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Antidiabetic evaluation of A*rtocarpus odoratissimus* (Moraceae) fruit

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Abstract

Background: Diabetes mellitus causes 4.2 million of deaths worldwide and 79% adults with diabetes are living in low- and middle-income countries. This research providing an alternative therapy through the prevention of postprandial hyperglycemia may help diabetic patients and provide a new utilization model of fruit peel. *Artocarpus odoratissimus*, commonly known as marang, is an edible fruit found in the southern part of the Philippines. Most of the weight of the fruit is discarded and treated as waste.

Objectives: This study aimed to utilize the by-products of marang fruit as a promising pharmaceutical agent by determining the phytochemicals present and *in vitro* antidiabetic activity of the different parts of the fruit.

Methods: Phytochemical screening of phenolics and flavonoids was done through thin layer chromatography. Ten concentrations (2-1000 µg/mL) of the extracts from the peel, pulp, and seeds were evaluated for the *in vitro* antidiabetic assay using alpha-glucosidase enzyme. Mean percent inhibition was calculated, and data was analyzed using ANOVA. The IC_{50} estimates were calculated using the program GraphPad Prism version 8.

Results: Extracts from the fruit parts of *A. odoratissimus* contained phenols and flavonoids and were active inhibitors of alpha-glucosidase enzyme. The fruit peel extract of marang was the most potent (IC_{50} = 48.19 µg/mL) compared to the seed extract, pulp extract, and the standard drug acarbose (p value = 0.035).

Conclusion: The fruit waste, the peel and seeds, has an intense activity against alpha-glucosidase enzyme because of their phenols and flavonoid contents.

Keywords: alpha-glucosidase, *Artocarpus,* diabetes, phenolics, fruit peel

1. Introduction

Diabetes mellitus (DM) is a metabolic condition of the endocrine system where there is an absolute absence or deficiency in insulin secretion or both. The disease now affects more than 100 million people worldwide and is predicted to reach 366 million by the year 2030. It is expected that one in every 10 people will be affected by diabetes in the next ten years. In the present data, China is considered to have the highest incidence of DM, affecting 94.8 million of its population and is closely followed by India and the United States of America (WHO, 2016). Antidiabetic drugs such as acarbose, a known alpha-glucosidase inhibitor, cause gastrointestinal disturbances. Thus, research is continuously being pursued to provide an alternative treatment to DM.

Plant-derived compounds for the management of diabetes have been used in folklore and traditional healing. The Philippines, being a tropical country, is rich in flora and fauna, which could promise a potential source of therapeutic agents. In spite of this, bioactive compounds must be thoroughly investigated to identify their specific mechanism of action concerning diabetes mellitus (Parveen, et al., 2018).

The *Artocarpus* covers about 50 species of deciduous fruit-bearing trees. The name *Artocarpus* is originated from two Greek words, "*artos"* and *"karpus,"* which directly translate to breadfruit (Akinloye, et al., 2015). The genus is known as a source of edible fruits and is widely used as traditional medicines. The genus is a scientific interest since the members contain medicinally important secondary metabolites that possess pharmacological activities. The extracts from the aerial and underground plant parts are used in traditional medicines for the treatment of diabetes, diarrhea, malaria, tapeworm infections, and other ailments (Bapat & Jagtap, 2010).

