Lapitan et al., 2011). The mountain's flora comprises a large number of species, including the family of Moraceae that is one of the endemic families. This includes the *Ficus* genus, where various species can be found in its different sub watersheds of the mountain (Fernando et al., 2004). *Ficus* species serves as a medicinal plant used for a mild laxative, anti-rheumatic, anti-dysentery, hypotensive, antioxidant, anti-inflammatory, anti-helminthic, and anti-diabetic (Lansky et al., 2008). Anti-diabetic properties have been reported in active compounds from various *Ficus* species (Deepa et al., 2018). In addition to this, a recent study reported that an ethanolic extract of *F. nota* exhibits an α-glucosidase inhibitory activity (Cruz et al., 2018). With these reported folkloric uses and studies, *Ficus* is being recognized as an option for Filipino diabetic patients.

Diabetes mellitus is a chronic disease caused by an inherited or acquired deficiency in insulin production or ineffective insulin production. Diabetes was responsible for 1.6 million deaths worldwide, and it is expected to be the seventh leading cause of death by 2030. By 2025, Asia will have seen the most significant growth in diabetes cases (WHO, 2017). The Philippines, an Asian country, reported around 3,721,900 diabetes cases in 2017 and an adult prevalence rate of 6.2. Diabetes is the sixth leading cause of death in the Philippines, affecting more than 6 million people (DOH, 2017). This statistic signifies that the percentage of Filipinos who have diabetes escalates rapidly. Biguanides, sulfonylurea, and blood glucose monitoring are the only medications and basic technology available in primary care centers for diabetes mellitus (WHO, 2016). However, these currently available medications may cause hypoglycemia as a severe adverse effect (Whalen et al., 2015). One of the oral medications for diabetes mellitus is alpha-glucosidase inhibitor, which inhibits carbohydrate absorption and thus prevents postprandial hypertension (Goodman et al., 2006). Unfortunately, these medications may produce gastrointestinal adverse effects such as flatulence, diarrhea, and abdominal cramping in the patient (Katzung et al., 2018).

As a result, diabetes and its complications remain one of the major medical issues, despite the availability of various anti-diabetic medications on the market. Because of the undesirable side effects and high costs of treatment, not only international researchers but also Filipino researchers want to provide safer, more effective, and more affordable medicines using our local resources. Thus, the purpose of this study is to conduct a preliminary investigation into the mammalian alpha-glucosidase inhibitory activity of selected *Ficus* species collected in Mount Makiling Laguna, Philippines.

## REVIEW OF RELATED LITERATURE

### Screening for Drug Discovery Process

Random screening and knowledge-based approaches can be used to discover new pharmacologically active plant compounds. The random selection of test material may reveal unexpected bioactivities that could not be predicted based on prior knowledge. For this reason, the knowledge-based approach was born (Atanasov et al., 2015).

Ethnopharmacology is a classic knowledge-based strategy. The selection of test samples is based on the folkloric use of plants. It involves observing, describing, and testing traditional drugs' bioactivities (Atanasov et al., 2015). Drug discovery for herbal medicines involves identifying new chemical entities of potential therapeutic value that can be isolated from natural sources (Katiyar et al., 2012).

Most of our modern-day products came from different plant sources. Herbal medicine research takes 5–6 years whereas synthetic drug research takes 10–15 years. The process is similar to synthesized drugs. It also includes various processes. Before FDA registration, it must pass pre-clinical and human clinical trials (Maramba-Lazarte, 2013). The researchers will first survey traditional healers on herbal medicines to collect folkloric data and then conduct scientific tests to validate the traditional healers' claims. Pre-clinical studies will begin once researchers find an herbal medicine to study.

Pre-clinical studies focus on acute, subchronic, and chronic toxicity, safety pharmacology, mutagenicity, clastogenicity, heavy metal content, and active compound isolation (Villamor et al., 2017). This determines whether a drug is safe for human use and effective against a disease target in chemical or animal tests (Dunne et al., 2013). After pre-clinical testing, researchers evaluate the results and decide whether to test a drug on humans in clinical or human trials. Clinical trials typically progress from small-scale Phase 1 studies to large-scale Phase 3 studies (US FDA, 2018).

Phase 1 typically involves 20 to 80 healthy or sick volunteers. It's all about drug safety. Phase 2 usually includes 100-300 people with the disease/condition. Thus, the trials shift from safety to efficacy. Finally, Phase 3 involves 1,000-3,000 volunteers with the disease/condition to show or confirm therapeutic benefit for a specific patient population (Remington, 2013). A patent application will be filed with the Philippine Intellectual Property Office (IPO) after the herbal medicine has been tested for safety and efficacy. Pharmaceutical companies will then manufacture and market herbal medicine (Villamor et al., 2017).

### Mount Makiling

The Philippines is one of the world's most important countries for preserving biodiversity (Ong et al., 2002). One of the mountains found in the Philippines is Mount Makiling. Mount Makiling is located at 14°08'N 121°11'E, straddling the provinces of Laguna and Batangas in Luzon, Philippines. It rises to an elevation of 3,580 feet above sea level (Tiburan, 2002). The mountain's peak is 1090 masl. (Lapitan et al., 2011). The reserve is located 65 kilometers south of Metro Manila and encompasses the municipalities of Los Baños, Bay, and Calamba in Laguna and Sto. Tomas in Batangas, Philippines.