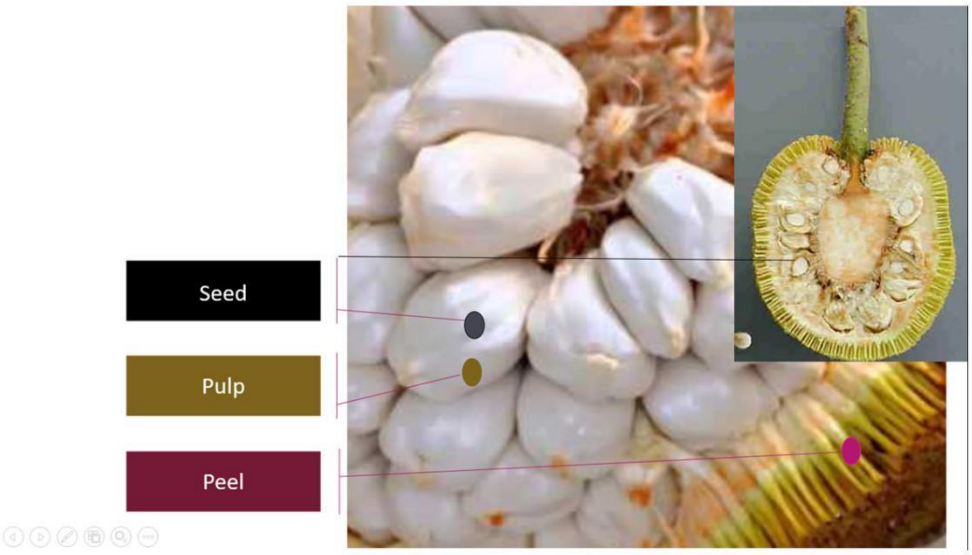

Figure 1. Fruit parts of *Artocarpus odoratissimus*

Artocarpus odoratissimus, locally known in the Philippines as marang, is a fruit native to the Mindanao islands. Locals and tourists commonly consume it because of its tasty and soft flavored pulp. Recent studies proved that the fruit parts displayed superior antioxidant properties (Bakar, et al., 2010). The fruits are sub-globose, measuring 20 cm in diameter, green-yellow and densely covered with stiff, hairy processes measured 1 cm long, borne at the end of long flexible branches, with a mass of seeds embedded in pulp (Godofredo U. Stuart Jr., 2017). The fruit is classified as a syncarp, a type of aggregate fruit that is multiple and made of fleshy fruits (Spjut & Thieret, 1989). The flesh is white, juicy, with a characteristic sweet odor, and edible. The fruits are covered in a stiff and hairy exocarp that represents 50% of the total weight

of the fruit, while the seeds represent 10% of the weight. From this, 60% of the total weight of the fruit is not utilized and considered to be a significant waste product (Bakar, et al., 2009).

To provide a new use to *Artocarpus odoratissimus* by-products and to contribute to the development of a new pharmaceutical product in the future, this research aimed to compare and investigate the presence of secondary metabolites in the different fruit parts of *A. odoratissimus*, namely the pulp, peel, and seed. The antidiabetic potential of the fruit parts was evaluated using the *in-vitro* assay by α-glucosidase enzyme inhibition.

2. Methods

2.1. Plant Extraction

The fruits of *Artocarpus odoratissimus* were harvested from a farm located at Brgy. Magsaysay, Marilog District, Davao City, Philippines. It was then brought and authenticated in the Herbarium of the Research Center for Natural and Applied Sciences, University of Santo Thomas, España, Manila, Philippines. Fifty (50) kilograms of matured fruit were separated into pulp, peel, and seeds and washed with distilled water. Fruit parts were dried using a fruit drier in a maintained temperature of 42°C until devoid of watery components. Fruit parts were ground using a homogenizer. Powdered fruit parts were percolated using separate percolator containing 95% ethanol (1:10 w/v ratio) as an extracting solvent. Resulting percolates were concentrated *in vacuo*, and crude extracts were stored at -20°C until further use. The color, odor, and nature of the extracts were examined organoleptically. The percentage yield was computed using the formula:

$$\%\ Yield = \frac{weight\ of\ extract}{weight\ of\ dried\ material} \quad \text{x } 100$$

2.2 Phytochemical Screening through Thin Layer Chromatography

The crude ethanolic extracts of *A. odoratissimus* fruit parts were used as a sample for the phytochemical screening through Thin Layer Chromatography (TLC). Approximately 5 mg of the extracts was dissolved in 1 mL dimethyl sulfoxide (DMSO). Samples were applied in commercially available Merck TLC Silica gel 60 F_{254} aluminum sheet plates measuring 20 x 65 cm.