It is also known as Mt. Makiling Forest Reserve (MMFR), a tropical rainforest with one of the most diverse ecosystems with high species diversity (Luna et al., 1999). MMFR is a

watershed and water source of the CALABARZON region's industrial, agricultural, and residential sectors (Lapitan et al., 2011). Furthermore, MMFR has four (4) major zones based on watershed boundaries namely Molawin-Dampalit, Cambantoc, Greater Sipit, and Tigbi (Tiburan et al., 2002).

The mountain's flora comprises a large number of endemic families, genera, and species that include many interesting forms. J.V. Pancho, a well-known Filipino botanist, found 949 genera and 2038 species of flowering plants and ferns representing 225 families on Mt. Makiling and its surroundings in 1983 (Lapitan et al., 2011). In Greater Sipit Subwatershed, the dominant *Ficus* species based on the computed relative dominance, basal area, and frequency is *Ficus septica*. In the Molawin-Dampalit watershed, Castillo et al., 2018 observed 44 indigenous species prospering in the area. A variety of *Ficus* species were identified among the 44 indigenous species that were discovered. *Diospyros blancoi* and *Parasborea malaanonan* are one of the critically endangered species that are found abundant in this subwatershed. Also, *Ficus subcordata* is one of the most encountered species (Lapitan et al., 2011).

### ***Ficus*** **Species**

*Ficus* (Moraceae) is one of the largest plant genera, with over 750 described species in the world, mostly in tropical countries (Serrato et al., 2004). It is a valuable plant resource with high economic and nutritional value, and it contributes to the rainforest ecosystem's biodiversity. The highest number of species in the Moraceae family (*Ficus*) indicates high moisture content and availability of shallow aquifers in the watershed (Palis et al., 2011). They are the most common genus in soil seed banks in tropical Asia (Tang et al., 2006).

*Ficus* is a diverse genus found in all forest types (Berg, 1989). *Ficus* is the fifth most diverse genus in the Philippines with 104 species. *F. septica, F. ulmifolia, F.elastica*, and *F.nota* are found in Luzon, Visayas, and Mindanao (Reyes et al., 2020).

*Ficus* figs, fruits, and trees are among the oldest and most successful higher plant species. Fruit tree leaves contain more antioxidants than fruit. Fruit tree leaves are less edible than fruits and more medicinal. Leaves, latex, bark, and roots, but mostly leaves, are used medicinally. The accessibility of these species produces more substances that are suitable for medicines, drugs, and pharmaceutical products (Lansky & Paavilainen, 2010). Active compounds derived from plants in the genus *Ficus* (Moraceae) have been used to treat diabetes and other chronic diseases since ancient times.

*F. septica*'s (Hauili) decoction of the root part can be used as a diuretic and prevention of asthma, while the fresh leaves are sudoforic and can be applied externally as antirheumatic (Ragasa et al., 2016). This plant's dichloromethane extract contains alkaloids, specifically β-sitosteryl-3β-glucopyranoside-6'-O-fatty acid esters, α-amyrin fatty acid ester, β-sitosterol, stigmasterol, β-amyrin, and long-chain saturated fatty alcohols (Ragasa et al. 2016). Alkaloids, quaternary base, tannins, 2-deoxysugars, and benzopyrone nucleus were found in the ethanolic extract (Vita, 2010).

*F. ulmifolia* (Is-is) leaves are used in the treatment of allergy, asthma, diarrhea, diabetes, tumor, and cancer. *F. ulmifolia* leaf's dichloromethane extract contains l-sitosteryl3Î2-glucopyranoside-6'- O-palmitate, squalene, lutein, Î±-amyrin acetate, lupeol acetate, and

Î2-carotene (Tsai et al., 2012). Phytochemical screening yielded terpenes, glycosides, and phenols (Herrera et al., 2010).

*F. elastica* (India Rubber Tree) leaves are used to cure skin diseases and allergies, moreover as diuretic and yielded two new compounds, *Ficus elastica* acid and (1'S,6'R) -8-O-β-D-glucopyranosyl abscisate sodium, along with 12 known compounds: feroxidin, quercitrin, kaempferin, myricitrin, syringin, citroside B, corchoionoside C, (6S,9R) - roseoside, oleanolic acid, ursolic acid, benzyl O-β-D-glucopyranoside, icariside F2 (Phan et al., 2012).

*F. nota* (Tibig) root and bark decoction can treat urinary tract infection, hypertension, and diabetes. Alkaloids, tannins, flavonoids, saponins, and anthraquinones were found in ethanolic extract of *F. nota* fruit and leaves. Ethanolic leaf extract yielded alkaloids, flavonoids, phenols, terpenoids, saponins, and tannins (Mapatac, 2015). Total phenolic and flavonoid content were 348.3 ± 3.2 mg GAE/g and 2.64 ± 0.06 mg QE/g, respectively (Santiago et al., 2017). The alpha-glucosidase inhibition assay was performed on rat intestinal enzymes. A moderate alpha-glucosidase inhibitory activity was observed with an IC50 of 219.9.359. Among all partitions, EAP had the lowest IC50, 243.49.494 g/mL lower than ECE. Flavonoids were found in EAP by phytochemical and TLC analysis (Cruz et al., 2018).

**Diabetes Mellitus**

The World Health Organization estimates that diabetes caused 1.6 million deaths in 2015 and will be the seventh leading cause of death in 2030. Asia is also predicted to have the greatest rise in diabetes cases by 2025. According to the International Diabetes Federation (IDF), 425 million people, one out of 11 adults, have diabetes by 2017. However, there are 212 million undiagnosed diabetics worldwide. Type 1 diabetes affects over a million kids and teens. Pregnancy hyperglycemia affects 1 in 6 births. The Western Pacific (WP) Region currently represents 28 diabetes organizations in 20 countries and two territories. It has the largest IDF population and accounts for 37% of global diabetes cases. The Philippines is one of the 22 IDF WP countries.

Over 6 million Filipinos have diabetes, according to the Philippine Center for Diabetes Education Foundation (Department of Health, 2017). It means that the number of Filipinos who have diabetes is rapidly rising. According to the 2014 Philippine Health Statistics, diabetes is the sixth leading cause of death in the Philippines (Department of Health, 2018).

Diabetes mellitus is a chronic disease caused by an inherited or acquired deficiency in insulin production or ineffective insulin production. An increase in glucose concentration/ insulin deficiency damages many of the body's systems, especially the blood vessels and nerves (World Health Organization, 2017).

The American Diabetes Association classified the diagnosis of diabetes mellitus into four categories: type 1 (formerly insulin-dependent diabetes mellitus), type 2 (formerly non–insulin-dependent diabetes mellitus), gestational diabetes mellitus, and other causes such as genetic defects or medications (Whalen et al., 2015).

Type 1 diabetes mellitus is caused by a severe lack of insulin due to beta-cell loss. Islet cell death is linked to genetics, autoimmunity, and the environment. Type 1 diabetes mellitus has immune-mediated (type 1A) and idiopathic causes (type 1B) (Mohan, 2018).

Type 2 diabetes mellitus is characterized by high blood glucose and low insulin. Genetic predisposition and environmental factors, mainly obesity and sedentary lifestyle lead to insulin resistance, pancreatic beta-cell dysfunction, and increased hepatic glucose production (Grossman & Porth, 2014). Type 2 diabetes has normal or even elevated insulin levels due to these factors. Insulin resistance decreases muscle, liver, and fat glucose uptake (Katzung et al., 2018).

Gestational diabetes mellitus is any glucose abnormality that occurs during pregnancy. The placenta and placental hormones cause insulin resistance in the last trimester (Katzung et al., 2018). About 4% of pregnant women develop DM due to metabolic changes during pregnancy. Although their blood sugar levels return to normal after delivery, these women are at risk of developing diabetes later in life (Mohan, 2018).

There are other specific types of diabetes mellitus which includes genetic defect of β-cell function due to mutations in various enzymes (e.g., hepatocyte nuclear transcription factor—HNF), genetic defects in insulin action, diseases of the exocrine pancreas (e.g., chronic pancreatitis), endocrinopathies (e.g., acromegaly), drug- or chemical-induced (e.g., thiazides, β-blockers, etc.), infections (e.g., cytomegalovirus), uncommon forms of immune-mediated DM (stiff-man syndrome), other genetic syndromes (e.g., Down's syndrome) (Dipiro et al., 2014; Katzung et al., 2018).

The goals of diabetes mellitus therapy are to reduce the symptoms, prevent acute and chronic complications, maintain normal blood glucose and lipid targets, intensive therapy for cardiovascular risk factors, and improve quality and quantity of life (Dipiro et al., 2014). Diabetes management and treatment can be categorized as pharmacologic or non-pharmacologic (Beers et al., 2003).

The non-pharmacological approach for diabetes mellitus includes weight loss, exercise, and dietary changes, which can reduce insulin resistance and correct hyperglycemia. Furthermore, frequent self-monitored blood glucose testing is required to achieve near-normal blood glucose concentrations and assess for hypoglycemia, especially in patients with type 1 diabetes mellitus.

The pharmacological approach for type 1 diabetes is treated with insulin, while type 2 diabetes is treated with oral antihyperglycemics and insulin (Dipiro et al., 2014). Diet alone cannot control blood sugar levels in people with type 2 diabetes. Several glucose-lowering agents are available for patients with type 2 diabetes, including sulfonylureas, meglitinides, d-phenylalanine derivatives, biguanides, thiazolidinediones, -glucosidase inhibitors, glucagon-like peptide-1 receptor agonists, dipeptidylpeptidase-4 [DPP-4] inhibitors, sodium-glucose co-transporter inhibitors [SGLTs], and other hypoglycemic agents (Katzung et al., 2018).

Sulfonylureas, meglitinides, and d-phenylalanine derivatives bind to sulfonylurea receptors and stimulate insulin receptors (Katzung et al., 2018). Major adverse effects of all these classes are weight gain, hyperinsulinemia, and hypoglycemia. Except for d-phenylalanine derivatives, they should be used with caution in patients with hepatic or renal insufficiency.