The spotted plates were placed in the equilibrated chamber containing the solvent system to develop the chromatogram. The chromatograms were visualized by inspecting under the ultraviolet (UV) light, short-wavelength (240 nm), and long-wavelength (365 nm) UV before spraying with the reagent for the desired constituents. The spray reagents, antimony (III) chloride, vanillin-sulfuric acid, and potassium ferricyanide-ferric chloride ($K_4Fe(CN)_6$), were

utilized to screen the phenolics and flavonoid phytochemicals present in the *A. odoratissimus* fruit extracts.

2.3 Alpha Glucosidase Assay

The ability of the extracts to inhibit alpha-glucosidase enzyme was evaluated using the method from Chen *et al*. in 2019, with minor modifications. Quantification was done colorimetrically by monitoring the glucose released from sucrose (Bnouham *et al*, 2018). Concentrations (2 µg/mL – 1,000 µg/mL) of each crude extract and acarbose were prepared as samples. A concentration of 50 mM phosphate buffer system (PBS), maintained at a pH of 6.8, was used as diluent for the Para-nitrophenyl- β-D-glucuronide (p-NPG) and alpha-glucosidase enzyme.

p-NPG was used as a chromogenic substrate for the alpha-glucosidase enzyme. The p-NPG solution was prepared in 10 mM concentration to screen the most potent fruit part extract. The enzyme hydrolyzed the p-NPG and yielded the chromogenic product ρ-nitrophenol, which was yellow and measured spectrophotometrically at 405 nm at the ultraviolet to the visible range.

The crude extracts of *A. odoratissimus* fruit peel, pulp, and seed were prepared in 10 concentrations using PBS as diluent: 2, 4, 6, 8, 16, 31, 63, 125, 250, 500, and 1000 µg/mL. The same concentrations were prepared for the positive control, Acarbose. A concentration of 0.017 units/mL of alpha-glucosidase enzyme was prepared in cold PBS. All mixtures were freshly prepared for the experiment.

In a 96-well plate, 120 µL of the sample was mixed with 20 µL of α-glucosidase enzyme solution and incubated at 37°C for 15 minutes. After incubation, 20 µL of 10 mM pNPG was added to each well to catalyze the reaction mixture. The plate was then placed in an incubator at 37°C for another 15 minutes. The reaction was stopped by placing 80 µL of 0.2 M sodium carbonate into each well. The mixture was measured spectrophotometrically using the SkanIt software set at 405 nm. Percent inhibition of α-glucosidase was calculated as follows:

$$\%\ Inhibition = \frac{Absorbance\ of\ Control\ -Absorbance\ of\ sample}{Absorbance\ of Control} x\ 100$$

All the sample, blank (PBS), and positive control (acarbose) concentrations were performed in triplicates. The mean and its standard error (SEM) were used to summarize the data from the experiment. One-factor analysis of variance (ANOVA) was used to determine the effect of different concentrations on the percent inhibition of the extracts (Table 2). The IC_{50} of the extracts was estimated using four-parameter logistic regression models (Table 3). Also, the IC_{50} values were computed to determine which of the extracts had the most potent activity. All

statistical tests were performed in SPSS version 20.0 and GraphPad Prism version 7.0. P-values less than 0.05 indicated significant differences.

3. Results and Discussion

3.1. Plant extraction

The extraction done by percolation with 95% ethanol resulted in the extracts of pulp, peel, and seeds. The weight of the pulp extract obtained was 595.2 g, with a yield of 27.8%. The pulp extract is yellow, has an oily consistency and a sweet smell, resembling that of the fruit. The weight of the peel extract was 740.8 g with a yield of 46.95% and can be described as a green, syrupy consistency, and having a characteristic sweet smell. The seeds yielded a yellow, syrupy extract weighing 321.53 g (23.61%) and devoid of odor.