Biguanides and thiazolidinediones are classified as insulin sensitizers that lower glucose levels by their actions on the liver, muscle, and adipose tissue. It is contraindicated in renal

dysfunction due to the risk of lactic acidosis. It should be stopped if you have a heart attack, heart failure, sepsis, or other conditions that cause acute renal failure. TZDs include pioglitazone and rosiglitazone. The major adverse effect is weight gain. It increases subcutaneous fat and causes fluid retention, which can worsen heart failure.

Alpha-glucosidase inhibitors reduce the intestinal absorption of starch, dextrin, and disaccharides by inhibiting the action of α-glucosidase in the intestinal brush border. Inhibition of this enzyme slows carbohydrate absorption. Alpha-glucosidase inhibitors do not stimulate insulin release and therefore do not result in hypoglycemia (Goodman et al., 2006). Examples of this are acarbose and miglitol used for the treatment of type 2 diabetes (Whalen et al., 2015). Prominent adverse effects of α-glucosidase inhibitors include flatulence, diarrhea, and abdominal pain. In type 2 diabetic patients, miglitol in combination with metformin gives greater glycemic improvement than metformin monotherapy (Chiasson, 2001).

Sodium–glucose cotransporter 2 (SGLT2) inhibits the reabsorption of glucose in the kidney. Adverse reactions include female genital mycotic infections, UTIs, and urinary frequency. Hypotension has occurred, especially in the elderly or diuretic patients (Whalen et al., 2015).

Glucagon-like peptide-1 receptor (GLP-1) agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors mimic the incretin effect or prolong incretin action. GLP-1 receptor agonists cause constipation, diarrhea, nausea, vomiting, and increased risk of pancreatitis. The most common side effects of DPP-4 inhibitors are nasopharyngitis, upper respiratory infections, and headaches. Insulin secretagogues or insulin can cause hypoglycemia when combined with the drug (Katzung et al., 2018). All DPP-4 inhibitors have caused pancreatitis (Whalen et al., 2015).

Other oral diabetic agents used in diabetes mellitus namely Pramlintide, Bromocriptine, and Colesevelam. All of these agents cause gastrointestinal irritations. Colesevelam and bromocriptine have very modest efficacy in lowering glucose levels, and their use for this purpose is questionable (Katzung et al., 2018).

### *In Vitro* Alpha-Glucosidase Inhibitory Assay

Alpha-glucosidase enzymes are located on the intestinal brush border and are responsible for the breakdown of carbohydrates into glucose and other easily absorbed simple sugars (Whalen et al., 2015). When these enzymes are inhibited, carbohydrate absorption is slowed. As a result, both normal and diabetic individuals experience a decreased postprandial rise in plasma glucose (Goodman et al., 2006).

Alpha-glucosidase hydrolyzes the terminal, non-reducing 1,4-linked α-D-glucose residues with the release of α-D-glucose (Martinez et al., 2013). The enzyme alpha-glucosidase converts starch and disaccharides to glucose (Nair et al., 2013). An assay by Martinez et al (2013) utilizes p-nitrophenyl-α-D-glucopyranoside that is hydrolyzed specifically by alpha-glucosidase into a yellow-colored product by enzymatic hydrolysis of the substrate correlated with the activity of the sample when compared with the negative control. The absorbance of released p-nitrophenol at 410 nm was measured. Based on the kinetic reaction, the reaction rate is directly proportional to enzyme activity.

Alpha-glucosidase inhibitory activity can be measured in vitro by determining the reducing

glucose resulting from sucrose hydrolysis by alpha-glucosidase enzyme isolated from rat small intestine (Matsui et al., 2001). For crude extracts dissolution, extracts were dissolved in various concentrations of 100% dimethylsulfoxide (DMSO) (Kamantigue et al., 2017). In preparation of mammalian enzymes, 1000 mg of rat intestinal acetone powder was dissolved in 30 mL of 50 mM pH 6.8 phosphate-buffered saline to make the enzyme. The mixture was vortexed, sonicated for 5 minutes, and centrifuged at 6,000 rpm for 30 minutes before being kept at -4°C. The supernatant liquid that resulted was employed in the test (Kang et al., 2016). The powdered rat intestine (200 mg) was dissolved in 4 ml of 50 mM ice cold phosphate buffer and sonicated for 15 minutes at 4°C. The suspension was centrifuged (10,000 g, 4°C, 30 minutes) after vigorous vortexing for 20 minutes, and the resultant supernatant was utilized for the test. A reaction mixture containing 50 µl of phosphate buffer (50 mM; pH 6.8), 10 µl of yeast or Rat α-glucosidase (1 U/ml), and 20 µl of different amounts of plant extract was pre-incubated for 5 minutes at 37°C before adding 20 µl of 1 mM PNPG as a substrate. After another 30 minutes at 37°C, the process was stopped by adding 50 µl of Na2CO3 (0.1 M). The same buffer was used to make all of the enzyme, inhibitor, and substrate solutions. Acarbose served as a positive control, while water served as a negative control. The absorbance at 405 nm was measured in a microtiter plate reader to determine enzymatic activity (Bio-TEK, USA). Experiments were carried out in triplicate. The percentage of enzyme inhibition was calculated by the following formula:

$$\% \, Inhibition = \frac{(Ac - Ab) - (As - Ab)}{Ac - Ab} \, x \, 100$$

where AC is the control absorbance and AS is the tested sample absorbance. The IC50 value is defined as the concentration of an inhibitor required to inhibit 50% of enzyme activity under the specified assay conditions (Mohamed et al., 2011).