3.2 Phytochemical Screening through Thin Layer Chromatography

Extracts of *A. odoratissimus* were screened for the presence of phenolics and flavonoids. The pulp extract chromatogram was developed using the solvent system chloroform-acetic acid-methanol (4:1:2), while the seed and peel extracts were developed using DCM-Methanol (19:2) solvent system. All developed chromatograms were sprayed with different spray reagents. Antimony (III) chloride was used to screen flavonoids and steroids. A positive result for antimony (III) chloride was in the presence of intense yellow to orange visible zones that were also fluorescent under long ultraviolet light. The pulp, peel, and seed yielded 2, 3, and 4 spots for antimony (III) chloride, respectively. Vanillin-sulfuric acid confirmed the presence of higher alcohols, phenols, steroids, and essential oils. The positive result for this spray reagent was the appearance of blue-violet colored spots. Four spots were noted for the pulp and peel, while the seed extract afforded 2 spots. The last solvent system was potassium ferricyanide – ferric chloride ($K_4Fe(CN)_6$). The display of blue spots denoted positive results. The pulp, peel, and seed extracts identified 3 positive spots for $K_4Fe(CN)_6$ spray reagent. From these results, all extracts of the fruit *A. odoratissimus* were positive for the presence of phenolics and flavonoids.

Table 1. Phytochemical screening of *Artocarpus odoratissimus* fruit parts

Extract	Solvent System	Spray Reagents	Number of Spots Identified Positive
Pulp	4 Chloroform: 1 Acetic Acid: 2 Methanol	Antimony Chloride	2
		Vanillin-Sulfuric acid	4
		$K_4Fe(CN)_6$	3
Peel	19 DCM: 2 Methanol	Antimony Chloride	3
		Vanillin-Sulfuric acid	4
		$K_4Fe(CN)_6$	3
Seed	19 DCM: 2 Methanol	Antimony Chloride	4
		Vanillin-Sulfuric acid	2
		$K_4Fe(CN)_6$	3

Phenolic acids, such as ferulic and *p*-coumaric acids, are known potent antioxidants and anticancer activities against colon cancer. Ferulic acid was detected in the seed of *A. odoratissimus* (444.40 ± 23.13 µg/g) while none was detected in the flesh (Alkhalidy, et al., 2015). Diosmin, on the other hand, is a flavonoid that could be detected only from the fruit by-product and is used pharmaceutically as an active ingredient for hemorrhoidal preparations. Diosmin was found in the seeds of *A. odoratissimus* with 288.90 ± 70.88 µg/g quantity (Bakar, et al., 2015). Artocarpin is a flavonoid previously isolated in *A. odoratissimus* that can be used in cosmetic products due to its activity on the inhibition of tyrosinase and melanogenesis (Chan, et al., 2018). The phytochemicals which may contribute to the antidiabetic activity are the phenols and flavonoids. The pharmacological activity can be contributed to the reactive phenol moiety, which can scavenge free radicals (Bakar, et al., 2009). Scavenging the free radicals that affect several pathological pathways contributing to hyperglycemia is the target of phytochemicals (Parveen, et al., 2018)

3.3 Alpha Glucosidase Assay

The highest percent inhibition was seen in the 1,000 µg/mL concentration among all samples. The seed extract attained the highest inhibition of alpha-glucosidase enzyme (98.25±0.16%), followed by the pulp extract (96.32 ± 0.08%), then the peel extract (95.91 ± 0.08%), and lastly, acarbose (76.07 ± 1.64%). The results are promising because all extracts exhibit a comparable anti-alpha glucosidase activity to the positive control acarbose (*p>0.05).*