In the same study, it was also stated the use of GraphPad Prism software, including the t-test or one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test. P values less than 0.05 were deemed statistically significant (Mohamed et al., 2011).
A reported work of Kamantigue et al. (2017) about in vitro mammalian intestinal alpha-glucosidase inhibition was conducted in Siba-o, Calabanga, Camarines Sur, Philippines to develop new herbal drug candidates that are effective, safe, and affordable. The phytoconstituents were extracted from the plant matrix by exhaustive maceration with absolute ethanol. The IC50 was calculated from the generated linear regression extrapolated from concentrations-percent inhibitions plot. The presence of flavonoids, tannins, essential oil, reducing sugar, coumarin, anthraquinones, anthrones, steroids, alkaloids, and peptides was determined using TLC bioautography (thin layer chromatography). The ethanolic extracts of *Melothria sp*. stem with leaves showed concentration-dependent inhibition activity against mammalian a-glucosidase from rat intestinal acetone powder with IC50 values of 49.24 ppm from the 98 crude plant samples extracted. TLC bioautography detected tannins, flavonoids, essential oils, and indoles which may be responsible for bioactivity. The findings showed that some plant samples could be used as an alternative herbal drug. However, only *Melothria sp*. crude leaves and stem extract (SB32LS) showed concentration-dependent activity, requiring further research to identify the active metabolites.

## PURPOSE OF THE STUDY

The main purpose of this study is to screen the alpha-glucosidase inhibitory activity of selected *Ficus* species collected from Mount Makiling, Laguna, Philippines. Specifically, the study is guided with these specific questions:

1. Which has the more significant percentage yield between the aqueous and ethanolic crude extract of selected *Ficus* species leaves?
2. What are the phytochemical constituents present in the aqueous and ethanolic crude extract of selected *Ficus* species leaves?
3. What is the result of alpha-glucosidase inhibitory activity in terms of % enzyme inhibition & half-maximal inhibitory concentration (IC50) of selected *Ficus* species using in vitro mammalian alpha-glucosidase inhibitory assay?
4. Which among the extract has the most significant alpha-glucosidase inhibitory activity?

There is no significant difference in the half-maximal inhibitory concentration (IC50) of selected *Ficus* species from Mt. Makiling, Laguna, Philippines.

## METHODOLOGY

### Collection, Identification, and Preparation of Plant Materials

All plant samples were obtained at the Molawin-Dampalit subwatershed of Mt. Makiling Laguna, Philippines. In a study, Castillo et al. (2018) discovered 44 endemic species thriving in the Molawin-Dampalit watershed. Among the 44 endemic species, a variety of *Ficus* species were discovered. The selected *Ficus* species are *Ficus septica, Ficus elastica, Ficus ulmifolia*, and *Ficus nota*. The leaves of *F. ulmifolia* and *F. septica* were plucked straight off the branches whereas the leaves of *F. nota* and *F. elastica* were sheared from the tree first.

All collected leaves were sorted, rinsed three times with tap water, and dried for a week. To dry the leaves thoroughly, they were placed in a 30-40°C oven for 10-15 minutes. In a Nutribullet blender, the leaves were ground into a fine powder and sieved 36 times with mesh no. 20. The fine powder was transferred into a clean, dry, tightly closed jar.

### Extraction of Selected Ficus Species Leaves

The powdered plant samples were macerated with 95% ethanol for three (3) days with constant shaking to produce the ethanolic crude extract. The powdered plant samples were decocted for 30 minutes to produce the aqueous crude extract. Both crude extracts were filtered and concentrated in a rotating vacuum evaporator (Heidolph) at 40°C with 90 rpm. The extracts were stored in Type 1 borosilicate glass and sent to the UPLB BIOTECH National Institute for freeze-drying. It was freeze-dried at -40°C to -45°C using VirTis Freezemobile 25SL. The freeze-dried powder was transferred into a clean, dry, tightly closed jar.

### Phytochemical Analysis of selected Ficus species leaves

The phytochemical analysis of aqueous and ethanolic crude extracts was performed by the UP National Institutes of Health using their standard phytochemical methods.