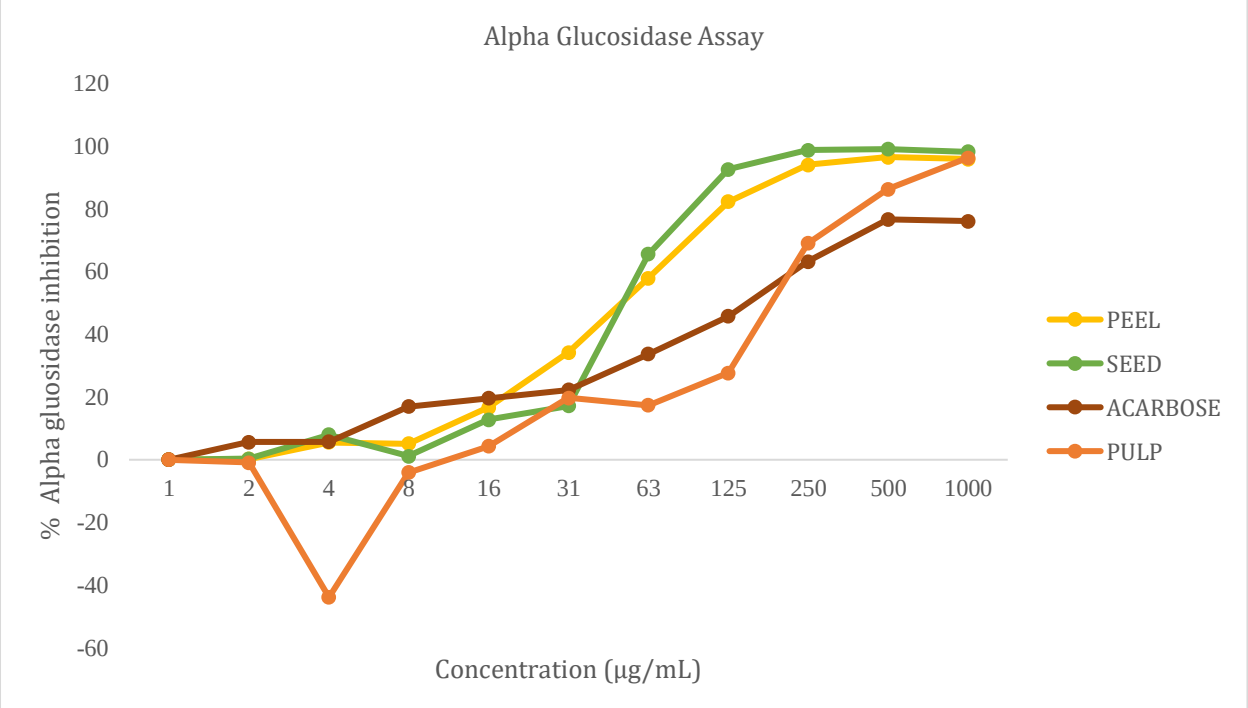

Figure 2. Inhibition activity on α-glucosidase enzyme from *A. odoratissimus* fruit extracts compared to acarbose in different concentrations. Results are reported as mean ± SEM (*n=3; p*>0.05*)* inhibition of the enzyme.

The mean was used to compare the percent inhibition of α-glucosidase and the extracts of *A. odoratissimus* fruit parts. The results reveal that the pulp, peel, and seed extract effects are comparable with the effect of the standard drug, Acarbose. Therefore, the peel and seeds of *A. odoratissimus* that are often considered as food waste can be a promising source of effectively natural alpha-glucosidase inhibitors.

The standard error of the mean was used to compare the concentrations of the extracts and the inhibitions. There is a significant interaction effect [F=3.190, p<0.05] between the extract and the concentrations, indicating that the activity of the extracts is dose-dependent. The concentration of 1000 µg/mL exhibits the highest inhibitory activity against α-glucosidase enzyme.