### *In vitro* Mammalian Alpha-glucosidase Inhibitory Assay

The dissolution of crude extract was adapted from the method described by Kamantigue et al. (2017). The aqueous and ethanolic crude extracts were dissolved with 50% dimethylsulfoxide (DMSO) at varying concentrations from 1500 µg/mL to 11.719 µg/mL. To prepare the substrate, 180.750 mg of p-nitrophenyl-a-d- glucopyranoside (p-NPG) is dissolved in 60 ml of 50 mM pH phosphate buffer saline. The mixture was vortex mixed and sonicated for 5 minutes. The modified mammalian enzyme preparation was adapted from the reported work of Kang et al., (2016). To prepare the enzyme, dissolve 1000 mg of rat intestinal acetone powder in 30 mL of 50 mM pH 6.8 phosphate-buffered saline. The mixture was vortex mixed, sonicated for 5 minutes, and centrifuged for 30 minutes at 6,000 rpm, stored at -4°C. The resulting supernatant liquid was used for the assay.

The mammalian alpha-glucosidase inhibitory assay was adapted from the method described by Mohamed et al., (2011). Fifty microliter (50 µL) of phosphate buffer saline solution and 25 µL of crude plant extracts at various concentrations were added to 96-well flat-bottom microplates, followed by 50 µL of 1 mM substrate (pNPG). The microplates were then incubated for 5 minutes at 37°C with the solutions in each well inside the microplate reader. After incubation, the sample plate was removed and added with 25µL of 33.33 mg/mL α-glucosidase from rat intestine acetone extract. The reaction mixture was re-incubated at 37°C, and the reaction was terminated with the addition of 50 µL of 0.1M sodium carbonate solution. Phosphate buffer saline and 50% DMSO with enzyme and substrate were used as a negative control. Acarbose (Glucobay®), a secondary standard according to European Pharmacopeia, was used as a positive control. The alpha-glucosidase inhibitory activity was determined by measuring the yellow-colored p-nitrophenol released from pNPG at 405 nm. The wells containing buffer and test samples were assigned with separate wells to subtract the absorbance due to colored plant extracts. By adding separated wells containing buffer and enzymes, the absorbance due to the yellow color of the crude mammalian enzyme was also eliminated. The spectral changes were monitored using a BMG® microplate reader to analyze the para-nitrophenol emitted from the substrate at 405 nm, and the data was obtained using the Omega program version 3.10 R6. The assay was performed in triplicate.

### Statistical Analysis of Data

The statistical analysis of mammalian alpha-glucosidase inhibitory assay was adapted from the method described by Mohamed et al. (2011). The percentage inhibition of alpha-glucosidase activity is calculated using this equation:

$$\% \, Inhibition = \frac{(Ac - Ab) - (As - Ab)}{Ac - Ab} \, x \, 100$$

Where Ac = absorbance of the negative control, As = absorbance of the sample, and Ab = absorbance of the background to eliminate absorbance caused by plant pigments in crude plant extract and yellowish appearance caused by mammalian enzyme.

The percentage inhibitions of alpha-glucosidase in the various test samples were compared using the Analysis of Variance (ANOVA). At p0.05, the results were considered significant. The IC50 value of the treatments was computed using GraphPad Prism 8

employing a nonlinear regression curve fit on the computed percent enzyme inhibition per concentration. All tests were run in triplicate for three trials.

## RESULTS

### RQ1. Comparison of the aqueous and ethanolic crude extract of selected *Ficus* species leaves in terms of percentage yield

Aqueous crude extracts of the individual plant samples yielded a higher percentage in comparison with ethanolic extracts. To specify, aqueous and ethanolic crude extract of *F. nota* leaves had the highest percentage yielding 9.99% and 7.19%, respectively. The lowest percentages were obtained from *F. ulmifolia* aqueous crude extract and *F. elastica* ethanolic crude extract, at 4.87% and 3.23%, respectively.

Table 1 shows the percentage yield of the aqueous and ethanolic extract of selected *Ficus* species.

| Plant Sample | Crude Extract | Wt. of Plant Sample (g) | Wt. of Lyophilized Extract (g) | Percentage Yield (%) |
|---|---|---|---|---|
| *F. elastica* | Aqueous | 150 g | 8.00 g | 5.33% |
| | Ethanolic | 150 g | 4.85 g | 3.23% |
| *F. nota* | Aqueous | 150 g | 14.99 g | 9.99% |
| | Ethanolic | 150 g | 10.79 g | 7.19% |
| *F. septica* | Aqueous | 150 g | 8.90 g | 5.94% |
| | Ethanolic | 150 g | 7.85 g | 5.24% |
| *F. ulmifolia* | Aqueous | 150 g | 7.31 g | 4. 87% |
| | Ethanolic | 150 g | 7.19 g | 4.79% |

*Table 1. Percentage yield of aqueous crude extract (ACE) and ethanolic crude extract (ECE) of selected Ficus species.*

### RQ2. The phytochemical constituents present in aqueous and ethanolic crude extract of selected *Ficus* species leaves

Flavonoids, carbohydrates, and saponins were present in all plant samples, but alkaloids, anthraquinones, proteins, or resins were not present. Reducing sugars were found in the aqueous extracts of only a few *Ficus* species. The ethanolic crude extracts of *F. elastica* and *F. ulmifolia* leaves had no tannins, while the rest of the plant samples did. The aqueous extracts of *F. septica, F. ulmifolia,* and *F. nota* did not contain glycosides. Phytosterol was only absent in the aqueous crude extract of *F. ulmifolia* leaves and the ethanolic extracts of *F. elastica* and *F. ulmifolia* leaves. The presence of flavonoids, saponins, and tannins enhances the alpha-glucosidase inhibitory activity of plant samples.