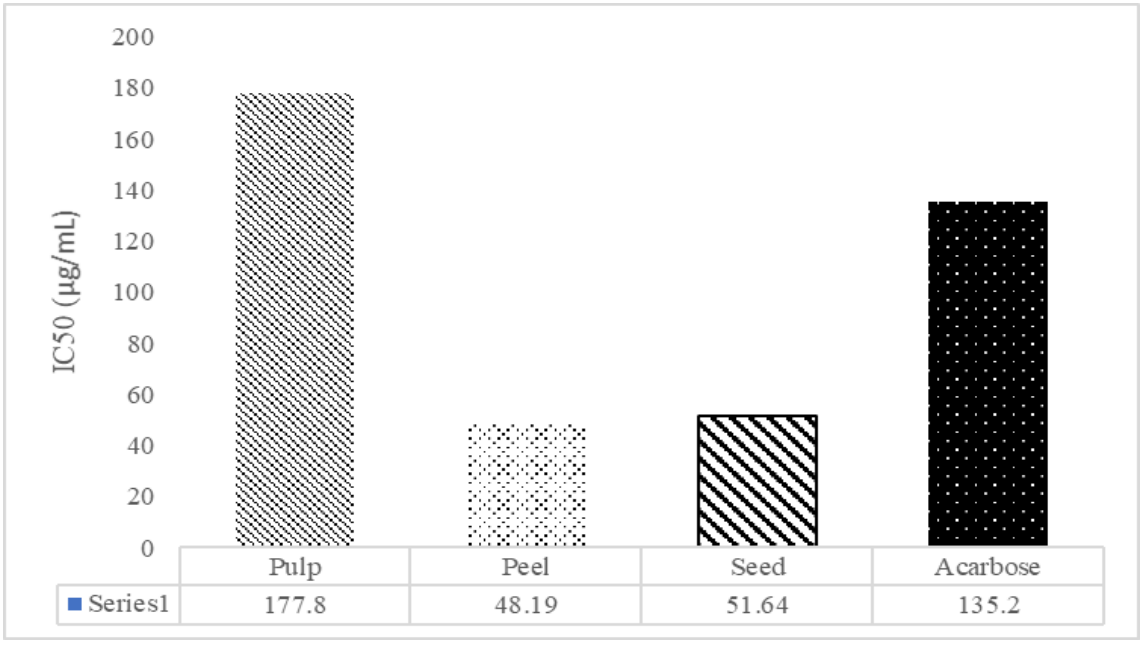

Figure 3. IC_{50} (µg/mL) estimates of extracts from the three fruit parts of *A. odoratissimus* and acarbose. Each value represents the mean (*n=3).*

The estimation of IC_{50} showed that the peel extract was the most effective with a value of 48.19 µg/mL. The extracts from the seed, pulp and acarbose yielded IC_{50} values of 51.64 µg/mL, 177.8 µg/mL, 135.2 µg/mL, respectively.

Alpha glucosidase enzyme digests carbohydrates and increases the postprandial glucose level among patients suffering from diabetes mellitus. The inhibition of alpha glucosidase enzyme is an *in vitro* model to reduce the risk of developing diabetes (Parani & Poovitha, 2016). The present study is able to establish the *in vitro* antidiabetic activity from the peel of *A. odoratissimus.* Further research is required to develop a novel drug from fruit peel of *A. odoratissimus.*

The presence of phenolics and flavonoids from the fruit peel of *A. odoratissimus* is a challenge for the discovery and development of antidiabetic molecules. Isolation of

pharmaceutically active compounds against diabetes mellitus should be considered for future studies (Firdous, 2014).

4. Conclusion

The pulp, peel, and seed of *A. odoratissimus* displayed a notable inhibitory activity against alpha-glucosidase enzymes *in vitro*. The highest activity was observed in the seed extract. The pharmacological activity of the fruit parts of *A. odoratissimus* is attributed to the phenolic and flavonoid content of the fruit parts. This study has proven that the peel, which forms about 60% of the *A. odoratissimus* weight, normally underutilized and discarded as a waste product, can be a potential source of antidiabetic agent.

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