Table 2 shows the tabulated results of the phytochemical analysis of an aqueous and ethanolic crude extract of selected *Ficus* species.

| Phytochemical Constituent | *F. elastica* | | *F. nota* | | *F. septica* | | *F. ulmifolia* | |
|---|---|---|---|---|---|---|---|---|
| | ACE | ECE | ACE | ECE | ACE | ECE | ACE | ECE |
| Flavonoids | + | + | + | + | + | + | + | + |
| Carbohydrates | + | + | + | + | + | + | + | + |
| Saponins | + | + | + | + | + | + | + | + |
| Alkaloids | - | - | - | - | - | - | - | - |
| Resins | - | - | - | - | - | - | - | - |
| Anthraquinone | - | - | - | - | - | - | - | - |
| Proteins | - | - | - | - | - | - | - | - |
| Reducing Sugars | + | - | + | - | + | - | + | - |
| Tannins | + | - | + | + | + | + | + | - |
| Glycosides | + | + | - | + | - | + | - | + |
| Phytosterols | + | - | + | + | + | + | - | - |

Legend: ACE – Aqueous Crude Extract; ECE – Ethanolic Crude Extract; (+): present; (-): not detected;

*Table 2. Phytochemical Analysis of aqueous crude extract (ACE) and ethanolic crude extract (ECE) of selected Ficus species.*

**RQ3. Assessment of the alpha-glucosidase inhibitory activity, in terms of % enzyme inhibition & half-maximal inhibitory concentration (IC50), of selected *Ficus* species through in vitro mammalian alpha-glucosidase inhibitory assay**

The aqueous extract of *F. nota* exhibited the highest percent inhibition (42.020%) and the highest IC50 value (65.318 µg/mL). In comparison to the positive control, acarbose, which has an 82.360% and an IC50 of 168.172 µg/mL. Whereas, the ethanolic crude extract of *F. nota* leaves has the lowest IC50 value of 8.866 µg/mL with 26.122%. The ethanolic crude extract of *F. elastica* leaves has the lowest percent inhibition at 8.240% and an IC50 value of 23.517 µg/mL.

Table 3 shows the result of the mammalian alpha-glucosidase inhibitory activity, in terms of % enzyme inhibition and half-maximal inhibitory concentration (IC50) value of Acarbose (positive control), and aqueous and ethanolic extract of selected *Ficus* species leaves.

| Treatment | Crude Extract | % Enzyme Inhibition | IC50 value (µg/mL) |
|---|---|---|---|
| *Acarbose* | | 82.360% | 168.172 µg/mL |
| *F. elastica* | Aqueous | 11.302% | 358.582µg/mL |
| | Ethanolic | 8.240% | 23.517 µg/mL |
| *F. nota* | Aqueous | 42.020% | 65.318 µg/mL |
| | Ethanolic | 26.122% | 8.866 µg/mL |
| *F. septica* | Aqueous | 36.136% | 350.880 µg/mL |
| | Ethanolic | 28.713% | 1000.684 µg/mL |
| *F. ulmifolia* | Aqueous | 32.283% | 773.494 µg/mL |
| | Ethanolic | 29.745% | 680.871 µg/mL |

*Table 3. Results of the mammalian alpha-glucosidase inhibitory activity, expressed in % enzyme inibition and IC50 value (µg/mL) of Acarbose, aqueous, and ethanolic extract of selected Ficus species leaves.*

**R4. Which among the extract has the most significant alpha-glucosidase inhibitory activity**

The aqueous crude extract of *F. nota* leaves exhibited the highest percent inhibition with 42.020% and IC50 value of 65.318 µg/mL. In comparison with the positive control which is Acarbose with 82.360% and IC50 of 168.172. Using commercially available Acarbose instead of the primary or secondary standard may contribute to this result. The ethanolic crude extract of *F. nota* leaves is the lowest IC50 value of 8.866 with 26.122%. The lowest percent inhibition is the ethanolic crude extract of *F. elastica* leaves with 8.240% with an IC50 value of 23.517. The preparation of plant materials and solvent choice can be a factor for the resulting inhibition. Overall, the ethanolic crude extract of *F. nota* leaves has the most significant inhibitory activity against mammalian alpha-glucosidase due to its lowest IC50 value of 8.866 (µg/mL).

**DISCUSSION**

The percentage yield of the aqueous and ethanolic crude extracts of selected *Ficus* species leaves were shown in Table 1. According to the table, the aqueous extract of *F. nota* leaves had the highest percentage yielding 9.99%. The ethanolic crude extract of *F. elastica* leaves, on the other hand, yielded the lowest percentage, with an average of 3.23%. It indicates that *F. nota* leaves have the highest extract yield after preparation. The preparation of plant materials and the solvent used may have an effect on this result (Wang et al., 2018).

Qualitative phytochemical analysis was conducted using freeze-dried aqueous and ethanolic crude extracts of selected *Ficus* species leaves. Table 3 shows the tabulated results of the phytochemical analysis of an aqueous and ethanolic crude extract of selected *Ficus* species leaves. Table 3 shows that the presence of secondary metabolites such as carbohydrates, flavonoids, and saponins are present in all extracts. The presence of phytochemicals such as flavonoids, saponins, and tannins in plant samples greatly contributes to the alpha-glucosidase inhibitory activity (Kazeem et al., 2013). Flavonoids can inhibit alpha-glucosidase activity and have antioxidant activity because of the hydroxyl groups. Glycosides were present in both crude extracts except aqueous crude extracts of *F. ulmifolia, F. nota, and F. septica*. Glycosides also have a role in inhibiting alpha-

glucosidase due to the same substrate structure (glucose) that allows glycosides to bind to the active site. Tannins were present in aqueous and ethanolic crude extracts except in ethanolic crude extracts of *F. elastica* and *F. ulmifolia*. Tannins have a role in inhibiting alpha-glucosidase because those can bind to protein make complexes. The hydroxylgroups in tannins have roles in inhibiting alpha-glucosidase and antioxidant activity (Zahratunnisa et al., 2017). Carbohydrates (Godard et al., 2010), flavonoids (Al-Ishaq et al., 2019), saponins (Sen & Chakraborty, 2010), tannins (McDougall et al., 2005) and glycosides (Liu et al., 2015) were found to produce significant anti-hyperglycemic activity. Whereas, alkaloids, resins, anthraquinones, and proteins were absent in all crude extracts. Although all of the aqueous crude extracts contained reducing sugar, none of the ethanolic crude extracts tested positive. Phytosterols were absent in both crude extracts of *F. ulmifolia* and ethanolic crude extract of *F. elastica*.

The mammalian alpha-glucosidase inhibitory assay for aqueous and ethanolic crude extracts was adapted from the method described by Mohamed et al., (2011). The percent enzyme inhibition per concentration for each sample was expressed in IC50 values derived from the calculated percent enzyme inhibition. The assay results showed that Acarbose (positive control) has a lower IC50 value compared with aqueous and ethanolic crude extracts of *F. septica* and *F. ulmifolia* leaves, as well as with aqueous crude extract of *F. elastica* leaves. It implies that these crude extracts inhibit alpha-glucosidase less effectively than the positive control. However, due to the synergism or additive activity of different compounds from crude extracts (Kamantigue, 2017), the aqueous crude extract of *F. nota* leaves and the ethanolic crude extracts of *F. elastica* and *F. nota* leaves were shown to have lower IC50 values than Acarbose. It implies that these crude extracts showed significant inhibitory activity against mammalian alpha-glucosidase when compared to the positive control. Among all the crude extracts from selected *Ficus* species, the ethanolic crude extract of *F. nota* leaves has the most significant inhibitory activity against mammalian alpha-glucosidase due to its lowest IC50 value.

The IC50 values of treatments were computed using GraphPad Prism 8 by employing a nonlinear regression curve fit on the computed percent enzyme inhibition per concentration. The statistical difference between the alpha-glucosidase percentage inhibitions of the different test samples was analyzed using Analysis of Variance. There is no significant difference in concentration levels between the aqueous and ethanolic crude extracts of *F. ulmifolia* and *F. elastica* leaves and the aqueous crude extracts of *F. septica* and *F.nota* leaves because their P-values are all greater than 0.05. The level of concentration in the ethanolic crude extract of *F. septica* leaves and the aqueous crude extract of *F.nota* leaves, on the other hand, differs significantly. The inhibition of acarbose varies greatly depending on concentration because the P-value is less than 0.01.

## CONCLUSION

The ethanolic crude extract of *Ficus nota* leaves showed the most significant alpha-glucosidase inhibitory activity due to its lowest IC50 value among other plant extracts. *F. nota* leaves showed the presence of carbohydrates, flavonoids, saponins, tannins, and glycosides, all of which were found to inhibit alpha-glucosidase and have anti-hyperglycemic activity Therefore, the ethanolic crude extract of *Ficus nota* leaves inhibited mammalian alpha-glucosidase and may have hypoglycemic properties.

However, further study on *Ficus nota* is needed to evaluate the phytochemical constituents using thin layer chromatography bioautography. Use of a lower concentration of DMSO in the alpha-glucosidase inhibitory assay must be conducted. Isolate the active compound(s) in the ethanolic crude extract of *F. nota* leaves, identify alpha-glucosidase inhibition, and elucidate its structure. Furthermore, using enzyme inhibition kinetics, characterize the mechanism of inhibition of the active compound(s) identified.

This research will benefit the biomedical community in the management of diabetes mellitus cases. The analysis will contribute to the goal of the Philippine Institute of Traditional and Alternative Health Care of exploring traditional and complementary medicine. The study aims to look for *Ficus* species that have significant alpha-glucosidase inhibitory activity. Thus, it will help pharmaceutical companies learn more about a plant that can be utilized to treat diabetes. Furthermore, this study seeks to promote the use of *Ficus* species as a possible anti-diabetic medication. The findings may also serve as a foundation for future research on *Ficus* species and diabetes, potentially leading to new therapeutic inventions.

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